OPEN ACCESS

Kashf Journal of Multidisciplinary Research
Vol: 02 - Issue 2 (2025)
P-ISSN: 3007-1992 E-ISSN: 3007-200X
https://kjmr.com.pk

CRISPR/CAS GENOME EDITING TO ENHANCE RESISTANCE LEADING TO ENHANCED PRODUCTIVITY IN CROPS

Afifa Marium*
Department of Biochemistry, Quaid I Azam University, Islamabad, Pakistan.
Sidra Tariq
Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar, Pakistan.
Hadeeqa Arshad
Department of Biochemistry, University of Agriculture Faisalabad, Pakistan.
Muhammad Salman Khalid
Department of Biomedical Engineering and Sciences, School of Mechanical and Manufacturing Engineering, National University of Sciences and Technology, Pakistan.
Bakhtawar Murtaza
Department of Biochemistry, The Islamia University of Bahawalpur, Pakistan.
Hira Aslam
Department of Plant Breeding and Genetics, PMAS-Arid Agriculture University Rawalpindi, Pakistan.
Akhtar Gul
Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar, Pakistan
Neelum Naheed
Department of Botany, PMAS-Arid Agriculture University Rawalpindi, Pakistan.
Aniqa Aziz
Department of Biological Sciences, University of Sialkot, Pakistan.
Ayesha Jabeen
Department of Biological sciences, Superior University Lahore.

Corresponding author: Afifa Marium (Afifamariumrwp@gmail.com)
DOI: *https://doi.org/10.71146/kjmr298*

Article Info

Abstract

CRISPR/Cas genome editing has revolutionized agricultural biotechnology by providing a precise, efficient, and cost-effective method for enhancing crop resistance against biotic and abiotic stresses. This technology enables targeted modifications in plant genomes, improving traits such as disease resistance, drought tolerance, and nutrient efficiency, ultimately leading to enhanced crop productivity. By knocking out susceptibility genes or introducing resistance-related genes, CRISPR/Cas facilitates the development of crops that can withstand pathogens, pests, and environmental challenges. One of the significant applications of CRISPR/Cas in agriculture is its role in combating plant pathogens, including bacteria, fungi, and viruses. By targeting genes associated with host susceptibility, scientists have successfully engineered resistance against devastating diseases such as bacterial blight in rice and powdery mildew in wheat. Additionally, CRISPR/Cas has been employed to enhance tolerance to abiotic stresses such as salinity, drought, and extreme temperatures, which are major constraints on global food security. Furthermore, genome editing can optimize nutrient use efficiency in crops, reducing reliance on chemical fertilizers and promoting sustainable agriculture. The precision of CRISPR/Cas minimizes unintended genetic modifications, ensuring the safety and stability of edited crops. However, regulatory challenges and ethical concerns regarding genome-edited crops remain a subject of debate.Overall, CRISPR/Cas technology presents a promising avenue for developing resilient, high-yielding crop varieties, addressing global food security challenges while promoting environmental sustainability. Continued research and policy advancements will be crucial in harnessing its full potential for future agricultural innovations.

Keywords:
CRISPR/Cas technology, Crop resistance, Abiotic and biotic stress tolerance, Sustainable agriculture

Introduction

Agriculture plays an important role to enhance the economic growth of any country [1]. It is predicted that by the year to 2050 the world population will reach upto 10 billion [2] [3] and 11.0 billion by 2100 [4] so it causes challenges for the world to achieve food security [5]. Due to rapidly increasing world population, global food demand increases by more than 50% [6]. As a result, the demand for agriculture production will increase upto more than 100% by 2050 [8] [9]. Feeding the rapidly growing global population has become a significant challenge, with the increasing demand for food posing a major concern. To address this issue, CRISPR/Cas technology has been developed to enhance agricultural productivity by improving crop yield and growth. The complete name of CRISPR/Cas9 is "Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9."(CRISPR/Cas9 applications and future prospectus in crop genetic improvement) and climate is the first causal factor of agricultural productivity [10] Climate change that directly affect agricultural production are increase in temperature, increase in level of CO2 which is requirement of photosynthesis, change in weather condition and frequency of precipitation. [11] It is expected that in the 21st century climate change is the reason of 25% reduction in world food production [12]. CRISPR-Cas technology is used to generate transgenic plants [13]. crops such as soybean, wheat, rice and maize very easily and precisely genetically modifed [15] genetically modified crops are sourse of sustainable agriculture [16] CRISPR/Cas9 is a molecular tool, it modify the important crop traits, such as, resistance to diseases, improve quality, and generate herbicide tolerance in plants, [17]. and high yield which is the most important advantage [18]. Genome Editing through CRISPR-Cas is efficient, simple and most accurate technique as compared to old methods such as MNs, ZFNs, and TALENs. [19] so it rises the hope to get global food security [20]. The fundamental purpose of this review article is to provide an in-depth analysis of CRISPR/Cas genome editing technology in the agricultural sector, with a specific focus on its potential to enhance resistance and alternatively productivity in crop plants.

Discovery of CRISPR Cas Systems:

More than 30 years after its discovery in archaea and Escherichia coli, CRISPR is doing just well [21] [22]. A link to CRISPR was discovered, beginning with the concept that cas proteins are involved in DNA repair [23]. Since these infections target cells with spacer sequences, some have proposed using CRISPR-Cas to eradicate them[24,25, 26]. According to studies on RNA-guided DNA cleavage, CRISPR-Cas could protect cells from harmful phages by acting as an adaptive immune system [27].

According to scientists, the first occurrence of a CRISPR array was discovered in the genome of Escherichia coli in 1987. Consisting of a sequence of repeated elements spaced uniformly apart, the array lacked an apparent purpose [15]. Since the regions that divide CRISPR repetitions match to specific places in these genomes, you could employ CRISPR arrays to target phage genomes that aren't currently used for vaccination [28]. Research into phage sequence matching and the existence of a big CRISPR-associated protein with a DNA-cleaving HNH domain led to the discovery that Streptococcus thermophilus is the host of the type II system and its defining protein, Cas9 [24]. Phages are developed to be resistant to specific phages with matching sequences because they are utilized as spacers in CRISPR arrays. Researchers identified this resistance by employing the S. thermophilus type II system. Another important function of cas genes is in vaccines and phage interference [27]. The seemingly unbelievable but actually true claim that CRISPR-Cas is an adaptive immune system in bacteria has been validated..

Classification of CRISPR Cas System:

An RNA guide, crRNA, and either a pair of Cas proteins (Class 1) or a single multi-domain Cas protein (Class 2) are required components of the "effector complex" in CRISPR-RNA Cas for RNA-guided DNA/RNA cleavage. Each class's subtypes are specified by the CRISPR-Cas locus architecture, which is based on finding the signature protein that cuts the target polymerase. The most current taxonomy of the species divides it into two main groups, six subgroups, and 33 subtypes [29].

Class 1 CRISPR-Cas systems are mainly categorized as I, III, and IV, as shown below [29]. [30]. A type I effector complex called CASCADE, which stands for CRISPR-associated complex for antiviral defense, is formed by a wide variety of Cas proteins and crRNA. (31, 32). Cascade activates Cas3 when it finds the DNA that needs cleavage [33]. Cas3 is a nuclease and helicase that causes major changes to DNA [34]. The selection and cleavage of transcriptionally active RNA in type III transcription is mediated by crRNA complementarity. In addition, it helps the single-stranded DNA inside the transcription bubble to break. Whereas Cas10 can only cleave single-stranded DNA, Cas7 can cleave RNA as well [35] [36] [37]. A proper immune response requires both of these things to be true [38, 39, 40, 41]. To further eliminate surplus nucleic acids, certain mechanisms cooperate with degradosome-nucleases. Plasmids and other analogous locations often harbor the fourth kind of CRISPR-Cas, which is restricted, does not contain adaptation proteins, and may help with plasmid preservation [42]. We still don't know which nuclease DinG helicase needs to avoid interfering with type IV-A plasmids [43]. references [44, 45].

There are three different types of CRISPR-Cas, and they are all included in this category. In order to damage its target DNA, the type II effector protein Cas9 uses the endonucleases HNH and RuvC to cut the DNA strands in half. There must be crRNA and another non-coding RNA called tracrRNA for DNA cleavage to occur in type II systems [46]. The effector protein Cas12 defines type V systems via staggered, sequence-specific DNA cleavage using its RuvC domain. The various subtypes of type V systems have different needs when it comes to target and guide RNA, crRNA usage, and crRNA-scout (short-complementarity untranslated) application [47] [48]. After binding to crRNA, the type VI signature nuclease Cas13 recognizes a complementary RNA target and triggers processive RNase activity through its HEPN domains. (49, 50).

Mechanism of CRISPR Cas system:

There are three distinct stages for CRISPR defense.

Adaptation: In the adaptation phase, a brief segment of DNA is removed from foreign DNA and precisely integrated into the CRISPR array, resulting in the formation of a new spacer. Established adaption mechanisms necessitate Cas1 and Cas2 for the acquisition and catalytic insertion of the spacer. Auxiliary proteins, including Cas4, Csn2, Cas9, and non-Cas proteins like integration host factor, are crucial for maintaining fidelity in specific subtypes. Type III systems utilizing reverse transcriptase-Cas1 fusion proteins are capable of acquiring spacers from RNA. [51]. Recent reviews have examined the specifics of adaptability. [52] [53]

crRNA Processing: Generally, the CRISPR array is translated into an elongated pre-crRNA, which complexes with Cas proteins for subsequent maturation into mature crRNAs. Class 1 systems necessitate Cas6 for the processing of pre-crRNA into distinct crRNA molecules [38] [39] [54]. Class 2 systems lack a characteristic Cas protein for crRNA processing and instead employ other methods. Type II systems rely on RNase III. [55] [56] Type V-A and type VI signature nucleases feature a unique active site for crRNA processing, with specific type V subtypes relying on host nucleases [57] [58] [59]. Certain type-II systems directly transcribe mature crRNAs utilizing specific promoters inside the CRISPR array [60].

Interference: This phase entails the sequence-specific identification and cleavage of exogenous DNA and/or RNA. The preceding classification section delineates pertinent proteins and cleavage types. Recent reviews have elucidated the structural and molecular aspects of CRISPR interference [61][62] Interference entails R-loop formation as the crRNA guide region hybridizes with target DNA or engages in base-pairing with target RNA. This is succeeded by the cleavage or degradation of the target.

CRISPR-Cas systems must differentiate between self and foreign DNA to prevent self-targeting. The criteria for intruder cleavage include (i) the presence of a protospacer adjacent motif (PAM), a DNA motif adjacent to the RNA-DNA complementary region in types I, II, and V; (ii) the lack of RNA complementarity between the 5′-tag of crRNA and the 3′ flank of the target RNA in type III; and (iii) the presence of a protospacer flanking sequence (PFS), an RNA motif within the target RNA, in type VI [63].

Delivery methods of CRISPR Cas System:DNA, RNA, and proteins are among the many viable modalities for CRISPR/Cas delivery. Cas and gRNA are transported as one plasmid when the delivery method is DNA. A distinct gRNA carries Cas mRNA in the RNA mode. The Cas protein is supplied as a ribonucleoprotein complex (RNP) with gRNA in the protein mode. There are some restrictions to each mode, but overall, they are successful.

Virus vectors and non-viral vectors are the two main categories of delivery vehicles. It is recommended to use viral vectors for CRISPR/Cas in vivo delivery. Vectors that do not utilize viruses have not yet seen the same level of use as those that do. Even though viral vectors get a lot of attention, non-viral vectors are just as important and are the subject of much study.

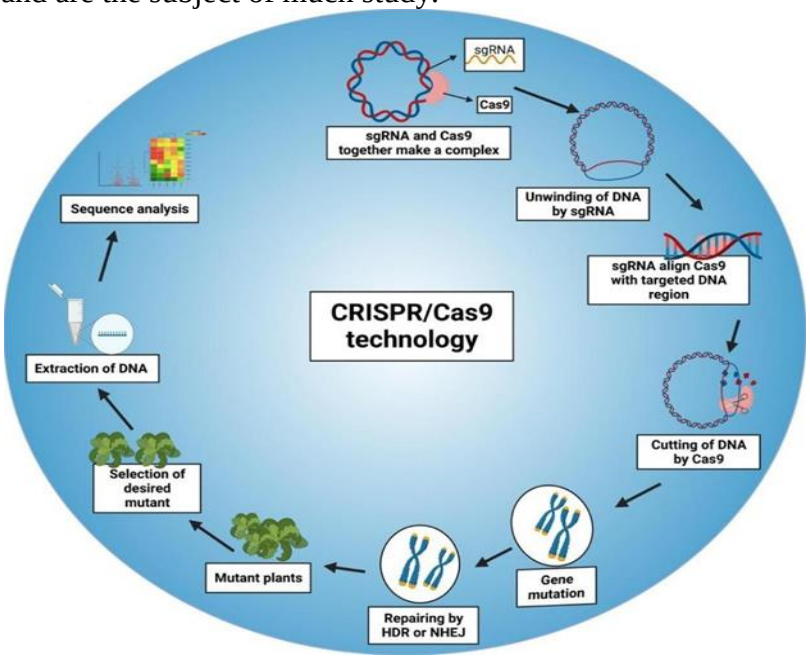

Figure 1: CRISPR/Cas Technology Mechanism

Viral based CRISPR/Cas gene editing and delivery:

To modify and transport genes using the CRISPR/Cas system, viruses are utilized. The three most common viral vectors used for this purpose are lentivirus, adenovirus, and AAV. Despite the high effectiveness of viral distribution in vivo, there are a few drawbacks, such as safety concerns. To infect host cells and release their viral genome is how these viruses function. Because of the importance of robust viral-host cell connections, viral delivery systems are more complex than their non-viral counterparts when tested in living organisms.

Lentivirus:

The lentivirus family of RNA viruses can infect both viable and non-viable cells. Lentiviruses are great vectors for transporting genes to cells that are difficult to transfect chemically. In addition, it possesses a vast packaging capacity of around 10.7 kilobytes [64]. It is able to contain several sgRNA sequences, which permits it to cause numerous gene edits simultaneously, thanks to this characteristic [65]. Many early gene-editing investigations used lentiviruses because of these benefits. When it comes to myeloid malignancy [66] and lung cancer [67], lentivirus administration has been used to create mouse models. A potential carcinogenic effect of integrating the viral genome is one of the drawbacks of lentivirus [68].

Improvements in lentiviral vector technology have led to the creation of integration-defcient lentiviral vectors (IDLVs), which mitigate these problems by limiting the viral genome's unintended integration with the host cell genome [69] [70]. Despite challenges in transfecting some cell types, IDLVs maintain their gene-editing capabilities [71] [72]. While it's true that IDLVs might alter genes in undesirable ways, the research also demonstrated that, by actively recruiting host HDR proteins, IDLVs can repair genetic

damage at specific sites [73]. The use of IDLVs could be enhanced by combining them with endonucleases that are less harmful, like SpCas9-HF or eSpCas9 [74] [75].

Adenovirus:

The adenovirus is a type of virus that uses double-stranded DNA. Infectiousness of dividing and non-dividing cells is shared by adenoviruses, just like lentiviruses. But adenoviruses don't produce potential of-target effects like lentiviruses do since they don't usually promote genome integration in the host DNA. Results from studies in adult mouse liver indicate that the Pcsk9 and Pten genes can be efficiently edited using the CRISPR/Cas system that is delivered by adenoviruses [76] [77]. Also, echinoderm microtubule-associated protein like 4-anaplastic lymphoma kinase (EML4-ALK)–driven lung cancer in vivo can be effectively induced using adenovirus-based administration [78]. But adenoviruses can make the immune system go into overdrive. Additionally, adenoviruses are not only difficult to mass-produce but also expensive. Problems like these restrict the use of adenovirus-mediated delivery to certain clinical gene therapy applications [76].

Adeno-associated virus:

AAVs, or adeno-associated viruses, are tiny ssDNA viruses. Since AAV-based administration causes minimal cytotoxicity and immunological responses, it is both safe and efficient when compared to lentivirus and adenovirus-based delivery [79, 80]. One advantage of AAVs for efficient gene editing is their large serotype diversity, which allows them to target a variety of tissues [81]. Swiech et al. [82] documented the first effective AAV-based CRISPR/Cas9 gene editing in the mouse brain, for instance. In a study conducted by Hung et al., identical methods were utilized to edit genes in the retina of adult mice, and the results showed promising editing effects [83]. Additionally, CRISPR/Cas components have been delivered to the central nervous system by AAV-based delivery to knockdown IGF [84]. The adoption of a self-complementary AAV (scAAV) method enhanced the effectiveness of CRISPR-Cas9 mediated gene editing in a DMD mouse model, as recently shown by Zhang et al. [85]. Another study that successfully deleted the MIR137 gene in a CRISPR/Cas9 knockin mice model of schizophrenia was conducted by Murlidharan et al. [86]. They used a chimeric AAV (AAV2g9) to deliver gRNAs targeting the MIR137 gene. Though there has been advancement in employing AAVs for CRISPR/Cas-based gene editing, their limited cargo capacity (<4.7 kb) might restrict their use. This means that a different vector system is typically needed when combining 4.2 kb standard SpCas9 with sgRNA. Researchers later created smaller Cas9 orthologs in bacteria including Staphylococcus aureus (SaCas9) [87], Campylobacter jejuni (CjCas9) [88], and Streptococcus thermophilus (StCas9) [89] to allow for in vivo gene editing with a single AAV vector.

Nonviral based CRISPR/Cas gene editing and delivery

DNA based delivery :

The CRISPR/Cas system is typically introduced into cells using DNA-based delivery rather than RNA-based delivery due to the stability of DNA. Compared to alternative gene editing approaches, CRISPR/Cas-encoding DNA allows for better efficiency [90] [91] [92]. As an example, a mouse model of tyrosinemia was able to achieve>6% gene correction in the liver cells following a single application of the CRISPR/Cas9 components, which were supplied in DNA form by tail-vein hydrodynamic injection [93]. In addition, Zhen et al. found that hydrodynamic injection of CRISPR/Cas9-encoding DNA successfully changes the hepatitis B viral DNA, rendering it incapable of replicating [94]. In addition to systemic delivery, a rat model of retinitis pigmentosa has been shown to undergo allele-specific gene editing using electroporation and subretinal injection of plasmid-based CRISPR/Cas components [95]. In a mouse model of retinitis pigmentosa, Latella et al. also discovered a comparable effect, which prevented serious visual dysfunction and significantly lowered levels of mutant proteins [96]. On top of that, Li et al. showed that in RhoP23H knock-in mice, the P23H allele—which has a single-nucleotide mutation—can be preferentially targeted using gene editing [97]. In addition, Shinmyo et al. [98] successfully performed in vivo brain-specific gene editing by introducing a plasmid containing CRISPR/Cas

components into the mouse brain using in utero electroporation. These studies proved that CRISPR/Cas9 delivered via DNA could be useful in living organisms.

RNA based delivery :

RNA-based delivery strategies significantly lessen the possibility of genome integration with the host. Although there are a few extra drawbacks to RNA-based delivery techniques, their effective time is relatively fast. For instance, this approach primarily addresses two issues: the requirement to supply the components (Cas mRNA and sgRNA) independently, and the stability of RNA. The CRISPR/Cas9 components were introduced using a delivery strategy demonstrated by Yin et al., wherein lipid nanoparticles carried the Cas9 mRNA and an AAV carried the sgRNA/HDR template. In a mouse model of familial tyrosinemia, they demonstrated effective repair of the Fah gene using this approach [99]. While this combined strategy does have some success, it is still dependent on viral codelivery, and RNA is far more prone to errors than DNA and protein. Additionally, editing efficiency may be drastically impacted by sgRNA degradation. To make these approaches even more effective, researchers need to find ways to make sgRNA more stable. Research has demonstrated that sgRNA stabilization can be improved by making modifications to sgRNA. In their modification of sgRNA, Yin et al. introduced phosphorothioate linkages and replaced the 2′OH group with 2′OMe and 2′F [100]. Researchers found that a single injection successfully edited Pcsk9 in mouse livers with an efficiency of over 80%. This suggests a potential modified approach to enhancing RNA stability and overcoming the challenges of RNA-based delivery. Furthermore, several teams have detailed a comparable investigation using lipid nanoparticles to transport modified sgRNA and mRNA expressing Cas9. The levels of the mouse Ttr (transthyretin) gene in the liver's serum protein decreased by almost 97% after just one injection. This research proved that gene editing is an effective method that can last for at least a year [101]. A different study found that using a lipid nanoparticle with disulfide bonds (BAMEAO16B) to transport Cas9 mRNA and sgRNA in vivo achieved an editing effectiveness of approximately 80% [102].

Protein based delivery : The most direct and efficient method for editing genes, which is also appropriate for in vivo therapeutic uses, is to deliver Cas protein with gRNA as a Cas9 RNP. A fusion protein including Cas9 and negatively charged proteins was developed to enable the delivery of Cas9 RNPs into target cells using transfection reagents designed with cationic lipids, such RNAiMAX [103]. Transgenic Atoh1 (atonal bHLH transcription factor 1)-GFP mice had a 13% decrease in GFP in their ears after receiving the Cas9 RNP/RNAiMAX complex through injection into their cochlea. When it comes to in vivo gene editing, Mangeot et al. [104] developed a nanoblade-based vector called MLV to transport Cas9 RNPs. There is also evidence of effective gene editing in a mouse model of Alzheimer's disease using an amphiphilic nanocomplex that was created to deliver Cas9 RNPs in vivo [105]. In addition, Cas9 RNP delivery in vivo was facilitated by the use of PEI polymers or a combination of PEI polymers and liposomes, which improved endosomal escape. Sun et al. introduced Cas9 RNPs into human cell nuclei by coating a DNA nanoclew with PEI polymers. The use of this vehicle allows for the disruption of target genes while simultaneously reducing cell viability [106]. An further way to improve editing efficacy, according to the paper, is to modify DNA nanoclew so that it is partially complimentary with the sgRNA. Direct cytoplasmic/nuclear transport of Cas9 RNP can be further enhanced by modifying the Cas9 protein. The Cas9En protein was created by Mout et al. [107]. It features an appended negatively charged oligo glutamic acid tag to the N-terminus of the Cas9 protein. The positively charged argininefunctionalized gold nanoparticles (Arg-AuNPs) were used to transport the Cas9En RNPs. Cas9 RNPs, which had the NLS attached, were transported directly to the cytosol, where they accumulated and achieved an editing efficiency of approximately 30%. Cas9 RNP delivery in vivo using this nanoassembled platform recently achieved>8% gene editing efficiency [108].

When contrasted with DNA and RNA-based delivery, protein-based administration often provides less immune response and fewer off-target consequences [109]. Cas9 RNPs are more effective because they prevent sgRNA degradation. Nevertheless, therapeutic benefits rely on Cas9 RNP trafficking into the cytosol or nucleus. Therefore, endosomal entrapment remains an important challenge [110].

Current approach of delivering the CRISPR/ Cas system in aquaculture

The CRISPR/Cas system has been utilized to successfully modify the genomes of numerous species in the aquaculture industry. These include zebrafish, Atlantic salmon, Nile tilapia, sea bream, catfish, carp, rainbow trout, Northern Chinese lamprey, and Pacific oyster. There has been gene editing in aquaculture species using CRISPR/Cas techniques that were designed for model species like zebrafish [111]. When working with aquatic species, microinjection is the go-to technique for gene transfer. One highly effective approach of gene editing is microinjection, which involves injecting the CRISPR/Cas complex into freshly fertilized eggs using specialized equipment [112]. Most mutations were induced by NHEJ, however HDR has been utilized successfully with rohu carp [113]. Genomic mosaicism, on the other hand, is possible if gene editing continues during early embryonic development. Addressing these difficulties is the primary goal of ongoing research that aims to facilitate the wider implementation of CRISPR/Cas technology in aquaculture. There have been advancements in aquaculture species sterility, growth, and disease resistance through the use of CRISPR/Cas technology. One goal of inducing sterility in fish is to conserve domesticated strains by stopping gene flow. We have employed CRISPR/Cas approaches to induce sterility in Atlantic salmon, for example [114]. An increasing number of studies have shown that channel catfish and common carp, among others, can have their myostatin genes edited using the CRISPR/Cas method to increase their growth rates [115,116]. Investigations on channel catfish, rohu carp, and grass carp immunity and disease resistance have also made use of the CRISPR/Cas method [113] [117] [118]. Rohu carp, by having its tlr22 gene disrupted, become a model for immunology research, proving that CRISPR/Cas can help find better treatments for aquaculture. Strengthening disease resistance, decreasing disease incidence, and improving species resilience in aquaculture can be achieved by understanding the underlying processes of transcription and translation through CRISPR/Cas-based procedures. For several reasons, CRISPR/Cas gene editing is a brilliant tool for aquaculture. Through the use of thousands of embryos that have been externally fertilized, microinjection can be performed by hand, allowing for enormous sample numbers without causing excessive costs. The huge sample size allows for fair comparisons of altered samples to controls and evaluations of disease resistance. Additionally, since broad phenotypes are feasible with a high sample size, well-formed disease challenge models can be built. Aquaculture technology is progressing to the point that it is easier to research gene function, increase disease resistance, and create new strains with desirable traits that enhance economic value.

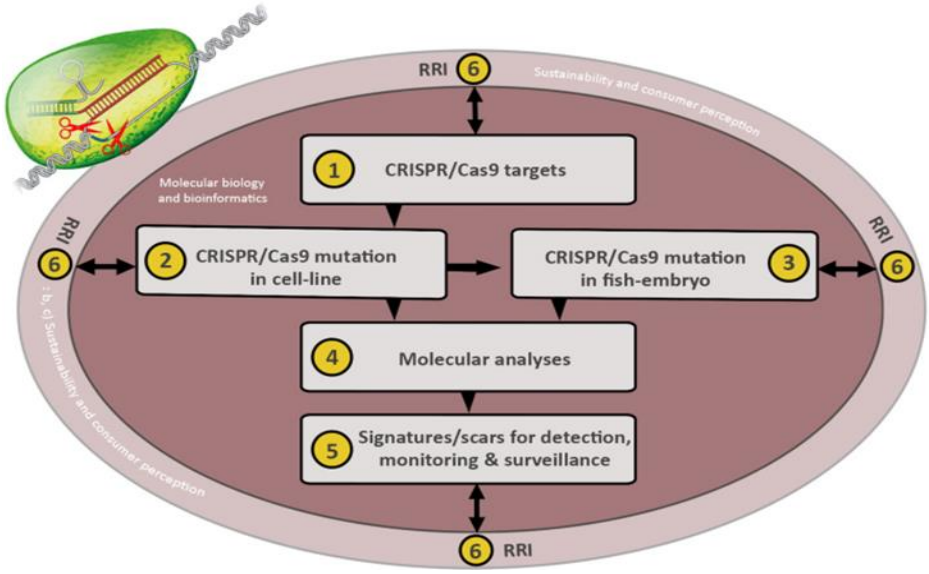

Figure 2: A schematic representation of the CRISPR/Cas9 workflow, illustrating the process from target selection to molecular analysis and detection. The diagram highlights key stages, including identifying CRISPR/Cas9 targets (Step 1), inducing mutations in cell lines (Step 2) and fish embryos (Step 3), performing molecular analyses (Step 4), and detecting genetic signatures for

monitoring and surveillance (Step 5). The outer framework emphasizes Responsible Research and Innovation (RRI), considering sustainability, consumer perception, and ethical considerations in CRISPR-based genome editing.

Current approach of delivering the CRISPR/ Cas system in plants

We have seen that the CRISPR/Cas system works very well to alter the genomes of animals. Proof of its capacity to alter plant genomes has also been found in research. Traditional methods for plant CRISPR/Cas system expression involve a mixed dual promoter system. To express Cas protein in mixed dual promoter systems, RNA polymerase II promoters are utilized. For gRNA, RNA polymerase III promoters that are particular to plants, such as AtU6 for tomato or Arabidopsis, TaU6 for wheat, and OsU6 or OsU3 for rice, are employed [119] [120] [121] [122]. But to use CRISPR/Cas9 technology to engineer novel plant features, it is crucial to efficiently distribute the CRISPR/Cas system into cells. Both direct and indirect ways of distribution are used by plants. Some approaches involve using viruses or bacteria in plants to introduce DNA constructs into specific plant cells. These methods are known as indirect methods. One key difference is that direct distribution does not include any living beings acting as intermediaries. Two popular direct approaches are protoplast transfection and biolistic particle delivery. Because of its adaptability and ease of usage, agroinfiltration has seen extensive use as a one-time test [123] [124, 125] [126] [127]. For stable editing, the usual tools are agroinfection, biolistic particle delivery, and viral infection. You can employ protoplast transfection for both temporary and permanent editing.

Transient events Indirect method Agroinfiltration: Agrobacterium spp. are plant pathogens. Agrobacterium tumefaciens generates tumor-like growth on the aerial parts of plants (crown gall), whereas Agrobacterium rhizogenes causes root tumors. Agrobacteria possess a substantial plasmid (surpassing 200 kb), designated Ti for A. tumefaciens and Ri for A. rhizogenes, which facilitates the transfer of a particular DNA segment (transfer DNA or T-DNA) into the infected plant cells, allowing for the integration of T-DNA into the host genome. These two strains of Agrobacterium have been engineered to possess a disarmed Ti/Ri plasmid from which tumor-inducing genes have been excised. The critical components of the T-DNA, border repeats (25 bp), are requisite for plant transformation and are utilized to produce transgenic plants. Agroinfiltration is a temporary procedure wherein a culture of A. tumefaciens, bearing mutated T-DNA, is directly injected into plant leaves[128] [129] [130]. A. rhizogenes is utilized for root hair transformation to assess editing efficiency in plant root hairs, predominantly in legume species like Medicago and soybean [131-133].

Method of direct approach Transfection of protoplasts:

Protoplast transfection is a technique utilized for transfection and transient experiments. This technique enzymatically degrades the cell walls of plant tissues and employs polyethyleneglycol (PEG) for transfection or electroporation for distribution. The identical protoplasts can convey several DNA structures. Protoplast transfection has been demonstrated to effectively introduce the CRISPR/Cas system, resulting in gene editing in Arabidopsis thaliana, Nicotiana benthamiana, rice, wheat, and maize, among other species. [120] [134] [135] [136] [137] [138] [139].

Stable events Indirect approachAgroinfection: Agrobacterium-mediated DNA transfer is the predominant technique employed for nearly all model plant species, major crop species, vegetable and fruit commodities, as well as forest crops. Agrobacterium, akin to agroinfiltration, can generate transgenic plants through the integration of its genome into the plant's nuclear DNA [140].

Viral infection:

The inaugural viral vector employed in plants was the tobacco mosaic virus (TMV). Investigators employed TMV to inhibit a gene in N. benthamiana [141]. The predominant category of plant viruses consists of RNA viruses characterized by single-stranded RNA genomes, which can be manufactured in vitro for plant inoculation or generated in vivo as DNA viruses from plasmids delivered directly into plants by mechanical methods for gene delivery [142]. To expedite the delivery process, the viral genome may be included as a cDNA fragment into a binary vector, therefore facilitating agroinfection-mediated distribution into a plant cell.

Tobacco rattle virus (TRV) is a single-stranded RNA virus including two genomic components, TRV1 (RNA1) and TRV2 (RNA2). Both genomic components are essential for inoculation. Plants modified by RNA viruses do not demonstrate germ line transmission of modifications. Ali et al. employed agroinfection to introduce the RNA1 genomic component of TRV and a vector derived from TRV RNA2, which contains targeting gRNA, into the leaves of N. benthamiana that overexpresses Cas9 for gene editing in plant cells [143].

Geminiviruses, in contrast to TRV, do not necessitate in vitro transcription before inoculation. Geminiviruses possess a circular single-stranded DNA genome. Geminiviruses lack a gene for DNA polymerase; hence, their ssDNA genomes are transformed into dsDNA genomes by host DNA polymerases within the nucleus. The dsDNA genome serves as a template for viral transcription and rolling circle replication. The replication initiator protein (Rep) is crucial for the commencement of rolling-circle replication. Rolling circle replication can either transform ssDNA genomes into dsDNA genomes or encapsulate ssDNA genomes into virions. Plasmodesmata routes in plants enable the translocation of virions to neighboring cells [144] [145]. The bean yellow dwarf virus (BeYDV), classified as a geminivirus, has been utilized to administer the CRISPR/Cas system [144]. Research has shown gene editing utilizing BeYDV in tomato (anthocyanin mutant 1 gene, ANT1), while a modified cabbage leaf curl virus (CaLCuV) has been employed in tobacco [120] [146]. These methodologies have been utilized in wheat, with researchers improving the efficacy of this technique through the development of an improved wheat dwarf virus (WDV) system [147].

Direct method Protoplast transfection: In contrast to the ephemeral approach of protoplast transfection, the stable transformation strategy produced targeted genomic alterations in whole plants regenerated from gene-edited protoplasts [137, 138]. Two advantages of protoplast transfection are the capacity to supply several components and to do so in substantial quantities. This technique is very effective for gene editing via donor template repair. A substantial number of transfected cells can enhance gene editing recovery through donor template repair. Nonetheless, a drawback of protoplast transfection is the regeneration rate of monocotyledonous plants. Protoplast transfection has been employed for gene editing in potato[148], tobacco, and lettuce [138].

Biolistic particle delivery :

Biolistic particle delivery is achieved through the transfection of cells via bombardment. Gene guns utilize physical force to breach the cell wall of plant cells for DNA delivery. This approach is prevalent in plant transformation because to its efficiency and capacity to introduce several DNA constructs concurrently [149]. Crucially, there are no restrictions on plant species for biolistic particle-based administration. The primary drawback of this strategy is that the introduction of numerous DNA copies in the target plants may lead to unintended consequences, such as gene suppression, in the resultant transgenic plants. Biolistic particle delivery has been employed for gene editing in rice, wheat, soybean, and maize via the CRISPR/Cas system [150] [151] [152]. This approach is utilized to administer CRISPR/Cas9 RNPs for gene editing in crops, including hexaploid wheat and maize [153] [152].

Crispr Cas Genome Editing on Different Stresses

Abiotic stress

Ion toxicity, salt, heat, drought, flooding, and radiation are the primary causes of abiotic stress in the modern world, which contributes to over half of the yearly crop loss [154]. Excessive production of greenhouse gases raises global temperatures, which in turn increases yearly crop loss by 20%. The mutant variant of transgenic plants may become more susceptible to resistant transgenes as a result of their enhanced ability to withstand abiotic stress [155]. As a result, new technology have opened up even more avenues for addressing abiotic stress in plants.

CRISPR/Cas on drought stress tolerance

The plant community is severely impacted by drought, the most common abiotic stressor caused by global warming. In recent years, researchers have used CRISPR-Cas9 technology to study plant communities' responses to drought stress and find ways to adapt to it. Zea mays[156][157][158],

Triticum[159][160][161][162], Oryza sativa[163,164][165][166], and many more crops have had drought tolerance QTLs (Quantitative trait loci) mapped. Abscisic acid (ABA) is a stress hormone that is essential for controlling salt and drought stress. Stomatal closure, caused by abscisic acid, decreases water loss in plants. An essential component of ABA-dependent development, a process in plants that is essentially a response to hyperosmotic stress, is SNF 1-related protein kinase 2 (SnRK2). The loss-of-function mutation of ABA-activated protein kinase 2 in rice plants is caused by CRISPR/Cas9. This makes the plants more vulnerable to drought.[167]

Corn drought tolerance genes are caused by the ethylene response pathway negative regulator gene ARGOS8, which has a low sensitivity to ethylene. Although a large number of drought-resistant natural genotypes were identified against ARGOS8, their expression levels were quite low. As a result, a CRISPR-Cas9-dependent approach to producing ARGOS8 has been well-established under the less-expressing constitutive promoter GOS2 [168].

Tomatoes mutated for the SlNPR1 gene using CRISPR/Cas9-dependent technology showed a marked reduction in drought stress. The SlNPR1 gene, which is involved in drought stress reduction, is also thought to regulate the SlGST, SlDHN, and SlDREB genes simultaneously [169]. Tomatoes were shown to have SlMAPK3 as a drought stress modulator when MAPKs were edited using the CRISPR/Cas technique [170] [171]. Reduced antioxidant enzyme activities, elevated hydrogen peroxide content, increased wilting, and enhanced membrane damage under drought stress are all characteristics of the mutant line that results from deleting the third exon of SlMAPK3. Additionally, SlMAPK3 prevents oxidative damage to cell membranes by regulating stress-related genes.

Through the use of a truncated gRNA (tru-gRNA)/Cas9 combination, researchers in Arabidopsis were able to create new alleles for the OPEN STOMATA 2 (OST 2) gene, which showed altered stomatal closure in reaction to environmental stress [172]. Evidence suggests that ABA-induced transcription repressors (AITRs) have a role in stress tolerance via modulating ABA-regulating signals. In order to increase plants' stress resistance, CRISPR/Cas9 technology produces quintuple mutants, as seen in the Arabidopsis aitr2aitr5aitr6 (aitr256) triple mutant, which expresses their functions in drought and salt tolerance [173].

CRISPR/Cas on salinity stress tolerance

In recent years, salinity stress has been further accelerated by the detrimental effects of climate change [174]. Soil salts build up as a result of water evaporation, transpiration, or drift from the sea. These salts impede plant development and growth by preventing the roots from taking in nutrients. The capacity of a plant to sustain its biomass and/or yield equilibrium in the face of salt stress is known as salinity tolerance [175]. Soil salinity can be caused by a variety of human actions, including the use of fertilizers and irrigation with salt water. There are three forms of tolerance that plants use to protect themselves: ion tolerance, osmotic tolerance, and tissue tolerance [176]. There are 51 genes in rice that make it resistant to salt [177].

To regulate cytokinin signal transduction, the OsRR22 gene is crucial in Oryza sativa. Increased tolerance to salt is the result of a decrease in OsRR22 transcription [178]. The OsRR22 gene was altered using CRISPR/Cas9 technology. Two homologous T2 generations later, there was no discernible difference between the modified and wild type lines in terms of salinity tolerance [179]. The OsRAV2 gene in rice was able to withstand high salt concentrations when engineered using CRISPR-Cas [180]. A plant that is both taller and more tolerant of salt was created by employing the CRISPR-Cas9 technique to generate the Osnac041 mutant [181]. The rice plant showed resilience to drought and salt in mutant alleles produced by CRISPR-Cas [182].

Pumpkins have a lot of salt-tolerant genes, including RBOHD, AHA1, GRF12, and HAK5, which are proteins involved in potassium transport and plasma membrane H+ ATPase. Plants become salt sensitive after CRISPR/Cas9 technology knocked down NADPH oxidase (RBOHD). The hypothesised importance of RBOHD-dependent H2O2 signaling in root apex salt tolerance was therefore advanced [183]. One of the mechanisms by which plants are able to withstand salt stress is the microRNA (miRNA) [184]. In

CRISPR-transformed plants, the GmMYB118 gene from soy beans responds to drought, salt, and temperature stress by increasing the production of genes related to stress, regulating the body's osmotic balance, and facilitating the antioxidant defense mechanism, all of which work together to keep cells in a state of homeostasis [185]. Another discovery regarding the SlARF4 gene is its role in tomato salinity tolerance and osmotic stress retardation [186].

CRISPR/Cas on cold stress tolerance

A number of symptoms, including quick wilting, sunken pith development, and necrotic areas in tissues, are observed in plants that are subjected to cold stress [187]. There are two forms of cold stress that can affect a plant's growth and development: chilling stress, which occurs between 0 and 20 °C, and freezing stress, which occurs at temperatures of 0 °C or below [188].

In a study conducted on wild-type tomato plants, the knock-out method of CRISPR/Cas9 in SlCBFs was found to delay chilling injuries [189]. Silencing the thermosensitive chlorophyll deficient mutant 10 (tcd 10) using the CRISPR/Cas9 method can improve the chilling stress in rice [190]. Chilling tolerance in rice is produced by the OsAnn3 gene, which is knocked out using the CRISPR/Cas9 technology [191]. Proteins rich in proline (PRPs) aid in chlorophyll production, reduce nutrient leakage, and boost antioxidant activity; they also aid in low-temperature tolerance. External salicylic acid (SA) therapy preserves chilling sensitivity after OsPrp1 gene knockout using CRISPR/Cas9 technology [192]. Concurrently, chilling tolerance is decreased when the OsAnn3 gene is knocked out in rice plants using CRISPR/Cas9 technology [191]. The overexpression of the PtPYRL1 and PtPYRL5 genes activates the ABA signaling system, which improves cold stress resistance [193]. Regulating the C-repeat binding transcription factors to produce cold tolerant Arabidopsis plants has also been achieved by the application of CRISPR-Cas mediated technology [194]. Mediator of CRISPR-based base editing It has been shown that when cold stress is applied to rice plants, the white stripe leaf 5 (wsl5) mutant line suppresses the genes responsible for photosynthesis, resulting in a phenotype of white-striped leaves [195, 196].

CRISPR/Cas on heat stress tolerance

The plant's ability to grow and develop is severely impaired when it experiences heat stress [197]. Heat stress impacts not just plant development but also phonological phases such as anthesis and grain filling [198]. Plants can be made heat resistant by raising the amounts of osmolytes and protecting cell proteins from damage by overexpressing heat shock transcription factors like the hsps gene [199]. Genetic engineering has allowed for the synthesis of glycine betaine, which has led to the development of heat stress tolerant Arabidopsis [200]. One way to make Oryza sativa more heat tolerant is to suppress the OsMDHAR4 gene [201]. Additionally, the heat stress tolerance activity in rice is enhanced by the transcription factor OsbZIP46CA1 and SAPK6 (protein kinase) [202]. The rice plant's ability to withstand heat stress is enhanced by a protein disulfide isomerase gene that was identified from Methanothermobacter thermautitrophicus [199].

Biotic stress

Many different kinds of illnesses can infect plants because of their susceptibility to diverse pathogens. An alternative that pollutes the environment indirectly and directly is the use of various herbicides, pesticides, and fungicides [203] [204] [205]. The plant metabolic pathway is likewise impacted by these substances [206] [207]. In order to protect plants from harmful organisms such as weeds, insects, nematodes, fungus, and bacteria, scientists are using CRISPR/Cas9 technology.

CRISPR/Cas on developing weed tolerance

Plants are most severely affected by weeds. They make it harder for plants to get the nutrients, water, sunlight, shelter, food, and space they need, which stunts their development. Some plants can cause disease by invading their host plants and infecting both their roots and their aerial parts. Some examples of parasitic plants that infest host plants and cause significant harm to agro-economic society are Cuscuta sp., Arceuthobium sp., Orobanche sp., and Phelipanche aegyptiaca. The pollen and seeds of many weeds contain harmful compounds that have an effect on the host plant in some way. There are a lot of efficient and inexpensive herbicides used to combat weeds, however reusing the same herbicide over and over

again causes weeds to become resistant [208]. The current base editing method may be the key to creating herbicide-resistant plants. Modern technology allows for base-level modifications to DNA and RNA within live cells, a process known as base editing. Editors of base pairs are nucleotide deaminases that are attached to Cas nucleases, which are catalytically disabled.

By modifying the upstream open reading frame (uORF) of LsGGP2, researchers were able to create paraquat-resistant lettuce. This type of lettuce is resistant to oxidative stress, produces a large amount of ascorbic acid, and has proven to be an effective herbicide resistance crop [209]. Herbicide resistance is conferred in Arabidopsis thaliana by means of missense point mutations in acetolactate syntetase (ALS) [210]. Resistance to the herbicide bispyribacsodium was initiated by inducing point mutations in the 548th and 627th amino acid positions of the rice ALS gene [211]. The roots of nearly all commercially significant plants in the groups Fabaceae, Solanaceae, Apiaceae, Brassicaceae, etc. are infested by the obligatory parasitic weeds Orobanche and Phelipanche [212] [213]. A plant hormone called strigolactones (SLs) was modified in a tomato crop using CRISPR/Cas9 technology. This modification inhibited the germination of parasite plant seeds. A weed-resistant tomato crop was developed via mutation of the tomato plant's CCD8 gene using CRISPR/Cas9 technology [214]. The CRISPR/Cas9 technology was used to construct herbicide-resistant rice plants by modifying the NHEJ intron targeting mutation [215].

CRISPR-Cas for insect resistance

It is challenging to generate insect resistance in plants using the CRISPR/Cas9 technology due to a lack of guidance in the insect's genomic sequence and the availability of embryonic microinjection systems. One way to make Arabidopsis resistant to lepidopteran insect pathogens is to use CRISPR/Cas9 to knock-in the potato protease inhibitor II (pinII) gene [216] [217]. Spodoptera litura, a lepidopteran pest, had abnormalities in its larval development phases after being injected with Cas9 messenger RNA and Slabd-A-specific single guide RNA (sgRNA) [218]. Transgenic peanuts engineered with the PR1 promoter and the Cry1Ac transgene, originally from Bacillus thuringiensis, are resistant to Spodoptera litura [219]. One of the most well-known promoters for aphid resistance in transgenic plants is the PR1 promoter [220]. Broccoli is protected from the pest Plutella xylostella by the Cry1Ab gene, which is expressed in response to the PR-1a promoter [221].

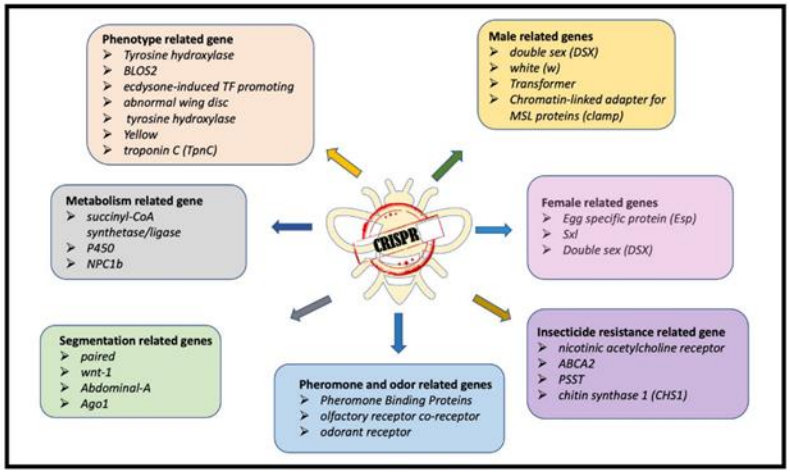

Figure 3: A graphical representation of CRISPR-based gene editing applications in various biological processes.

Bacterial infection

Because of factors such as the slow diagnosis of illnesses like mosaic, spots, and blights, the persistent development of plant genes, and the lack of effective chemical controls, dealing with the pathogenicity of bacteria in plants has historically been a challenging task. referenced in [222] [223]

By utilizing the CRISPR/Cas9 editing technique, the sucrose transporter gene OsSWEET13 conferred resistance to Xanthomonas oryzae pv. oryzae [224]. Editing the rice SWEET11, SWEET13, and SWEET14 genes using a CRISPR-Cas method conferred broad-spectrum resistance to Xanthomonas

oryzae pv. Oryzae [225]. This resistance to Xanthomonas citri illness was brought about by the CRISPR/Cas9 technology through effectors-triggered immunity (ETI). Without affecting the plant's phenotype, mutations in the CsLOB1 (lateral organ boundaries 1) promoter slowed rice's capacity to detect and react to bacterial effectors, resulting in resistance to the bacterium [226]. To combat the infection Pseudomonas syringae, scientists have employed CRISPR/Cas9 technology to silence the downy mildew resistance 6 (DMR6) genes in tomatoes, which function as a check on the plant's immune response [227] [228]. This study aimed to increase plant resistance to the disease Phytophthora capsici by knocking out the SlDMR6-1 gene, which is an ortholog of the tomato plant [229]. Similar findings were also noted when the DMR6 gene was altered in Arabidopsis thaliana, leading researchers to conclude that an increase in salicylic acid levels in plant defense resulted in better resistance to the phytopathogen [228]. The application of CRISPR/Cas9 technology to modify the Jasmonate ZIM-domain-2 ortholog (SlJAZ2) gene in tomato plants makes them resistant to bacterial speck disease, which is caused by Pseudomonas syringae. [230]

Viral infection

When viruses infect plants, they drastically reduce crop productivity [231] [232]. Viruses are obligatory parasites. For viral replication and proliferation, the machinery of the plant system is essential. Hence, in order to establish virus resistance, one can either alter the host's sensitivity or target the genes associated with pathogenicity [233]. By specifically targeting and cleaving viral genomes, the CRISPRCas method confers resistance to plants againstRNA and DNA viruses directly [234] [235].

In model species such as Nicotiana benthamiana and Arabidopsis thaliana, resistance to DNA viruses was initially established using CRISPR/Cas9 technology. These viruses include gemini viruses, which in this case are tomato leaf curl virus (TYLCV), bean yellow dwarf virus (BeYDV), and beet severe curled top virus (BSCTV). Here are the references: [236], [237], and [238]. Mutations in the coding and non-coding areas of hypopathogenic viruses, such as Cotton leaf curl Kokhran virus (CLCuKoV) and Tomato yellow leaf curl virus (TYLCV), were induced in plants by CRISPR/Cas9 based gene editing [239]. Also, CRISPR/Cas9 technology was used to combat the Begomoviruses that cause cotton plant leaf curl disease [240]. Transgenic cassava lines resistant to the African cassava mosaic virus (ACMV) were genetically engineered using the CRISPR/Cas9 system [241]. By rendering the targeted viral sequence inactive using the CRISPR/Cas9 technology, transgenic banana lines were able to establish resistance to endogenous banana streak virus (eBSV) [242]. An incompatibility with Cas9 made early resistance to the RNA virus challenging. After then, two further Cas nucleases were created, one from Francisella novicida (FnCas9) and the other from Leptotrichia wadei (LwaCas13a), both of which can detect viral RNA [243] [244]. Resistance to RNA viruses such as tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV) was shown in Arabidopsis and Nicotiana benthamiana by a variation of the CRISPR/Cas9 system (Fncas9) method [245]. The Cas13a/sgRNA transgenic potato lines exhibited high levels of resistance to the potato virus Y (PVY) [246]. Additionally, this method has been utilized to provide resistance to the following viruses: Arabidopsis turnip mosaic virus [247], rice tungro spherical virus (RTSV) [248], and banana streak virus (eBSV) [249].

Fungal infection

The fungal pathogen's ability to adapt its genes makes it a real threat to plants. A significant decrease in crop productivity can be caused by fungal diseases such as mildew, smut, rot, and others [250] [251] [231]. The mycotoxins produced by some phytopathogenic fungus can infect not only humans but also animals [231].

To enhance resistance to Oidium neolycopersici, researchers used CRISPR/Cas9 technology to target the ortholog SlPMR4, which is involved in Powdery Mildew Resistance 4 (PMR4) gene deposition. But, when the PMR4 gene became inactive as a result of an inversion mutation, the salicylic acid levels rose, triggering the HR response in the host plant. Consequently, pathogen resistance is conferred by either overexpressing PRR or by making use of malfunctioning S genes [233]. In order to improve the resistance of wheat, grapevine, and tomato plants to the powdery mildew disease caused by the fungus Blumeria

gaminis f. sp. Tritici, the CRISPR/Cas9 technology was used to modify the mildew resistance locus O (MLO), a famous host S gene the encodes a transmembrane protein (PRR). References: [171] [252, 253] [170]. The grapevine gene VvMLO7 was able to confer resistance to fungi when engineered using ribonucleoprotein (RNP) technology through CRISPR/Cas9 [252]. The 'Tomelo' tomato variety, created using CRISPR/Cas9 technology, is resistant to powdery mildew [253]. Ethylene Response Factor 922 (OsERF922) and enhanced disease resistance 1 (EDR1) were genetically engineered in Magnaporthe oryzae using CRISPR/Cas9 technology to develop resistance to blast-causing fungal pathogens and ethylene signaling, respectively, without affecting the crop's typical growth [254] [181] [255]. The Ossec3a gene mutation demonstrated an increase in salicylic acid levels and an upregulation of pathogenesis; nevertheless, this change resulted in smaller plants relative to the wild mutant [255]. An ortholog of EDR, Taedr1 was mutated using CRISPR/Cas9 technology in wheat to increase resistance to the phytopathogen fungus Erysiphe cichoracearum, among other diseases [256]. When the VvWRKY52 gene, a transcription factor in grapevines, was regulated, the plants developed resistance to Botrytis cinerea, which changed their appearance in comparison to the wild type [181].

How CRISPR/Cas influences key growth and developmental processes in Plants:

Knockout-Mediated Crop Trait Improvement:

Eradicating detrimental components is a viable approach for genetic enhancement. Consequently, the deletion of genes that impart undesired characteristics is the most straightforward and prevalent application of CRISPR/Cas9. Traits enhanced to date by CRISPR/Cas9 encompass yield, quality, and resistance to biotic and abiotic stresses. This strategy has also improved hybrid-breeding procedures and various other critical factors influencing crop output.

Increasing yields:

The necessity for enhanced food security renders yield the principal objective of gene editing in crop enhancement. Yield is a multifaceted characteristic influenced by numerous factors. Eliminating negative regulators that influence yield-determining factors, including grain number (OsGn1a), grain size (OsGS3), grain weight (TaGW2, OsGW5, OsGLW2, or TaGASR7), panicle size (OsDEP1, TaDEP1), and tiller number (OsAAP3), resulted in the anticipated phenotypes in plants with loss-of-function mutations in these genes, thereby illustrating that CRISPR/Cas9 is a potent instrument for enhancing yield-related characteristics. [257] [258] [259] [260] [261] [262]. The concurrent deletion of three genes associated with grain weight (GW2, GW5, and TGW6) in rice resulted in trait pyramiding, significantly enhancing grain weight. Nevertheless, as the majority of yield-related variables are quantitative and regulated by quantitative trait loci, merely eliminating individual components may prove inadequate for enhancing field production. Huang et al. [264] have devised a methodology for the extensive identification of genes associated with complicated quantitative variables, such as yield, using the integration of pedigree analysis, whole-genome sequencing, and CRISPR/Cas9 technology. The authors analyzed 30 cultivars of the progenitors and progeny of the Green Revolution rice variety IR8 and identified 57 genes conserved across all high-yielding lines for gene editing through deletion or knockdown with Cas9 or dCas9. Phenotypic study indicated that numerous genes are crucial for rice yield. This study elucidated the mechanism of yield development and may enhance molecular breeding for superior rice varieties.

Improving quality:

Different breeding needs call for different quality features. Genome editing has improved crop quality in several ways, including starch content, aroma, nutritional value, and storage quality. The deletion of Waxy by CRISPR/Cas9 resulted in rice with a low amylose concentration, which improved the eating and cooking quality of rice [151] [265]. A commercially viable CRISPR/Cas9 knockout waxy corn line was developed by DuPont Pioneer [266]. Patients with diet-related noninfectious chronic diseases should benefit from ingesting high-amylose foods; CRISPR/Cas9 was also utilized to mutate the starch branching enzyme gene SBEIIb, resulting in resistant starch rice with high amylose content [267].

The economic value of rice types is higher when they release pleasant aromas when cooked, since fragrance is a key quality attribute of rice. The primary aroma component of fragrant rice, 2-acetyl-1-pyrroline, is synthesized when there is a mutation in the betaine aldehyde dehydrogenase 2 (BADH2) gene. Our lab has developed a fragrant rice line that mimics the natural mutant fragrant rice variety in terms of 2-acetyl-1-pyrroline content (0.35-0.75 mg/kg) by using TALEN-targeted disruption of OsBADH2 [268].

Celiac disease affects over 7% of the Western population due to gluten proteins found in grain crops. Nearly 100 genes or pseudogenes make up the α-gliadin gene family, which is the main wheat gene family responsible for encoding gluten. CRISPR/Cas9 editing provides a novel approach to changing characteristics regulated by huge gene families that have redundant roles. True, scientists have produced low-gluten wheat by deleting the majority of α-gliadin family members' conserved domains all at once [269].

Other high-quality crops produced by CRISPR/Cas9 editing include seeds with high oleic acid oil in Camelina sativa [270] [271], and Brassica napus [272],Tomatoes with an extended shelf life [273] [274], high-value tomatoes with enhanced lycopene [275] or γ-aminobutyric acid content [276] [277], and potatoes (hairy roots) with reduced levels of harmful steroidal glycoalkaloids [278] are among the other high-quality crops created by CRISPR/Cas9 editing.

Speeding hybrid breeding:

This method is highly effective in boosting agricultural yields. A male-sterile maternal line is essential for creating a top-notch hybrid variety. Significant strides have been achieved in the creation of male-sterile lines through the use of CRISPR/Cas-mediated gene deletion. These lines include thermosensitive male-sterile tm5 in rice [279] and maize [280], photosensitive genic male-sterile csa in rice [281], and ms45 in wheat [282]. The primary problem with using heterosis in breeding is hybrid sterility. In order to get through the obstacles to reproduction in japonica-indica hybrids, scientists affected the SaF/SaM gene at the sterility locus Sa [283] and the OgTPR1 gene at the S1 locus [284]. A study conducted by Shen et al. [285] discovered that male fertility in hybrids between japonica and indica may be restored by deleting one or two copies of the Sc gene from the indica allele Sc-I. Hybrids of japonica and indica improved fertility when the toxin gene ORF2, responsible for rice's newly found selfish-gene suicide mechanism, was knocked out, as demonstrated by Yu et al. [286]. Recent genome editing has resulted in the replacement of meiosis with mitosis in rice by deleting three essential genes involved in meiosis: REC8, PAIR1, and OSD1. Asexual propagation lines were created by two separate groups. One group activated BBM1 in the egg cell at the same time [287]; the other group knocked down MTL [288], allowing hybrids to be fixed through seed propagation despite their heterozygosity.

To satisfy the needs of breeders, genome editing has been shown to be an effective method for improving a wide range of features, including haploid breeding[287] [289], growth rates[290], resistance to silique shatter [291], and self-incompatibility in diploid potatoes [292].

Crop Trait Improvement via Knock-In and Replacement:

Numerous agronomic characteristics are attributed to single-nucleotide mutations, alterations in gene expression, or the introduction of novel gene functions. Accurate gene alterations, including knock-ins and replacements, enhance breeding by incorporating novel alleles devoid of linkage drag or producing allelic variations absent in nature [293]. Furthermore, knock-in technology can be employed to modify various superior qualities by integrating genes inside a single variety. Consequently, knock-ins and replacements hold significant importance for the enhancement of crop traits.

Regrettably, due to HDR being a rare DNA repair mechanism, these procedures remain atypical, resulting in their limited application in trait enhancement to date. Shi et al. [294] employed CRISPR/Cas9-mediated gene editing to enhance drought tolerance in maize. ARGOS8 encodes a negative regulator of ethylene responses and is expressed at minimal levels in the majority of inbred maize lines. The authors enhanced ARGOS8 expression by replacing the native ARGOS8 promoter with the GOS2 promoter by HDR to facilitate ARGOS8 expression [294]. The modified ARGOS8 variants exhibited heightened ARGOS8

transcript levels and improved yields under drought conditions. Yu et al. [295] developed a tomato line with extended shelf life by introducing a T317A substitution in the ALC gene.

To enhance HDR efficacy, a geminivirus-based DNA replicon has been employed to augment the quantity of repair templates, hence improving gene-targeting efficiency in potato [296], tomato [134], [297], rice [298], wheat [147], and cassava [299]. Cermák et al. [134] utilized geminivirus replicons to attain a tenfold enhancement in the insertion frequency of the cauliflower mosaic virus 35S promoter upstream of ANT1 in tomato, resulting in the constitutive expression of ANT1 and the development of a purple tomato with elevated anthocyanin levels. Dahan-Meir et al. [297] devised a very efficient gene-targeting method devoid of selection and reporter systems, utilizing replicon-amplified donor fragments to effectively repair a fast-neutron-induced crtiso allele in tomato, which harbored a 281-base pair deletion, achieving an efficiency rate of up to 25%.

Herbicide selection facilitates the enhancement of gene-targeting events, making the endogenous acetolactate synthase (ALS) and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) genes prevalent targets for gene editing. Alteration of critical amino acids in the conserved domains of ALS and EPSPS can impart resistance to sulfonylurea herbicides or glyphosate. soybean [211], maize [152], and rice [300] [301, 302] resistant to sulfonylurea-based herbicides were produced via HDR-induced nucleotide alterations in ALS. Likewise, the T102I/P106S (TIPS) and T102I/P106A dual amino acid substitutions of EPSPS were incorporated into flax (Linum usitatissimum) [303] and cassava [299] through selection for HDR-induced glyphosate resistance. Due to the poor HDR rate, our team partnered with Li's group to develop TIPS EPSPS glyphosate-resistant rice by an intron-targeting approach utilizing NHEJ-mediated gene replacement and insertion [304]. While indels may occur at the junctions of the recombination sites within the targeted intron, the resultant gene transcript remains unaffected. This approach serves as a high-frequency alternative to HDR-mediated gene targeting in plants.

CRIPSR/Cas genome editing improves plant growth and development

The CRISPR/Cas system has been extensively utilized to investigate gene function in plant growth and development, and it has also served as a powerful tool for modifying plant morphology to promote growth and development. Arginase (ARG) is a crucial enzyme that regulates root development by inhibiting nitric oxide synthase (NOS). The overexpression of ARG impeded lateral root development [305]. Wang and colleagues (2017) have utilized CRISPR/Cas9 technology to delete the ARG gene in cotton. In the T1 generation of genome knockout lines, regardless of nitrogen medium concentration, the ARG knockout lines exhibit a considerable increase of 25% in the number of lateral roots and a 52% increase in total root surface area [306]. This indicates that CRISPR/Cas9-edited ARG plants exhibit superior root development, which will improve the capacity of genome-edited plants to take water and nutrients from the soil, thereby promoting plant growth, development, and resilience to various environmental challenges [307].

CRISPR/Cas9-mediated genome editing revealed the critical regulatory roles of the MADS box transcription factor genes MADS78 and MADS79 in endosperm cellularization and early seed development in rice. The single knockout mutants of MADS78 or MADS79 exhibited premature endosperm cellularization, while the double mutants impeded seed development and resulted in non-viable seeds in rice [308]. Knockout of the COPII components sarib and saric genes also modified pollen formation and induced male sterility in Arabidopsis [309]. Bnspl3 mutants, produced with CRISPR/Cas9 technology, displayed a developmental delay phenotype in allotetraploid oilseed rape [310]. The CRISPR/Cas9 deletion of the terminal flower1 (tfl1) gene modified the phase transition and blooming schedule in Brassica napus. The knockout of the tfl1 gene modified the plant architecture, specifically affecting plant height and branching in Brassica napus [311]. The application of CRISPR/Cas9 technology to induce mutations in the S and SP promoters enhanced inflorescence and plant architecture in tomato [312].

Ecological Impact of CRISPR Modified Crops

The technology of CRISPR gene-editing has been utilized worldwide by scientists to fulfill the need for food and feed of the highly growing population, as it can directly modify and insert DNA with high specificity and easy implementation [324, 329]. Although CRISPR can be used to bring resistance in crops against biotic and abiotic stresses, there are some long-term impacts on ecological systems. On one hand, it's a cure for many problems, but on the other hand, it may give rise to harmful genetic mutations [320, 323]. One of the most adverse impacts of using CRISPR/Cas9 on the ecological system is the loss of diversity. Its widespread use can greatly reduce diversity, to the extent that a single disease can destroy the whole population of the same cultivated crop variety [321, 322]. Some communities in Africa are against the CRISPR/Cas9-based genetically edited crops because it may impact cultural values, traditional farming, and local food systems [330]. In many countries, the edited crops are termed genetically modified organisms, and their commercialization is an issue in those countries due to regulatory authorities [321]. There is an adverse effect of CRISPR/Cas9-modified crops on ecosystem dynamics; if a modified crop is engineered to be more resistant to a specific pest, this can lead to a reduction of that pest population, which could be an alarming condition in the food chain for the predators depending on that pest [327, 329]. Despite the accurate and precise activity of CRISPR/Cas9, there is always a chance of off-target mutations, which can potentially affect plant physiology and lead to unexpected physiological impacts [313, 325]. The use of CRISPR/Cas9 can cause an issue called gene drive, in which the gene passes on to subsequent generations, raising concerns that the modified gene could spread uncontrollably in the environment [316, 327].

Ethical Considerations of Genetically Modified Crops:

There are many concerns and continuous debates on the widespread use of CRISPR technology, which has resulted in many ethical concerns along with promising advancements. The ethical considerations not only involve humans but also all other species [315]. There is a wide range of ethical concerns with using CRISPR technology, including environmental risks. There is a great chance of releasing GMOs into the environment, which can be hazardous microorganisms that spread disease and disturb food webs, as is the case with GMO microalgae [314]. In plants, certain herbicide-resistant GMOs can cross with wild types, creating a severe problem [319]. Another important ethical concern is the ownership of genetic resources. Patented CRISPR-modified crops have the potential to lead to monopolies, which adversely affect farmers' access to seeds, their right to save them, and their ability to replant them [318, 326]. An ethical concern about biopiracy has also been raised, where companies modify and patent crops that native people often grow without giving proper credit for their efforts, thus stealing benefits from these communities for negligible compensation [331]. The intensive use of CRISPR-modified crops can reduce traditional farming practices and food security systems if cultural and regional considerations are ignored during implementation [317]. There should be a regulatory authority to closely monitor CRISPR technology to ensure public trust and safety, and to control the environmental release of GMOs specifically.

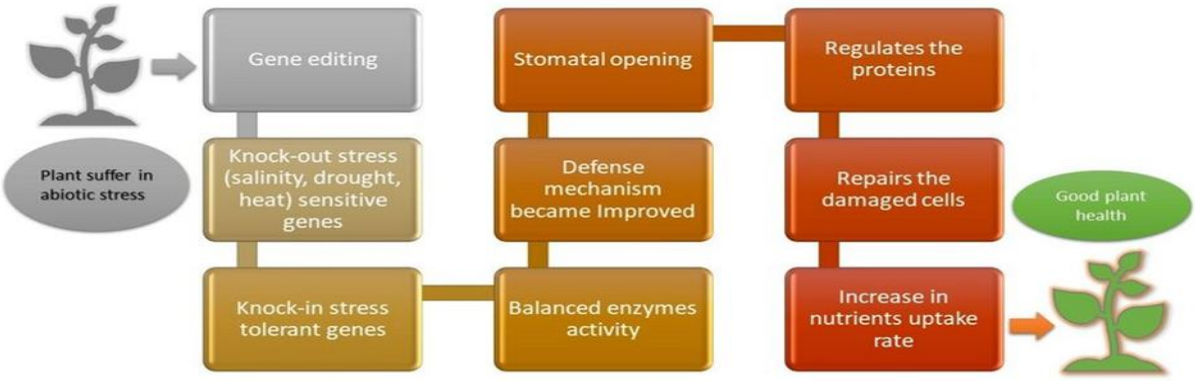

Figure 4: A schematic representation of gene editing strategies to enhance plant resilience against abiotic stress.

Conclusion and Future Perspective

The utilization of CRISPR-Cas technology is a novel strategy for addressing agricultural problems caused by the increasing worldwide need for food and the effects of climate change. It exceeds previous genetic modification approaches in its ability to improve crop yield, disease resistance, and overall productivity by enabling precise genome editing in crops including rice, wheat, and maize. Sustainable agriculture is supported by its ability to enhance characteristics like herbicide tolerance. The primary technique in aquaculture for delivering the CRISPR/Cas complex is microinjection, which has improved growth rates and disease resistance in species like salmon and catfish. Various delivery methods, including as agroinfiltration and protoplast transfection, have successfully improved desired characteristics in various crops. In order to address issues like pest resistance and drought, CRISPR/Cas9 has greatly increased crops resistance to biotic and abiotic stressors, improving food security. The ability to selectively target genes promotes crop resilience and helps up hybrid breeding. Even though CRISPR technology has a lot of potential, there are some ethical and ecological issues with it, such as decreased biodiversity and debates over who owns the genetic resources. Thus, in order to completely gain its benefits over time, cautious usage and careful regulatory control are necessary.

In the future, we should give preference to strategies that not only maximize efficiency in crop productivity but also save time, while ensuring genetic conservation and maintaining genetic diversity. CRISPR/Cas9 has shown promising results in enhancing stress tolerance in many crops (e.g., wheat, tomato, and rice), and we have great potential to expand its use to other economically important crops. The under-researched crops should be explored to ensure more global security and gain worldwide advantages from CRISPR technology in agriculture. Many research studies focus on single genes responsible for specific traits (e.g., drought, salinity, or pathogen resistance). In the future, we should focus on multi-trait gene editing in a single crop to enhance resistance against various biotic and abiotic factors. Crops produced this way would be more tolerant of harsh environmental conditions. Currently, most research is laboratory-based. In the future, we should focus on large-scale field trials to understand the possible consequences of CRISPR-edited crops on the environment. This strategy will help us assess the long-term advantages, possible ecological impacts, and interactions between CRISPR-edited crops and native species and the environment. Furthermore, the ongoing research objective is not only to develop strategies that can improve the efficacy but also the durability of modified resistance traits by utilizing CRISPR/Cas9 with other breeding techniques, such as marker-assisted selection and RNA interference (RNAi), to develop new and efficient resistance mechanisms in plants against biotic and abiotic stresses.

References:

Sharma, A., et al., Machine learning applications for precision agriculture: A comprehensive review. IEEE Access, 2020. 9: p. 4843-4873.

Rasheed, A., et al., A critical review: recent advancements in the use of CRISPR/Cas9 technology to enhance crops and alleviate global food crises. Current Issues in Molecular Biology, 2021. 43(3): p. 1950-1976.

Garcia, S.N., B.I. Osburn, and M.T. Jay-Russell, One health for food safety, food security, and sustainable food production. Frontiers in Sustainable Food Systems, 2020. 4: p. 1.

Sadigov, R., Rapid growth of the world population and its socioeconomic results. The Scientific World Journal, 2022. 2022(1): p. 8110229.

Barrett, C.B., Overcoming global food security challenges through science and solidarity. American Journal of Agricultural Economics, 2021. 103(2): p. 422-447.

Dhankher, O.P. and C.H. Foyer, Climate resilient crops for improving global food security and safety. 2018, Wiley Online Library. p. 877-884.

Powell, N., et al., Yield stability for cereals in a changing climate. Functional Plant Biology, 2012. 39(7): p. 539-552.

Ray, D.K., et al., Yield trends are insufficient to double global crop production by 2050. PloS one, 2013. 8(6): p. e66428.

Hunter, M.C., et al., Agriculture in 2050: recalibrating targets for sustainable intensification. Bioscience, 2017. 67(4): p. 386-391.

Adams, R.M., et al., Effects of global climate change on agriculture: an interpretative review. Climate research, 1998. 11(1): p. 19-30.

Nastis, S.A., A. Michailidis, and F. Chatzitheodoridis, Climate change and agricultural productivity. Afr. J. Agric. Res, 2012. 7(35): p. 4885-4893.

McKenzie, F.C. and J. Williams, Sustainable food production: constraints, challenges and choices by 2050. Food Security, 2015. 7: p. 221-233.

Wang, Y., et al., Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nature biotechnology, 2014. 32(9): p. 947-951.

Ntsomboh-Ntsefong, G., et al., CRISPR-Cas-based genome editing for crop improvement: progress, challenges and future prospects. Glob J Bot Sci, 2023. 11: p. 1-6.

Zhang, F., Development of CRISPR-Cas systems for genome editing and beyond. Quarterly Reviews of Biophysics, 2019. 52: p. e6.

Belhaj, K., et al., Editing plant genomes with CRISPR/Cas9. Current opinion in biotechnology, 2015. 32: p. 76-84.

Kumar, S., et al., CRISPR-Cas in agriculture: Opportunities and challenges. Frontiers in Plant Science, 2021. 12: p. 672329.

Ray, S., K. Sneha, and C. Jangid, CRISPR-Cas9 for Sustainable Food Production: Impacts, Recent Advancements and Future Perspectives. Food and Humanity, 2023.

Khan, Z., S.H. Khan, and A. Ahmad, Challenges and future perspective of CRISPR/Cas technology for crop improvement. CRISPR Crops: The Future of Food Security, 2021: p. 289-306.

Mushtaq, M., et al., CRISPR-based genome editing tools: Insights into technological breakthroughs and future challenges. Genes, 2021. 12(6): p. 797.

Ishino, Y., et al., Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. 1987. 169(12): p. 5429-5433.

Mojica, F.J., G. Juez, and F.J.M.m. Rodriguez-Valera, Transcription at different salinities of Haloferax mediterranei sequences adjacent to partially modified PstI sites. 1993. 9(3): p. 613-621.

Jansen, R., et al., Identification of genes that are associated with DNA repeats in prokaryotes. Molecular microbiology, 2002. 43(6): p. 1565-1575.

Bolotin, A., et al., Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology, 2005. 151(8): p. 2551-2561.

Mojica, F.J., et al., Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. Journal of molecular evolution, 2005. 60: p. 174-182.

Makarova, K.S., et al., A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. Biology direct, 2006. 1: p. 1-26.

Barrangou, R., et al., CRISPR provides acquired resistance against viruses in prokaryotes. Science, 2007. 315(5819): p. 1709-1712.

Mojica, F.J. and F. Rodriguez-Valera, The discovery of CRISPR in archaea and bacteria. The FEBS journal, 2016. 283(17): p. 3162-3169.

Makarova, K.S., et al., Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants. 2020. 18(2): p. 67-83.

Makarova, K.S., et al., An updated evolutionary classification of CRISPR–Cas systems. 2015. 13(11): p. 722-736.

Brouns, S.J., et al., Small CRISPR RNAs guide antiviral defense in prokaryotes. 2008. 321(5891): p. 960-964.

Jore, M.M., et al., Structural basis for CRISPR RNA-guided DNA recognition by Cascade. 2011. 18(5): p. 529-536.

Sinkunas, T., et al., Cas3 is a single-stranded DNA nuclease and ATP-dependent helicase in the CRISPR/Cas immune system. 2011. 30(7): p. 1335-1342.

Huo, Y., et al., Structures of CRISPR Cas3 offer mechanistic insights into Cascade-activated DNA unwinding and degradation. 2014. 21(9): p. 771-777.

Taylor, D.W., et al., Structures of the CRISPR-Cmr complex reveal mode of RNA target positioning. 2015. 348(6234): p. 581-585.

Mogila, I., et al., Genetic dissection of the type III-A CRISPR-Cas system csm complex reveals roles of individual subunits. Cell reports, 2019. 26(10): p. 2753-2765. e4.

You, L., et al., Structure studies of the CRISPR-Csm complex reveal mechanism of co-transcriptional interference. Cell, 2019. 176(1): p. 239-253. e16.

Staals, R.H., et al., Structure and activity of the RNA-targeting Type III-B CRISPR-Cas complex of Thermus thermophilus. Molecular cell, 2013. 52(1): p. 135-145.

Staals, R.H., et al., RNA targeting by the type III-A CRISPR-Cas Csm complex of Thermus thermophilus. Molecular cell, 2014. 56(4): p. 518-530.

Samai, P., et al., Co-transcriptional DNA and RNA cleavage during type III CRISPR-Cas immunity. Cell, 2015. 161(5): p. 1164-1174.

Estrella, M.A., F.-T. Kuo, and S. Bailey, RNA-activated DNA cleavage by the Type III-B CRISPR–Cas effector complex. Genes & development, 2016. 30(4): p. 460-470.

Makarova, K.S., et al., Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants. Nature Reviews Microbiology, 2020. 18(2): p. 67-83.

Crowley, V.M., et al., A type IV-A CRISPR-Cas system in Pseudomonas aeruginosa mediates RNA-guided plasmid interference in vivo. The CRISPR journal, 2019. 2(6): p. 434-440.

Özcan, A., et al., Type IV CRISPR RNA processing and effector complex formation in Aromatoleum aromaticum. Nature microbiology, 2019. 4(1): p. 89-96.

Pinilla-Redondo, R., et al., Type IV CRISPR–Cas systems are highly diverse and involved in competition between plasmids. Nucleic acids research, 2020. 48(4): p. 2000-2012.

Jinek, M., et al., A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. science, 2012. 337(6096): p. 816-821.

Yan, W.X., et al., Functionally diverse type V CRISPR-Cas systems. Science, 2019. 363(6422): p. 88-91.

Harrington, L.B., et al., A scoutRNA is required for some type V CRISPR-Cas systems. Molecular cell, 2020. 79(3): p. 416-424. e5.

Abudayyeh, O.O., et al., C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science, 2016. 353(6299): p. aaf5573.

O'Connell, M.R., Molecular mechanisms of RNA targeting by Cas13-containing type VI CRISPR–Cas systems. Journal of molecular biology, 2019. 431(1): p. 66-87.

Silas, S., et al., Direct CRISPR spacer acquisition from RNA by a natural reverse transcriptase–Cas1 fusion protein. Science, 2016. 351(6276): p. aad4234.

Mosterd, C., G.M. Rousseau, and S. Moineau, A short overview of the CRISPR-Cas adaptation stage. Canadian journal of microbiology, 2021. 67(1): p. 1-12.

Sternberg, S.H., et al., Adaptation in CRISPR-Cas systems. Molecular cell, 2016. 61(6): p. 797-808.

Taylor, H.N., et al., Structural basis of Type IV CRISPR RNA biogenesis by a Cas6 endoribonuclease. RNA biology, 2019. 16(10): p. 1438-1447.

Deltcheva, E., et al., CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature, 2011. 471(7340): p. 602-607.

Charpentier, E., et al., Biogenesis pathways of RNA guides in archaeal and bacterial CRISPR-Cas adaptive immunity. FEMS microbiology reviews, 2015. 39(3): p. 428-441.

East-Seletsky, A., et al., Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection. Nature, 2016. 538(7624): p. 270-273.

Fonfara, I., et al., The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. Nature, 2016. 532(7600): p. 517-521.

Liu, L., et al., Two distant catalytic sites are responsible for C2c2 RNase activities. Cell, 2017. 168(1): p. 121-134. e12.

Zhang, Y., et al., Processing-independent CRISPR RNAs limit natural transformation in Neisseria meningitidis. Molecular cell, 2013. 50(4): p. 488-503.

Murugan, K., et al., The revolution continues: newly discovered systems expand the CRISPR-Cas toolkit. Molecular cell, 2017. 68(1): p. 15-25.

Liu, T.Y. and J.A. Doudna, Chemistry of Class 1 CRISPR-Cas effectors: Binding, editing, and regulation. Journal of Biological Chemistry, 2020. 295(42): p. 14473-14487.

Gleditzsch, D., et al., PAM identification by CRISPR-Cas effector complexes: diversified mechanisms and structures. RNA biology, 2019. 16(4): p. 504-517.

Rittiner, J.E., et al., Gene-editing technologies paired with viral vectors for translational research into neurodegenerative diseases. Frontiers in Molecular Neuroscience, 2020. 13: p. 148.

Kabadi, A.M., et al., Multiplex CRISPR/Cas9-based genome engineering from a single lentiviral vector. Nucleic acids research, 2014. 42(19): p. e147-e147.

Heckl, D., et al., Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing. Nature biotechnology, 2014. 32(9): p. 941-946.

Sánchez-Rivera, F.J., et al., Rapid modelling of cooperating genetic events in cancer through somatic genome editing. Nature, 2014. 516(7531): p. 428-431.

Zhou, S., et al., Evaluating the safety of retroviral vectors based on insertional oncogene activation and blocked differentiation in cultured thymocytes. Molecular Therapy, 2016. 24(6): p. 1090-1099.

Joglekar, A.V., et al., Integrase-defective lentiviral vectors as a delivery platform for targeted modification of adenosine deaminase locus. Molecular Therapy, 2013. 21(9): p. 1705-1717.

Wanisch, K. and R.J. Yáñez-Muñoz, Integration-deficient lentiviral vectors: a slow coming of age. Molecular therapy, 2009. 17(8): p. 1316-1332.

Lombardo, A., et al., Gene editing in human stem cells using zinc finger nucleases and integrase-defective lentiviral vector delivery. Blood Cells, Molecules, and Diseases, 2008. 40(2): p. 278.

Sessa, M., et al., Lentiviral haemopoietic stem-cell gene therapy in early-onset metachromatic leukodystrophy: an ad-hoc analysis of a non-randomised, open-label, phase 1/2 trial. The Lancet, 2016. 388(10043): p. 476-487.

Wang, Y., et al., Integration-defective lentiviral vector mediates efficient gene editing through homology-directed repair in human embryonic stem cells. Nucleic Acids Research, 2017. 45(5): p. e29-e29.

Slaymaker, I.M., et al., Rationally engineered Cas9 nucleases with improved specificity. Science, 2016. 351(6268): p. 84-88.

Kleinstiver, B.P., et al., High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. Nature, 2016. 529(7587): p. 490-495.

Wang, D., et al., Adenovirus-mediated somatic genome editing of Pten by CRISPR/Cas9 in mouse liver in spite of Cas9-specific immune responses. Human gene therapy, 2015. 26(7): p. 432-442.

Ding, Q., et al., Permanent alteration of PCSK9 with in vivo CRISPR-Cas9 genome editing. Circulation research, 2014. 115(5): p. 488-492.

Maddalo, D., et al., In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system. Nature, 2014. 516(7531): p. 423-427.

Kotterman, M.A. and D.V. Schaffer, Engineering adeno-associated viruses for clinical gene therapy. Nature Reviews Genetics, 2014. 15(7): p. 445-451.

Nathwani, A.C., et al., Long-term safety and efficacy of factor IX gene therapy in hemophilia B. New England Journal of Medicine, 2014. 371(21): p. 1994-2004.

Nelson, C.E. and C.A. Gersbach, Engineering delivery vehicles for genome editing. Annual review of chemical and biomolecular engineering, 2016. 7(1): p. 637-662.

Swiech, L., et al., In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9. Nature biotechnology, 2015. 33(1): p. 102-106.

Hung, S.S., et al., AAV-mediated CRISPR/Cas gene editing of retinal cells in vivo. Investigative ophthalmology & visual science, 2016. 57(7): p. 3470-3476.

Lin, H., et al., Intramuscular delivery of scAAV9-hIGF1 prolongs survival in the hSOD1 G93A ALS mouse model via upregulation of D-amino acid oxidase. Molecular Neurobiology, 2018. 55: p. 682-695.

Zhang, Y., et al., Enhanced CRISPR-Cas9 correction of Duchenne muscular dystrophy in mice by a self-complementary AAV delivery system. Science advances, 2020. 6(8): p. eaay6812.

Murlidharan, G., et al., CNS-restricted transduction and CRISPR/Cas9-mediated gene deletion with an engineered AAV vector. Molecular Therapy-Nucleic Acids, 2016. 5.

Ran, F.A., et al., In vivo genome editing using Staphylococcus aureus Cas9. Nature, 2015. 520(7546): p. 186-191.

Kim, E., et al., In vivo genome editing with a small Cas9 orthologue derived from Campylobacter jejuni. Nature communications, 2017. 8(1): p. 14500.

Müller, M., et al., Streptococcus thermophilus CRISPR-Cas9 systems enable specific editing of the human genome. Molecular Therapy, 2016. 24(3): p. 636-644.

Patsali, P., M. Kleanthous, and C.W. Lederer, Disruptive technology: CRISPR/Cas-based tools and approaches. Molecular Diagnosis & Therapy, 2019. 23(2): p. 187-200.

Manghwar, H., et al., CRISPR/Cas systems in genome editing: methodologies and tools for sgRNA design, off-target evaluation, and strategies to mitigate off-target effects. Advanced science, 2020. 7(6): p. 1902312.

Mali, P., K.M. Esvelt, and G.M. Church, Cas9 as a versatile tool for engineering biology. Nature methods, 2013. 10(10): p. 957-963.

Yin, H., et al., Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nature biotechnology, 2014. 32(6): p. 551-553.

Zhen, S., et al., Harnessing the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated Cas9 system to disrupt the hepatitis B virus. Gene therapy, 2015. 22(5): p. 404-412.

Bakondi, B., et al., In vivo CRISPR/Cas9 gene editing corrects retinal dystrophy in the S334ter-3 rat model of autosomal dominant retinitis pigmentosa. Molecular Therapy, 2016. 24(3): p. 556-563.

Latella, M.C., et al., In vivo editing of the human mutant rhodopsin gene by electroporation of plasmid-based CRISPR/Cas9 in the mouse retina. Molecular Therapy-Nucleic Acids, 2016. 5.

Li, P., et al., Allele-specific CRISPR-Cas9 genome editing of the single-base P23H mutation for rhodopsin-associated dominant retinitis pigmentosa. The CRISPR journal, 2018. 1(1): p. 55-64.

Shinmyo, Y. and H. Kawasaki, CRISPR/Cas9-Mediated gene knockout in the mouse brain using in utero electroporation. Current Protocols in Neuroscience, 2017. 79(1): p. 3.32. 1-3.32. 11.

Yin, H., et al., Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. Nature biotechnology, 2016. 34(3): p. 328-333.

Yin, H., et al., Structure-guided chemical modification of guide RNA enables potent non-viral in vivo genome editing. Nature biotechnology, 2017. 35(12): p. 1179-1187.

Finn, J.D., et al., A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. Cell reports, 2018. 22(9): p. 2227-2235.

Liu, J., et al., Fast and efficient CRISPR/Cas9 genome editing in vivo enabled by bioreducible lipid and messenger RNA nanoparticles. Advanced Materials, 2019. 31(33): p. 1902575.

Zuris, J.A., et al., Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo. Nature biotechnology, 2015. 33(1): p. 73-80.

Mangeot, P.E., et al., Genome editing in primary cells and in vivo using viral-derived Nanoblades loaded with Cas9-sgRNA ribonucleoproteins. Nature communications, 2019. 10(1): p. 45.

Park, H., et al., In vivo neuronal gene editing via CRISPR–Cas9 amphiphilic nanocomplexes alleviates deficits in mouse models of Alzheimer's disease. Nature neuroscience, 2019. 22(4): p. 524-528.

Sun, W., et al., Self-assembled DNA nanoclews for the efficient delivery of CRISPR–Cas9 for genome editing. Angewandte Chemie, 2015. 127(41): p. 12197-12201.

Mout, R., et al., Direct cytosolic delivery of CRISPR/Cas9-ribonucleoprotein for efficient gene editing. ACS nano, 2017. 11(3): p. 2452-2458.

Lee, Y.W., et al., In vivo editing of macrophages through systemic delivery of CRISPR-Cas9-ribonucleoprotein-nanoparticle nanoassemblies. Advanced therapeutics, 2019. 2(10): p. 1900041.

Mout, R., et al., In vivo delivery of CRISPR/Cas9 for therapeutic gene editing: progress and challenges. Bioconjugate chemistry, 2017. 28(4): p. 880-884.

Bareford, L.M. and P.W. Swaan, Endocytic mechanisms for targeted drug delivery. Advanced drug delivery reviews, 2007. 59(8): p. 748-758.

Jao, L.-E., S.R. Wente, and W. Chen, Efficient multiplex biallelic zebrafish genome editing using a CRISPR nuclease system. Proceedings of the National Academy of Sciences, 2013. 110(34): p. 13904-13909.

Goto, R., et al., Microinjection of marine fish eggs. Microinjection: Methods and Protocols, 2019: p. 475-487.

Chakrapani, V., et al., Establishing targeted carp TLR22 gene disruption via homologous recombination using CRISPR/Cas9. Developmental & Comparative Immunology, 2016. 61: p. 242-247.

Wargelius, A., et al., Dnd knockout ablates germ cells and demonstrates germ cell independent sex differentiation in Atlantic salmon. Scientific reports, 2016. 6(1): p. 21284.

Zhu, M., et al., Cas9 Technology for Enhancing Desirable Traits of Fish Species in Aquaculture. Int. J. Mol. Sci, 2024. 25: p. 9299.

Zhong, Z., et al., Targeted disruption of sp7 and myostatin with CRISPR-Cas9 results in severe bone defects and more muscular cells in common carp. Scientific Reports, 2016. 6(1): p. 22953.

Elaswad, A., et al., Effects of CRISPR/Cas9 dosage on TICAM1 and RBL gene mutation rate, embryonic development, hatchability and fry survival in channel catfish. Scientific reports, 2018. 8(1): p. 16499.

Ma, J., et al., Efficient resistance to grass carp reovirus infection in JAM-A knockout cells using CRISPR/Cas9. Fish & shellfish immunology, 2018. 76: p. 206-215.

Kim, J.-S., Precision genome engineering through adenine and cytosine base editing. Nature Plants, 2018. 4(3): p. 148-151.

Shan, Q., et al., Genome editing in rice and wheat using the CRISPR/Cas system. Nature protocols, 2014. 9(10): p. 2395-2410.

Shimatani, Z., et al., Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. Nature biotechnology, 2017. 35(5): p. 441-443.

Xie, K. and Y. Yang, RNA-guided genome editing in plants using a CRISPR–Cas system. Molecular plant, 2013. 6(6): p. 1975-1983.

Bhaskar, P.B., et al., Agrobacterium-mediated transient gene expression and silencing: a rapid tool for functional gene assay in potato. PLoS One, 2009. 4(6): p. e5812.

Circelli, P., et al., Efficient Agrobacterium-based transient expression system for the production of biopharmaceuticals in plants. Bioengineered bugs, 2010. 1(3): p. 221-224.

Figueiredo, J.F., et al., Agrobacterium-mediated transient expression in citrus leaves: a rapid tool for gene expression and functional gene assay. Plant cell reports, 2011. 30: p. 1339-1345.

Kim, M.J., K. Baek, and C.-M. Park, Optimization of conditions for transient Agrobacterium-mediated gene expression assays in Arabidopsis. Plant Cell Reports, 2009. 28: p. 1159-1167.

Zheng, L., et al., A versatile Agrobacterium-mediated transient gene expression system for herbaceous plants and trees. Biochemical genetics, 2012. 50: p. 761-769.

Belhaj, K., et al., Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. Plant methods, 2013. 9: p. 1-10.

Feng, Z., et al., Efficient genome editing in plants using a CRISPR/Cas system. Cell research, 2013. 23(10): p. 1229-1232.

Piatek, A., et al., RNA-guided transcriptional regulation in planta via synthetic dC as9-based transcription factors. Plant biotechnology journal, 2015. 13(4): p. 578-589.

Zhang, H., et al., Efficient generation of CRISPR/Cas9-mediated homozygous/biallelic Medicago truncatula mutants using a hairy root system. Frontiers in Plant Science, 2020. 11: p. 294.

Michno, J.-M., et al., CRISPR/Cas mutagenesis of soybean and Medicago truncatula using a new web-tool and a modified Cas9 enzyme. GM crops & food, 2015. 6(4): p. 243-252.

Zhang, S., É. Kondorosi, and A. Kereszt, An anthocyanin marker for direct visualization of plant transformation and its use to study nitrogen-fixing nodule development. Journal of plant research, 2019. 132: p. 695-703.

Čermák, T., et al., High-frequency, precise modification of the tomato genome. Genome biology, 2015. 16: p. 1-15.

Liang, Z., et al., Targeted mutagenesis in Zea mays using TALENs and the CRISPR/Cas system. Journal of Genetics and Genomics, 2014. 41(2): p. 63-68.

Lin, Q., et al., Prime genome editing in rice and wheat. Nature biotechnology, 2020. 38(5): p. 582-585.

Park, J. and S. Choe, DNA-free genome editing with preassembled CRISPR/Cas9 ribonucleoproteins in plants. Transgenic Research, 2019. 28(Suppl 2): p. 61-64.

Woo, J.W., et al., DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. Nature biotechnology, 2015. 33(11): p. 1162-1164.

Lin, C.S., et al., Application of protoplast technology to CRISPR/Cas9 mutagenesis: from single-cell mutation detection to mutant plant regeneration. Plant biotechnology journal, 2018. 16(7): p. 1295-1310.

Hwang, H.-H., M. Yu, and E.-M. Lai, Agrobacterium-mediated plant transformation: biology and applications. The arabidopsis book, 2017. 15.

Kumagai, M.H., et al., Cytoplasmic inhibition of carotenoid biosynthesis with virus-derived RNA. Proceedings of the National Academy of Sciences, 1995. 92(5): p. 1679-1683.

Nagyová, A. and Z. Subr, Infectious full-length clones of plant viruses and their use for construction of viral vectors. Acta virologica, 2007. 51(4): p. 223.

Ali, Z., et al., Efficient virus-mediated genome editing in plants using the CRISPR/Cas9 system. Molecular plant, 2015. 8(8): p. 1288-1291.

Baltes, N.J., et al., DNA replicons for plant genome engineering. The Plant Cell, 2014. 26(1): p. 151-163.

Wawrzyniak, P., G. Płucienniczak, and D. Bartosik, The different faces of rolling-circle replication and its multifunctional initiator proteins. Frontiers in Microbiology, 2017. 8: p. 2353.

Yin, K., et al., A geminivirus-based guide RNA delivery system for CRISPR/Cas9 mediated plant genome editing. Scientific reports, 2015. 5(1): p. 14926.

Gil-Humanes, J., et al., High-efficiency gene targeting in hexaploid wheat using DNA replicons and CRISPR/Cas9. The Plant Journal, 2017. 89(6): p. 1251-1262.

Andersson, M., et al., Efficient targeted multiallelic mutagenesis in tetraploid potato (Solanum tuberosum) by transient CRISPR-Cas9 expression in protoplasts. Plant cell reports, 2017. 36: p. 117-128.

Kikkert, J.R., J.R. Vidal, and B.I. Reisch, Stable transformation of plant cells by particle bombardment/biolistics. Transgenic plants: methods and protocols, 2004: p. 61-78.

Shan, Q., et al., Targeted genome modification of crop plants using a CRISPR-Cas system. Nature biotechnology, 2013. 31(8): p. 686-688.

Ma, X., et al., A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. Molecular plant, 2015. 8(8): p. 1274-1284.

Svitashev, S., et al., Genome editing in maize directed by CRISPR–Cas9 ribonucleoprotein complexes. Nature communications, 2016. 7(1): p. 1-7.

Liang, Z., et al., Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. Nature communications, 2017. 8(1): p. 14261.

Pandey, P., et al., Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. Frontiers in plant science, 2017. 8: p. 537.

Parmar, N., et al., Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: a comprehensive review. 3 Biotech, 2017. 7: p. 1-35.

Hao, Z., et al., Meta-analysis of constitutive and adaptive QTL for drought tolerance in maize. Euphytica, 2010. 174(2): p. 165-177.

Almeida, G.D., et al., Molecular mapping across three populations reveals a QTL hotspot region on chromosome 3 for secondary traits associated with drought tolerance in tropical maize. Molecular breeding, 2014. 34: p. 701-715.

Trachsel, S., et al., Identification of QTL for early vigor and stay-green conferring tolerance to drought in two connected advanced backcross populations in tropical maize (Zea mays L.). PloS one, 2016. 11(3): p. e0149636.

Mathews, K.L., et al., Multi-environment QTL mixed models for drought stress adaptation in wheat. Theoretical and Applied Genetics, 2008. 117: p. 1077-1091.

Kirigwi, F., et al., Markers associated with a QTL for grain yield in wheat under drought. Molecular Breeding, 2007. 20: p. 401-413.

Gahlaut, V., et al., QTL mapping for nine drought-responsive agronomic traits in bread wheat under irrigated and rain-fed environments. PloS one, 2017. 12(8): p. e0182857.

Pinto, R.S., et al., Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. Theoretical and applied genetics, 2010. 121: p. 1001-1021.

Khowaja, F.S., et al., Improved resolution in the position of drought-related QTLs in a single mapping population of rice by meta-analysis. BMC genomics, 2009. 10: p. 1-14.

Kamoshita, A., et al., Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. Field crops research, 2008. 109(1-3): p. 1-23.

Swamy, B.M., et al., Meta-analysis of grain yield QTL identified during agricultural drought in grasses showed consensus. BMC genomics, 2011. 12: p. 1-18.

Suji, K., et al., Mapping QTLs for plant phenology and production traits using indica rice (Oryza sativa L.) lines adapted to rainfed environment. Molecular biotechnology, 2012. 52: p. 151-160.

Lou, D., et al., OsSAPK2 confers abscisic acid sensitivity and tolerance to drought stress in rice. Frontiers in plant science, 2017. 8: p. 993.

Upadhyay, S.K., Genome engineering for crop improvement. 2021: Wiley Online Library.

Li, R., et al., CRISPR/Cas9-Mediated SlNPR1 mutagenesis reduces tomato plant drought tolerance. BMC plant biology, 2019. 19: p. 1-13.

Wang, L., et al., Reduced drought tolerance by CRISPR/Cas9-mediated SlMAPK3 mutagenesis in tomato plants. Journal of agricultural and food chemistry, 2017. 65(39): p. 8674-8682.

Wang, X., et al., CRISPR/Cas9-mediated efficient targeted mutagenesis in grape in the first generation. Plant biotechnology journal, 2018. 16(4): p. 844-855.

Osakabe, Y., et al., Optimization of CRISPR/Cas9 genome editing to modify abiotic stress responses in plants. Scientific reports, 2016. 6(1): p. 26685.

Bhattacharya, A., V. Parkhi, and B. Char, CRISPR/Cas genome editing. 2020: Springer.

Zaman, M., et al., Introduction to soil salinity, sodicity and diagnostics techniques. Guideline for salinity assessment, mitigation and adaptation using nuclear and related techniques, 2018: p. 1-42.

Schmöckel, S.M., Salinity detection and control of sodium transport in Arabidopsis thaliana. 2014.

Roy, S.J., S. Negrão, and M. Tester, Salt resistant crop plants. Current opinion in Biotechnology, 2014. 26: p. 115-124.

Khan, I., et al., CRISPR-Cas technology based genome editing for modification of salinity stress tolerance responses in rice (Oryza sativa L.). Molecular Biology Reports, 2021. 48(4): p. 3605-3615.

Takagi, H., et al., MutMap accelerates breeding of a salt-tolerant rice cultivar. Nature biotechnology, 2015. 33(5): p. 445-449.

Zhang, A., et al., Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. Molecular breeding, 2019. 39: p. 1-10.

Liu, X., et al., The trihelix transcription factor OsGTγ-2 is involved adaption to salt stress in rice. Plant molecular biology, 2020. 103: p. 545-560.

Wang, F., et al., Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. PloS one, 2016. 11(4): p. e0154027.

Li, P., et al., Genome-wide identification and expression analysis of AP2/ERF transcription factors in sugarcane (Saccharum spontaneum L.). BMC genomics, 2020. 21: p. 1-17.

Huang, Y., et al., Tissue-specific respiratory burst oxidase homolog-dependent H2O2 signaling to the plasma membrane H+-ATPase confers potassium uptake and salinity tolerance in Cucurbitaceae. Journal of Experimental Botany, 2019. 70(20): p. 5879-5893.

Gao, S., et al., Transgenic sweetpotato plants expressing an LOS 5 gene are tolerant to salt stress. Plant Cell, Tissue and Organ Culture (PCTOC), 2011. 107: p. 205-213.

Du, Y.-T., et al., Identification and characterization of GmMYB118 responses to drought and salt stress. BMC Plant Biology, 2018. 18: p. 1-18.

Bouzroud, S., et al., Down regulation and loss of auxin response factor 4 function using CRISPR/Cas9 alters plant growth, stomatal function and improves tomato tolerance to salinity and osmotic stress. Genes, 2020. 11(3): p. 272.

Ahmad, P., Legumes under Environmental Stress: Yield, Improvement and Adaptations. 2014.

Kazemi-Shahandashti, S.-S. and R. Maali-Amiri, Global insights of protein responses to cold stress in plants: Signaling, defence, and degradation. Journal of Plant Physiology, 2018. 226: p. 123-135.

Li, R., et al., Reduction of tomato-plant chilling tolerance by CRISPR–Cas9-mediated SlCBF1 mutagenesis. Journal of agricultural and food chemistry, 2018. 66(34): p. 9042-9051.

Wu, L., et al., The rice pentatricopeptide repeat gene TCD10 is needed for chloroplast development under cold stress. Rice, 2016. 9: p. 1-13.

Shen, C., et al., Knock out of the annexin gene OsAnn3 via CRISPR/Cas9-mediated genome editing decreased cold tolerance in rice. Journal of Plant Biology, 2017. 60: p. 539-547.

Nawaz, G., et al., Knockout of OsPRP1, a gene encoding proline-rich protein, confers enhanced cold sensitivity in rice (Oryza sativa L.) at the seedling stage. 3 Biotech, 2019. 9: p. 1-18.

Yu, J., et al., Overexpression of pyrabactin resistance-like abscisic acid receptors enhances drought, osmotic, and cold tolerance in transgenic poplars. Frontiers in Plant Science, 2017. 8: p. 1752.

Jia, Y., et al., The cbfs triple mutants reveal the essential functions of CBF s in cold acclimation and allow the definition of CBF regulons in Arabidopsis. New Phytologist, 2016. 212(2): p. 345-353.

Mo, T., et al., The chloroplast pentatricopeptide repeat protein RCN22 regulates tiller number in rice by affecting sugar levels via the TB1-RCN22-RbcL module. Plant Communications, 2024.

Molla, K.A., J. Shih, and Y. Yang, Single-nucleotide editing for zebra3 and wsl5 phenotypes in rice using CRISPR/Cas9-mediated adenine base editors. Abiotech, 2020. 1(2): p. 106-118.

Pandey, G.K., M. Sharma, and G. Pandey, Elucidation of abiotic stress signaling in plants. 2015: Springer.

Hasanuzzaman, M., et al., Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. International journal of molecular sciences, 2013. 14(5): p. 9643-9684.

Debbarma, J., et al., Ethylene response factor (ERF) family proteins in abiotic stresses and CRISPR–Cas9 genome editing of ERFs for multiple abiotic stress tolerance in crop plants: a review. Molecular biotechnology, 2019. 61(2): p. 153-172.

Alia, et al., Enhancement of the tolerance of Arabidopsis to high temperatures by genetic engineering of the synthesis of glycinebetaine. The Plant Journal, 1998. 16(2): p. 155-161.

Liu, J., et al., Suppression of OsMDHAR4 enhances heat tolerance by mediating H 2 O 2-induced stomatal closure in rice plants. Rice, 2018. 11: p. 1-12.

Chang, Y., et al., Co-overexpression of the constitutively active form of OsbZIP46 and ABA-activated protein kinase SAPK6 improves drought and temperature stress resistance in rice. Frontiers in Plant Science, 2017. 8: p. 1102.

Isman, M.B. and M.L. Grieneisen, Botanical insecticide research: many publications, limited useful data. Trends in plant science, 2014. 19(3): p. 140-145.

Law, J.W.-F., et al., The potential of Streptomyces as biocontrol agents against the rice blast fungus, Magnaporthe oryzae (Pyricularia oryzae). Frontiers in microbiology, 2017. 8: p. 3.

Yoon, M.-Y., B. Cha, and J.-C. Kim, Recent trends in studies on botanical fungicides in agriculture. The plant pathology journal, 2013. 29(1): p. 1.

Aktar, W., D. Sengupta, and A. Chowdhury, Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary toxicology, 2009. 2(1): p. 1-12.

Kim, S.-R., Y. Kim, and G. An, Identification of methyl jasmonate and salicylic acid response elements from the nopaline synthase (nos) promoter. Plant physiology, 1993. 103(1): p. 97-103.

Shaner, D.L., The impact of glyphosate-tolerant crops on the use of other herbicides and on resistance management. Pest Management Science: formerly Pesticide Science, 2000. 56(4): p. 320-326.

Zhang, H., et al., Genome editing of upstream open reading frames enables translational control in plants. Nature Biotechnology, 2018. 36(9): p. 894-898.

Yang, W., et al., Programmed sequential cutting endows Cas9 versatile base substitution capability in plants. Science China Life sciences, 2020. 12(1): p. 388-397.

Li, Z., et al., Cas9-guide RNA directed genome editing in soybean. Plant physiology, 2015. 169(2): p. 960-970.

Westwood, J.H., et al., The evolution of parasitism in plants. Trends in plant science, 2010. 15(4): p. 227-235.

Joel, D., Direct infection of potato tubers by the root parasite Orobanche aegyptiaca. Weed Research, 2007. 47(4): p. 276-279.

Bari, V.K., et al., CRISPR/Cas9-mediated mutagenesis of CAROTENOID CLEAVAGE DIOXYGENASE 8 in tomato provides resistance against the parasitic weed Phelipanche aegyptiaca. Scientific reports, 2019. 9(1): p. 11438.

ZONG, Y., Genereplacementsand insertionsinricebyintrontargetingusingCRISPR-Cas9. NaturePlants, 2016. 2: p. 16139.

Bu, Q.Y., et al., Cloning of a potato proteinase inhibitor gene PINII-2x from diploid potato (Solanum phurejia L.) and transgenic investigation of its potential to confer insect resistance in rice. Journal of Integrative Plant Biology, 2006. 48(6): p. 732-739.

Liu, T.-H.A., L.C. Stephens, and D.J. Hannapel, Expression of a chimeric proteinase inhibitor II-GUS gene in transgenic Solanum brevidens plants. Journal of plant physiology, 1996. 149(5): p. 533-538.

Bi, H.L., et al., CRISPR/Cas9-mediated targeted gene mutagenesis in Spodoptera litura. Insect Science, 2016. 23(3): p. 469-477.

Zhu-Salzman, K., et al., Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. Plant physiology, 2004. 134(1): p. 420-431.

War, A.R., et al., Mechanisms of plant defense against insect herbivores. Plant signaling & behavior, 2012. 7(10): p. 1306-1320.

Cao, J., A.M. Shelton, and E.D. Earle, Gene expression and insect resistance in transgenic broccoli containing a Bacillus thuringiensis cry1Ab gene with the chemically inducible PR-1a promoter. Molecular Breeding, 2001. 8: p. 207-216.

Dogra, R., N. Kishore, and K. Kumar, Plant Disease Resistance: Concepts. Approaches and Trends in Plant Disease Management, 2014: p. 167.

Zeng, W., M. Melotto, and S.Y. He, Plant stomata: a checkpoint of host immunity and pathogen virulence. Current opinion in biotechnology, 2010. 21(5): p. 599-603.

Zhou, J., et al., Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. The Plant Journal, 2015. 82(4): p. 632-643.

Oliva, R., et al., Broad-spectrum resistance to bacterial blight in rice using genome editing. Nature biotechnology, 2019. 37(11): p. 1344-1350.

Jia, H., et al., Modification of the PthA4 effector binding elements in Type I Cs LOB 1 promoter using Cas9/sg RNA to produce transgenic Duncan grapefruit alleviating XccΔpthA4: dCs LOB 1.3 infection. Plant biotechnology journal, 2016. 14(5): p. 1291-1301.

Langner, T., S. Kamoun, and K. Belhaj, CRISPR crops: plant genome editing toward disease resistance. Annual review of phytopathology, 2018. 56(1): p. 479-512.

Zeilmaker, T., et al., DOWNY MILDEW RESISTANT 6 and DMR 6-LIKE OXYGENASE 1 are partially redundant but distinct suppressors of immunity in Arabidopsis. The Plant Journal, 2015. 81(2): p. 210-222.

Paula de Toledo Thomazella, D., et al., CRISPR-Cas9 mediated mutagenesis of a DMR6 ortholog in tomato confers broad-spectrum disease resistance. BioRxiv, 2016: p. 064824.

Ortigosa, A., et al., Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of Sl JAZ 2. Plant biotechnology journal, 2019. 17(3): p. 665-673.

Das, A., N. Sharma, and M. Prasad, CRISPR/Cas9: a novel weapon in the arsenal to combat plant diseases. Frontiers in Plant Science, 2019. 9: p. 2008.

Shahriar, S.A., et al., Control of plant viral diseases by CRISPR/Cas9: resistance mechanisms, strategies and challenges in food crops. Plants, 2021. 10(7): p. 1264.

Tyagi, S., et al., Engineering disease resistant plants through CRISPR-Cas9 technology. GM Crops & Food, 2021. 12(1): p. 125-144.

Mushtaq, M., et al., Tweaking genome-editing approaches for virus interference in crop plants. Plant Physiology and Biochemistry, 2020. 147: p. 242-250.

Rath, D., et al., The CRISPR-Cas immune system: biology, mechanisms and applications. Biochimie, 2015. 117: p. 119-128.

Ali, Z., et al., CRISPR/Cas9-mediated viral interference in plants. Genome biology, 2015. 16: p. 1-11.

Baltes, N.J., et al., Conferring resistance to geminiviruses with the CRISPR–Cas prokaryotic immune system. Nature Plants, 2015. 1(10): p. 1-4.

Hirano, H., et al., Structure and engineering of Francisella novicida Cas9. Cell, 2016. 164(5): p. 950-961.

Ali, Z., et al., CRISPR/Cas9-mediated immunity to geminiviruses: differential interference and evasion. Scientific reports, 2016. 6(1): p. 26912.

Kis, A., et al., Creating highly efficient resistance against wheat dwarf virus in barley by employing CRISPR/Cas9 system. Plant biotechnology journal, 2019. 17(6): p. 1004.

Mehta, D., et al., Linking CRISPR-Cas9 interference in cassava to the evolution of editing-resistant geminiviruses. Genome biology, 2019. 20: p. 1-10.

Tripathi, J.N., et al., CRISPR/Cas9-mediated editing of DMR6 orthologue in banana (Musa spp.) confers enhanced resistance to bacterial disease. Plant Biotechnology Journal, 2021. 19(7): p. 1291.

Aman, R., et al., RNA virus interference via CRISPR/Cas13a system in plants. Genome biology, 2018. 19: p. 1-9.

Zhang, Y.-Z., M. Shi, and E.C. Holmes, Using metagenomics to characterize an expanding virosphere. Cell, 2018. 172(6): p. 1168-1172.

Zhang, T., et al., Establishing RNA virus resistance in plants by harnessing CRISPR immune system. Plant biotechnology journal, 2018. 16(8): p. 1415-1423.

Zhan, X., et al., Generation of virus-resistant potato plants by RNA genome targeting. Plant biotechnology journal, 2019. 17(9): p. 1814-1822.

Pyott, D.E., E. Sheehan, and A. Molnar, Engineering of CRISPR/Cas9-mediated potyvirus resistance in transgene-free Arabidopsis plants. Molecular plant pathology, 2016. 17(8): p. 1276-1288.

Macovei, A., et al., Novel alleles of rice eIF4G generated by CRISPR/Cas9-targeted mutagenesis confer resistance to Rice tungro spherical virus. Plant biotechnology journal, 2018. 16(11): p. 1918-1927.

Tripathi, L., V.O. Ntui, and J.N. Tripathi, Application of genetic modification and genome editing for developing climate-smart banana. Food and Energy Security, 2019. 8(4): p. e00168.

Tyagi, S., et al., VOCs-mediated hormonal signaling and crosstalk with plant growth promoting microbes. Critical reviews in biotechnology, 2018. 38(8): p. 1277-1296.

Dong, O.X. and P.C. Ronald, Genetic engineering for disease resistance in plants: recent progress and future perspectives. Plant physiology, 2019. 180(1): p. 26-38.

Malnoy, M., et al., DNA-free genetically edited grapevine and apple protoplast using CRISPR/Cas9 ribonucleoproteins. Frontiers in plant science, 2016. 7: p. 1904.

Nekrasov, V., et al., Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. Scientific reports, 2017. 7(1): p. 482.

Huibers, R.P., et al., Powdery mildew resistance in tomato by impairment of SlPMR4 and SlDMR1. PLoS One, 2013. 8(6): p. e67467.

Ma, J., et al., Disruption of OsSEC3A increases the content of salicylic acid and induces plant defense responses in rice. Journal of experimental botany, 2018. 69(5): p. 1051-1064.

Zhang YunWei, Z.Y., et al., Simultaneous modification of three homoeologs of TaEDR1 by genome editing enhances powdery mildew resistance in wheat. 2017.

Li, M., et al., Reassessment of the four yield-related genes Gn1a, DEP1, GS3, and IPA1 in rice using a CRISPR/Cas9 system. Frontiers in plant science, 2016. 7: p. 377.

Li, S., et al., The OsmiR396c-OsGRF4-OsGIF1 regulatory module determines grain size and yield in rice. Plant biotechnology journal, 2016. 14(11): p. 2134-2146.

Liu, J., et al., GW5 acts in the brassinosteroid signalling pathway to regulate grain width and weight in rice. Nature plants, 2017. 3(5): p. 1-7.

Lu, K., et al., Blocking amino acid transporter Os AAP 3 improves grain yield by promoting outgrowth buds and increasing tiller number in rice. Plant Biotechnology Journal, 2018. 16(10): p. 1710-1722.

Zhang, Y., et al., Analysis of the functions of Ta GW 2 homoeologs in wheat grain weight and protein content traits. The Plant Journal, 2018. 94(5): p. 857-866.

Zhang, Y., et al., Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. Nature communications, 2016. 7(1): p. 12617.

Xu, R., et al., Rapid improvement of grain weight via highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. Journal of Genetics and Genomics= Yi chuan xue bao, 2016. 43(8): p. 529-532.

Huang, J., et al., Identifying a large number of high-yield genes in rice by pedigree analysis, whole-genome sequencing, and CRISPR-Cas9 gene knockout. Proceedings of the National Academy of Sciences, 2018. 115(32): p. E7559-E7567.

Zhang, J., et al., Generation of new glutinous rice by CRISPR/Cas9-targeted mutagenesis of the Waxy gene in elite rice varieties. Journal of integrative plant biology, 2018. 60(5): p. 369.

Waltz, E., CRISPR-edited crops free to enter market, skip regulation. Nature Biotechnology, 2016. 34(6): p. 582-583.

Sun, Y., et al., Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. Frontiers in plant science, 2017. 8: p. 298.

Shan, Q., et al., Creation of fragrant rice by targeted knockout of the Os BADH 2 gene using TALEN technology. Plant biotechnology journal, 2015. 13(6): p. 791-800.

Sánchez-León, S., et al., Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. Plant biotechnology journal, 2018. 16(4): p. 902-910.

Jiang, W.Z., et al., Significant enhancement of fatty acid composition in seeds of the allohexaploid, Camelina sativa, using CRISPR/Cas9 gene editing. Plant biotechnology journal, 2017. 15(5): p. 648-657.

Morineau, C., et al., Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid Camelina sativa. Plant biotechnology journal, 2017. 15(6): p. 729-739.

Okuzaki, A., et al., CRISPR/Cas9-mediated genome editing of the fatty acid desaturase 2 gene in Brassica napus. Plant Physiology and Biochemistry, 2018. 131: p. 63-69.

Ito, Y., et al., CRISPR/Cas9-mediated mutagenesis of the RIN locus that regulates tomato fruit ripening. Biochemical and biophysical research communications, 2015. 467(1): p. 76-82.

Li, R., et al., CRISPR/Cas9-mediated mutagenesis of lncRNA1459 alters tomato fruit ripening. The Plant Journal, 2018. 94(3): p. 513-524.

Li, X., et al., Lycopene is enriched in tomato fruit by CRISPR/Cas9-mediated multiplex genome editing. Frontiers in plant science, 2018. 9: p. 559.

Li, R., et al., Multiplexed CRISPR/Cas9-mediated metabolic engineering of γ-aminobutyric acid levels in Solanum lycopersicum. Plant biotechnology journal, 2018. 16(2): p. 415-427.

Nonaka, S., et al., Efficient increase of ɣ-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. Scientific reports, 2017. 7(1): p. 7057.

Nakayasu, M., et al., Generation of α-solanine-free hairy roots of potato by CRISPR/Cas9 mediated genome editing of the St16DOX gene. Plant Physiology and Biochemistry, 2018. 131: p. 70-77.

Zhou, H., et al., Development of commercial thermo-sensitive genic male sterile rice accelerates hybrid rice breeding using the CRISPR/Cas9-mediated TMS5 editing system. Scientific reports, 2016. 6(1): p. 37395.

Li, J., et al., Generation of thermosensitive male-sterile maize by targeted knockout of the ZmTMS5 gene. Journal of genetics and genomics= Yi chuan xue bao, 2017. 44(9): p. 465-468.

Li, Q., et al., Development of japonica photo-sensitive genic male sterile rice lines by editing carbon starved anther using CRISPR/Cas9. Journal of Genetics and Genomics= Yi chuan xue bao, 2016. 43(6): p. 415-419.

Singh, M., et al., Concurrent modifications in the three homeologs of Ms45 gene with CRISPR-Cas9 lead to rapid generation of male sterile bread wheat (Triticum aestivum L.). Plant molecular biology, 2018. 97: p. 371-383.

Xie, Y., et al., Suppression or knockout of SaF/SaM overcomes the Sa-mediated hybrid male sterility in rice. Journal of integrative plant biology, 2017. 59(9): p. 669-679.

Xie, Y., et al., Interspecific hybrid sterility in rice is mediated by OgTPR1 at the S1 locus encoding a peptidase-like protein. Molecular plant, 2017. 10(8): p. 1137-1140.

Shen, R., et al., Genomic structural variation-mediated allelic suppression causes hybrid male sterility in rice. Nature Communications, 2017. 8(1): p. 1310.

Yu, X., et al., A selfish genetic element confers non-Mendelian inheritance in rice. Science, 2018. 360(6393): p. 1130-1132.

Khanday, I., et al., A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. Nature, 2019. 565(7737): p. 91-95.

Wang, C., et al., Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. Nature biotechnology, 2019. 37(3): p. 283-286.

Yao, L., et al., OsMATL mutation induces haploid seed formation in indica rice. Nature plants, 2018. 4(8): p. 530-533.

Li, X., et al., High-efficiency breeding of early-maturing rice cultivars via CRISPR/Cas9-mediated genome editing. Journal of genetics and genomics= Yi chuan xue bao, 2017. 44(3): p. 175-178.

Braatz, J., et al., CRISPR-Cas9 targeted mutagenesis leads to simultaneous modification of different homoeologous gene copies in polyploid oilseed rape (Brassica napus). Plant physiology, 2017. 174(2): p. 935-942.

Ye, M., et al., Generation of self-compatible diploid potato by knockout of S-RNase. Nature Plants, 2018. 4(9): p. 651-654.

Luo, M., B. Gilbert, and M. Ayliffe, Applications of CRISPR/Cas9 technology for targeted mutagenesis, gene replacement and stacking of genes in higher plants. Plant Cell Reports, 2016. 35: p. 1439-1450.

Shi, J., et al., ARGOS 8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant biotechnology journal, 2017. 15(2): p. 207-216.

Yu, Q.-h., et al., CRISPR/Cas9-induced targeted mutagenesis and gene replacement to generate long-shelf life tomato lines. Scientific reports, 2017. 7(1): p. 11874.

Butler, N.M., et al., Geminivirus-mediated genome editing in potato (Solanum tuberosum L.) using sequence-specific nucleases. Frontiers in plant science, 2016. 7: p. 1045.

Dahan-Meir, T., et al., Efficient in planta gene targeting in tomato using geminiviral replicons and the CRISPR/Cas9 system. The Plant Journal, 2018. 95(1): p. 5-16.

Wang, M., et al., Gene targeting by homology-directed repair in rice using a geminivirus-based CRISPR/Cas9 system. Molecular plant, 2017. 10(7): p. 1007-1010.

Hummel, A.W., et al., Allele exchange at the EPSPS locus confers glyphosate tolerance in cassava. Plant biotechnology journal, 2018. 16(7): p. 1275-1282.

Butt, H., et al., Efficient CRISPR/Cas9-mediated genome editing using a chimeric single-guide RNA molecule. Frontiers in plant science, 2017. 8: p. 1441.

Endo, M., M. Mikami, and S. Toki, Biallelic gene targeting in rice. Plant physiology, 2016. 170(2): p. 667-677.

Sun, Y., et al., Engineering herbicide-resistant rice plants through CRISPR/Cas9-mediated homologous recombination of acetolactate synthase. Molecular plant, 2016. 9(4): p. 628-631.

Sauer, N.J., et al., Oligonucleotide-mediated genome editing provides precision and function to engineered nucleases and antibiotics in plants. Plant physiology, 2016. 170(4): p. 1917-1928.

Li, J., et al., Gene replacements and insertions in rice by intron targeting using CRISPR–Cas9. Nature plants, 2016. 2(10): p. 1-6.

Meng, Z., et al., Expression of the rice arginase gene OsARG in cotton influences the morphology and nitrogen transition of seedlings. PLoS One, 2015. 10(11): p. e0141530.

Wang, Y., et al., Increased lateral root formation by CRISPR/Cas9-mediated editing of arginase genes in cotton. Science China. Life Sciences, 2017. 60(5): p. 524.

Peng, R., et al., From sequencing to genome editing for cotton improvement. Trends in Biotechnology, 2021. 39(3): p. 221-224.

Paul, P., et al., MADS78 and MADS79 are essential regulators of early seed development in rice. Plant Physiology, 2020. 182(2): p. 933-948.

Liang, X., et al., COPII components Sar1b and Sar1c play distinct yet interchangeable roles in pollen development. Plant Physiology, 2020. 183(3): p. 974-985.

Li, C., et al., An efficient CRISPR/Cas9 platform for rapidly generating simultaneous mutagenesis of multiple gene homoeologs in allotetraploid oilseed rape. Frontiers in Plant Science, 2018. 9: p. 442.

Sriboon, S., et al., Knock-out of TERMINAL FLOWER 1 genes altered flowering time and plant architecture in Brassica napus. BMC genetics, 2020. 21: p. 1-13.

Rodríguez-Leal, D., et al., Engineering quantitative trait variation for crop improvement by genome editing. Cell, 2017. 171(2): p. 470-480. e8.

Ahmad, Niaz, Mehboob-ur Rahman, Zahid Mukhtar, Yusuf Zafar and Baohong Zhang 2020. A critical look on CRISPR-based genome editing in plants. Journal of Cellular Physiology 235: 666-682.

Beacham, Tracey A., Jeremy B. Sweet and Michael J. Allen 2017. Large scale cultivation of genetically modified microalgae: A new era for environmental risk assessment. Algal Research 25: 90-100. doi: https://doi.org/10.1016/j.algal.2017.04.028

Caplan, Arthur L, Brendan Parent, Michael Shen and Carolyn Plunkett 2015. No time to waste—the ethical challenges created by CRISPR. EMBO reports 16: 1421-1426. doi: https://doi.org/10.15252/embr.201541337

Collins, James P 2018. Gene drives in our future: challenges of and opportunities for using a self-sustaining technology in pest and vector management. In Gene drives in our future: challenges of and opportunities for using a self-sustaining technology in pest and vector management, BMC proceedings, 9: Springer.

Ferrari, Linda 2022. Farmers' attitude toward CRISPR/Cas9: The case of blast resistant rice. Agribusiness 38: 175-194.

Glenna, Leland L 2023. Are intellectual property policies for gene-edited crops fit for purpose? The perspectives of German scientists. Plants, People, Planet 5: 976-984.

Kumam, Yaiphabi, Harold N Trick, PV Vara Prasad and Mithila Jugulam 2023. Transformative Approaches for Sustainable Weed Management: The Power of Gene Drive and CRISPR-Cas9. Genes 14: 2176.

Lio, Aaron 2023. Assessing the Use of CRISPR-Cas9 in Agriculture. University of Toronto's Journal of Scientific Innovation: 45-56.

Liu, Q., F. Yang, J. Zhang, H. Liu, S. Rahman, S. Islam, W. Ma and M. She 2021a. Application of CRISPR/Cas9 in Crop Quality Improvement. Int J Mol Sci 22. doi: 10.3390/ijms22084206

Liu, Qier, Fan Yang, Jingjuan Zhang, Hang Liu, Shanjida Rahman, Shahidul Islam, Wujun Ma and Maoyun She 2021b. Application of CRISPR/Cas9 in crop quality improvement. International Journal of Molecular Sciences 22: 4206.

Maharajan, Theivanayagam, TP Ajeesh Krishna, Kasinathan Rakkammal, Stanislaus Antony Ceasar and Manikandan Ramesh 2022. Application of CRISPR/Cas system in cereal improvement for biotic and abiotic stress tolerance. Planta 256: 106.

Ndudzo, Abigarl, Angela Sibanda Makuvise, Sizo Moyo and Enetia Disberia Bobo 2024. CRISPR-Cas9 genome editing in crop breeding for climate change resilience: Implications for smallholder farmers in Africa. Journal of Agriculture and Food Research 16: 101132. doi: https://doi.org/10.1016/j.jafr.2024.101132

Noman, Ali, Muhammad Aqeel and Shuilin He 2016. CRISPR-Cas9: tool for qualitative and quantitative plant genome editing. Frontiers in plant science 7: 1740.

Peschard, Karine and Shalini Randeria 2020. 'Keeping seeds in our hands': the rise of seed activism. The Journal of Peasant Studies 47: 613-647.

Piergentili, Roberto, Alessandro Del Rio, Fabrizio Signore, Federica Umani Ronchi, Enrico Marinelli and Simona Zaami 2021. CRISPR-Cas and its wide-ranging applications: From human genome editing to environmental implications, technical limitations, hazards and bioethical issues. Cells 10: 969.

Pongpaiboon, Siwat 2022. Understanding the ethical and environmental implications of genetically modified crops through the Bt insecticidal gene. International Journal of Research in Engineering, Science and Management 5: 26-33.

Shew, Aaron M, L Lanier Nalley, Heather A Snell, Rodolfo M Nayga Jr and Bruce L Dixon 2018. CRISPR versus GMOs: Public acceptance and valuation. Global Food Security 19: 71-80.

Tripathi, Leena, Kanwarpal S Dhugga, Valentine O Ntui, Steven Runo, Easter D Syombua, Samwel Muiruri, Zhengyu Wen and Jaindra N Tripathi 2022. Genome editing for sustainable agriculture in Africa. Frontiers in Genome Editing 4: 876697.

Wynberg, Rachel 2023. Biopiracy: Crying wolf or a lever for equity and conservation? Research Policy 52: 104674. doi: https://doi.org/10.1016/j.respol.2022.104674