

Evaluating CRISPR/Cas9's Progressive Role in Crop Enhancement and Sustainable Agriculture

Abstract

This review paper provides a comprehensive exploration of the progressive effect of CRISPR/Cas9 genome altering innovation on functional genomics and crop enhancement. It highlights the evolution of CRISPR technology, emphasizing its versatile applications in plant breeding. The paper discusses the transition from gene knockouts to precise modifications and multiplex genome engineering, with a focus on enhancing plant nutrition, disease resistance, and drought tolerance. Traditional Cas9-gRNA delivery methods are compared with ascending CRISPR ribonucleoproteins (RNPs) as solutions to overcome limitations associated with plasmid-based systems. The booming worldwide claim for food crops underscores the possibility of CRISPR/Cas9-based genome editing for improving cereal features. Earlier innovations like zinc finger nucleases (ZFNs) and TALENs, CRISPR/Cas9 offers a more cost-effective and time-efficient approach. It enables the blueprint and cloning of numerous guide RNAs for accurate genome targeting, with enhanced specificity and efficiency achieved through modified Cas9 cassettes and enzymes from various bacterial species. The review also emphasizes CRISPR/Cas9's potential to develop non-genetically modified (Non-GMO) crops with certain needed traits, thereby boosting yield potential under various stress conditions. Furthermore, the article discusses problems and solutions analogous with CRISPR/Cas9-based genome editing, providing valuable insights for researchers in crop advancement.

Keywords: *Genome editing, ribonucleoproteins, Cas9-gRNA, ZFNs, TALENs and Non-GMO*

1. Introduction

The rising worldwide populace development has brought about a huge flood in the interest in food crops. In any case, conventional techniques for breeding harvests require broad work, assets, and time. Thus, the current harvest assortments and current agrarian practices will probably miss the mark concerning meeting the future worldwide food interest (Van Vu et al. 2022). The CRISPR/Cas framework has acquired far-reaching use for genome altering in plants and the age of freaks because of its straightforwardness and accommodation. It is progressively utilized to alter attributes in different plants, including vital harvests, and to make new hereditary assets. The normal CRISPR/Cas9 framework used for genome altering works by separating DNA through the direction of RNA, with the DNA endonuclease Cas9 (Gaillochet et al. 2021). The refinement of this framework improves the effectiveness and accuracy of quality adjustment, hence speeding up the course of plant breeding. Thus, CRISPR/Cas9 has continuously arisen as the most broadly used and good quality-altering framework. This survey fills in as a prologue to the CRISPR/Cas9 genome-altering framework, illustrating the advancement in related research, the hidden component, the connected innovation, and its appropriateness in crop improvement (Zegeye et al. 2022). Moreover, the survey tends to the impediments of the CRISPR/Cas9 framework and examines likely procedures to conquer these difficulties. Specialists will find basic bits of knowledge in this survey now relevant for the usage of CRISPR/Cas9 quality-altering innovation to improve crops and foster novel cultivars.

Commented [DV1]: Add "the"

Commented [DV2]: Write as guide RNA

Commented [DV3]: Modify the sentence in a easily understandable way

Commented [DV4]: Mention full form for the first time

Commented [DV5]: Write in a short form

Commented [DV6]: Modify this word as it is repetition of the first lines

Crop improvement is a multi-layered try with the overall objectives of upgrading crop yield, supporting obstruction against biotic and abiotic stresses, lifting quality, and increasing health benefits (Sinha et al. 2023). Different techniques **is** being used to upgrade different harvest qualities, including regular reproducing strategies (Wenefrida et al. 2013), substance and radiation-interceded transformation breeding (Lusser et al. 2012), sub-atomic marker-helped breeding (Ramesh et al. 2020) and hereditary designing breeding (Chaudhary et al. 2019); (Liu et al. 2021).

Commented [DV7]: Correct it as "are"

Lately, genome altering (GE) innovation has arisen as a groundbreaking way to deal with cereal improvement (Ahmar et al. 2020). GE empowers exact and unsurprising adjustment of plant genomes, bringing about inheritable transformations at explicit destinations. This innovation limits the gamble of askew impacts and wipes out the reconciliation of exogenous quality groupings (Rato et al. 2021). GE-interceded DNA adjustments envelop cancellations, additions, **single-nucleotide replacements (SNPs)**, and enormous section replacements. Four significant groups of **site-coordinated nucleases (SDNs)** are engaged with a nucleotide extraction instrument, including homing endonucleases (HEs) (Liu et al. 2021), (ZFNs) (Bibikova et al. 2002) and CRISPR-related protein (Cas) frameworks (Davidson et al. 2020). Most SDNs definitively target twofold strand format DNA to instigate twofold strand breaks (DSBs).

Commented [DV8]: Check for the corrected abbreviations

Commented [DV9]: Check for the corrected abbreviations

ZFNs and TALENs address the original of genome-altering nucleases, joining zinc finger DNA-restricting areas with the FokI endonuclease space or explicit DNA-restricting spaces from Story proteins, separately (Becker et al. 2021). In any case, these progressions are agitated about their confounded advancement processes, confining their wide application in plants. ZFNs and TALENs, while powerful quality-altering apparatuses have experienced difficulties in their broad execution in plants due to the multifaceted and asset-serious development processes included. Conversely, CRISPR, initially recognized in **E. coli** in 1987 as a protection system against attacking viral and plasmid DNA (Ishino et al. 1987), has ascended to unmistakable quality as a surprisingly productive and clear genome-altering innovation. The straightforwardness of CRISPR altering, which depends on the nucleotide complementarity of guide RNA to explicit successions, has extraordinarily worked with its far and wide usage in different harvests, as well major cereals (Montecillo et al. 2020), and **Solanum Lycopersicum** (Rajput et al. 2021). This convenience positions CRISPR as a main decision for specialists and reproducers trying to upgrade crop characteristics. The reception of GE in crop improvement has seen a significant increment, with specific accentuation on further developing yield quality. This survey typifies the new progressions in CRISPR/Cas9-intervened crop quality upgrade and dives into the likely future uses of genome altering in the domain of yield improvement.

Commented [DV10]: Scientific names should be in italics

Commented [DV11]: Scientific names should be in italics and species should be in small letters

1.2 History and evolution of CRISPR System

CRISPR is the critical component of RNA-intervened versatile resistance in prokaryotes, including microbes and archaea. It is introduced in **Escherichia coli** in 1980s and in this manner depicted by (Ishino et al. 1987). These DNA fragments were challenging to sequence at the time, taking many months, and researchers were uncertain about their origins or function within the bacterial cell (**Gostimskaya et al. 2022**). A new approach was developed using specific nucleases for targeted modifications. Sangamo Biosciences discovered zinc finger nucleases (ZFNs) that have non-specific FokI endonuclease cleavage domains and DNA binding motifs in

Commented [DV12]: Scientific names should be in italics

Commented [DV13]: Remove bold form

addition to restriction activities (Curtin *et al.* 2011). The discovery (TALENs), which divide DNA at particular locations, was made possible by the non-specific cleavage of ZFNs (Christian *et al.* 2010).

The disclosure that *Streptococcus thermophilus* can foster protection from a bacteriophage by embedding a piece of the irresistible infection's genome into the CRISPR locus has given the initial trial knowledge into the working of the CRISPR framework (Doudna and Charpentier 2014; Samsø *et al.* 2015). As a result of their discovery, Danisco began utilizing CRISPR systems to "vaccinate" bacterial cells in 2005, and the authors received one of the first patents in the field (Isaacson, 2021). A brief history and evolution of CRISPR System is illustrated in figure 1.

Commented [DV14]: Remove bold form

Commented [DV15]: Remove bold form

Commented [DV16]: Scientific names should be in italics

Commented [DV17]: Give space before "As"

Commented [DV18]: Remove bold form

Commented [DV19]: Give space before "A"



Figure 1: CRISPR/Cas9 technology has witnessed many important discoveries and developments.

1.1 Elucidating CRISPR/Cas Components and Functions

Figuring out the job of the CRISPR/Cas framework in microscopic organisms and archaea has divulged its true capacity as a genome-altering device. A progression of examinations, upheld by computational biological devices, has explained the various parts of CRISPR/Cas and their capability to give versatile resistance to bacterial cells. A CRISPR locus is described by bunches of CRISPR-related (Cas) qualities and CRISPR clusters, which act as stores of immunological memory (Zink et al. 2020). The CRISPR exhibit includes successions of 21-40 base pair rehash groupings (direct rehashes) mixed with 25-40 base pair variable arrangements (spacers) (Jansen et al. 2020). In 2005, three autonomous examination gatherings (Rawashdeh et al. 2022) proposed the job of spacer components as leftovers of past experiences with unfamiliar DNA, giving resistance against phage contaminations. They likewise noticed that share a typical end grouping, presently perceived as PAM (Protospacer Nearby Theme). Palacios Araya et al. (2021) tentatively showed association of CRISPR exhibits in giving protection from bacteriophages, related to Cas qualities.

Commented [DV20]: PAM stands for Protospacer adjacent motif

1.2 Potential usage of the CRISPR

Progression in CRISPR/Cas9 innovation is unrivaled. The greater part of the examination directed to date has basically centered around quality knockout or quality quieting instruments through Non-Homologous End Joining (NHEJ), which transcendent and below exact component. Notwithstanding, encouraging results have been seen in mammalian and plant cells while utilizing quality thump-in or quality substitution techniques, which include designated mutagenesis through Homology-Coordinated Fix (HDR). Before, accomplishing homology-driven fixes in plants was a moving errand because of its low proficiency and the wasteful conveyance of homologous giver groupings in transduced plant cells, as verified by Puchta and Fauser (2014) and Steinert et al. (2016). By and by, various methodologies have been created to improve the effectiveness of the homology-coordinated fix instrument, and these endeavors have yielded victories, as revealed by Sun et al. (2022). Leading genomic concentrates on in woody plants present remarkable difficulties due to their lengthy vegetative periods, low hereditary change productivity, and restricted accessibility of freaks.

Commented [DV21]: Check for spelling

Commented [DV22]: Add "is"

Commented [DV23]: Add "the"

Commented [DV24]: Check for spelling

It is quite significant that CRISPR/Cas9 innovation isn't restricted to higher plants; it has additionally been applied to bring down individuals from the plant realm, including green growth, bryophytes, and pteridophytes. Liverworts, for example, have arisen as a model animal category for concentrating on the development of land plants. CRISPR/Cas9-assigned mutagenesis has been used to investigate *Marchantia polymorpha* L.'s sub-nuclear innate characteristics, as demonstrated by Sauret Gueto et al. (2020). Past genome altering, CRISPR/Cas9 innovation is persistently advancing and tracking down applications in different spaces, adding to how we might interpret useful genomics and atomic science. The ongoing accentuation is on dropping-of-capability and achievement-of-capability investigation of single qualities, as well as the distinguishing proof of quality modules and hereditary articulation designs Li et al. (2020). Figure 2 represents the growing uses of the CRISPR/Cas9 framework, a considerable lot of which are yet to be investigated with regard to plants.

Commented [DV25]: Scientific names should be in italics

Besides, its utilized to create chromosomal erasures traversing numerous DNA base matches in plant species like *Arabidopsis* and *Nicotiana benthamiana*, among others Matsuo et al. (2020).

Commented [DV26]: Scientific names should be in italics

The CRISPR framework holds huge commitment in the field of horticulture, offering a scope of potential applications that could reform crop reproducing and creation. One huge area of use is crop improvement. By exactly focusing on and altering explicit qualities answerable for various characteristics obstruction, and abiotic stress resilience, CRISPR innovation empowers the improvement of harvests with upgraded ascribes. This not just can possibly increment food security by further developing harvest yields and quality but in addition to lessening the requirement for compound pesticides and manures, advancing more maintainable agrarian practices (Zafar et al. 2020).

Commented [DV27]: Add "in"

What's more, the CRISPR framework can be utilized for the fast advancement of new harvest assortments. Customary breeding strategies are tedious and frequently depend on the possibility of hereditary recombination. CRISPR innovation takes into consideration the immediate altering of target qualities, essentially speeding up the reproducing system. In addition, it empowers the presentation of advantageous characteristics from wild or related plant species into developed assortments, extending the hereditary variety accessible for crop improvement. With its capacity to make non-transgenic, hereditarily changed crops and to address explicit rural difficulties, CRISPR is ready to assume a critical part in forming the eventual fate of farming—(Saini et al. 2020).

Commented [DV28]: Add reasonable explanation

2. Visionary concepts of CRISPR/Cas9 technology

Throughout recent years, research in genome-altering devices has risen above customary limits, going from exact quality changes to the plan of eIF4E obstruction alleles, a basic player in infection opposition (Zaidi et al. 2020). The CRISPR-Cas9 innovation arisen the eventual fate of changing genome altering, offering powerful and compelling results. It has significantly changed the scene of quality altering in plants, including crops, and investigating the principal science of plant advancement and stress reactions prepares for the making of world-class and unrivaled yield assortments: (Gordon-Kamm et al. 2021)

Commented [DV29]: Change tense "arose"

Commented [DV30]: Correct the spelling

This innovation has shown its viability in combatting rice shoot sickness, with an explicit focus on the OsERF922 quality in rice prompting the distinguishing proof of 21 CRISPR-ERF922 actuated freaks from 50 T₀ transgenic plants (Távora et al. 2021). Moreover, high outputs can be accomplished by joining cytidine deaminase chemical with Cas9, considering the effective adjustment of target codons in rice (Komatsu et al. 2020). The combination of dCas9 with cytidine deaminase empowers the immediate transformation of cytidine to uridine, bringing about a change from C/G base matches to T/A base matches during replication in one little girl cell (Lau et al. 2020). While research is progressing quickly in this progressive field, the relentless test of askew impacts in plants remains, which can be tended to through entire genome sequencing. A few organizations are effectively using this innovation to create tip-top food and feed crops.

Commented [DV31]: Keep it in subscript

2.1 Revolutionizing Crop Breeding: A Journey from Crossbreeding to CRISPR-Cas9 Genome Editing"

Evolutionary trajectory of crop breeding techniques, examining the timetaking processes of crossbreeding, mutation breeding, and revolutionary approach of genome editing in **Figure 3**. Crossbreeding, a traditional method requires considerable time (8–10 years) to enhance advantageous characteristics in a specific organism

Commented [DV32]: Give space after time

Commented [DV33]: Remove bold form

Commented [DV34]: Correct the spelling

(Ahmar *et al.* 2020). This involves exchange of genetic material with a best variety line with a donor variety line, followed by multiple backcrossing cycles to eliminate undesirable traits and introduce new progeny with improved characteristics. Transgenic breeding, a well-known method, facilitates the improvement of crop traits (4–6 years) by introducing exogenous genes into elite varieties (Salonia *et al.* 2020). The most recent innovation, genome editing, emerges as a game-changer, enabling the rapid enhancement of targeted traits in elite varieties within an unprecedented timeframe of 2–3 years (Serba *et al.* 2020). This technique involves precise revisions to the target gene or regulatory sequence, marking a paradigm shift in crop breeding efficiency and precision. The review provides an in-depth analysis of these techniques, highlighting their strengths, limitations. Scientists at The Novo Nordisk Foundation Centre for Biosustainability (DTU Biosustain) have created a procedure that significantly accelerates the development of bacteria for various bioproduction applications, as well as expanding the number of genomic regions that can be edited (Jensen *et al.* 2019). This innovation was not possible with earlier CRISPR systems. Thus, it utilizes modified Cas9 enzymes along with CBE or ABE editor modules, which function as molecular pencils to accurately control gene activity by altering specific DNA nucleotides (Tong *et al.* 2019). Pablo Casasso has set a new benchmark in CRISPR-Cas technology by developing a toolkit that enables accurate and reversible DNA modifications in bacteria; this method goes beyond the limitations of traditional CRISPR technology and significantly enhances industry and research capabilities to create bacterial cell factories. pAbloCasso facilitates the development of bacteria for various bioproduction applications, including pharmaceuticals and biofuels, aligned with sustainable production objectives, by enabling quick and accurate editing (Kozaeva *et al.* 2024).

Commented [DV35]: Remove bold form

Commented [DV36]: Remove bold form

Commented [DV37]: Remove bold form

Commented [DV38]: Remove bold form

2.2 The "pAblo-pCasso" toolset for unconstrained alteration of cytidine and adenine bases in *Pseudomonas* species, with self-relieving vector capabilities.

This research introduces a novel usage of SpCas9 variant, Deft, which has minimal PAM requirements, the substitution of nucleotides in Gram-negative bacterial structures. Additionally, the study describes the enhancement of a plasmid toolkit named pAblo-pCasso for effective base modification. Both the conventional SpnCas9 and Agile, which have very low PAM requirements, were employed along with various management modules such as CBE and ABE. It was discovered that the Nimble variant targets almost every PAM in the genetic material, preferring 5'-NRN-3' over 5'-NYN-3', where R stands for A or G and Y for C or T. Moreover, to enhance the variety of instruments for metabolic designing applications, a bunch of enlistment-reliant, self-easing, and standardized vectors were created, showing contingent replication for simple plasmid re-establishing (Kozaeva *et al.* 2024).

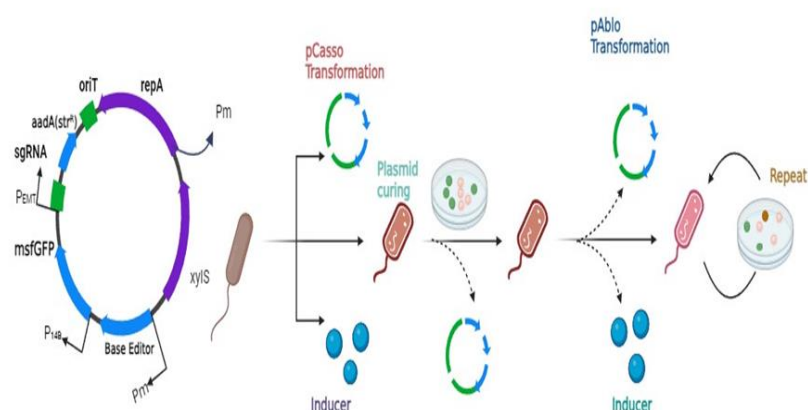


Figure 3: Graphical Representation of the pAbo-pCasso self-curing vector toolset for unconstrained cytidine and adenine base-editing in Gram-negative bacteria.

Table 1: List of genomic approaches viz. CRISPR/Cas9 in biofortification in cereals

Crop	Genome-editing	Nutrients	Vectors used	Reference
Rice	CRISPR/Cas9	Carotenoid	–	Dong <i>et al.</i> 2020
		High amylose	<i>pCXUN-Cas9</i>	Sun <i>et al.</i> 2017
		Low phytic acid	<i>pH_itpk6</i>	Jiang <i>et al.</i> 2019
		Beta- carotene	–	Endo <i>et al.</i> , 2019
		Amylose	<i>CRISPR/Cas9</i> <i>vector</i>	Zhang <i>et al.</i> 2018a, b
		Sucrose efflux transporter	<i>pTOPO/D</i>	Jiang <i>et al.</i> 2019
		Amylase synthase	<i>pCAMBIA1300</i>	Ma <i>et al.</i> 2015
Wheat	CRISPR/Cas9	Low gluten	<i>pANIC-6E</i> <i>destination vector</i>	Navarro-León <i>et al.</i> 2018
		Fe, mg	<i>pBract202</i>	Connorton <i>et al.</i> 2017
Maize	CRISPR/Cas9	Carotenoid	<i>pMD18-T</i>	Zhu <i>et al.</i> 2016
		Low phytic acid content	<i>pEasy blunt vector</i>	Liang <i>et al.</i> 2014

Table 2: A use of CRISPR/Cas9 in genome editing in legumes

Legume crop	Targeted genes	References
<i>Glycine max</i>	Phytoene desaturase	Al Amin <i>et al.</i> 2019
	glutamine synthase	Bao <i>et al.</i> 2019
	chalcone-flavanone isomerase	Bao <i>et al.</i> 2020
<i>Cicer arietinum</i>	4-coumarate ligase (4CL)	Badhan <i>et al.</i> 2021
	Reveille 7 (RVE7)	
<i>Medicago truncatula</i>	Hua enhancer1	Curtin <i>et al.</i> 2018
	phytoene desaturase	Meng <i>et al.</i> 2017
	symbiosis receptor-like kinase	Wolabu <i>et al.</i> 2020
<i>Vigna unguiculata</i>	Symbiosis receptor-like kinase	Che <i>et al.</i> 2021
	VuSPO11-1	Ji <i>et al.</i> 2019
<i>Lotus japonicus</i>	glucosyltransferase	Curtin <i>et al.</i> 2018
	Lotus histidine kinase 1 (LHK1)-interacting protein (LjCZF1-2)	Wang <i>et al.</i> 2019

3. CRISPR Applications for Trait Improvement in Crop Plants

Eliminating undesirable traits is a potential strategy for genetically enhancing plants. Moreover, the most common use of CRISPR/Cas is thought to be theoretically possible: the removal of genes that exhibit harmful characteristics in plants. Enhancement of nutrition, quality, yield, and biotic and abiotic stress tolerance are among the phenotypic and genotypic features that have been improved with CRISPR/Cas9. Hybrid breeding, which has also been accomplished using CRISPR technology, is one of the other key areas for increasing crop productivity (Chen *et al.* 2019). Agrobacterium-mediated transformation was the primary method used to successfully carry out most CRISPR knock-out studies in plants. Other methods included PEG-mediated (Andersson *et al.* 2017), particle bombardment (Zhang *et al.* 2018b), electroporation (Gago *et al.* 2017), and ribonucleoprotein delivery (Andersson *et al.* 2018; González *et al.* 2020).

3.1 Yield Enhancement

CRISPR is an effective method that can be used to increase rice output and possibly other commercially significant crops as well. Like this, triplet gene knockout significantly increased the length, width, and TGW of rice grains by 11.69, 8.47, and 12.68% in double mutants and increased these morphological parameters to 25.3, 20.5, and 29.8% in triple mutants. These triplet genes are grain weight 2 (GW2), grain weight (GW5), and thousand grain weight 6 (TGW6) (Xu *et al.* 2016). Though since most of the genes affecting yield are quantitative and controlled by quantitative trait loci, a straightforward gene knockout would not be sufficient to reveal changes linked to yield augmentation. 57 genes from 30 cultivars of the Green Revolution miracle rice variety IR8 were

Commented [DV39]: Mention the full form

identified by Huang et al. 2018a, who also discovered that these genes modified using CRISPR/Cas9 or dCas9 are of high-yielding kinds. Additionally, the phenotypic research demonstrated that these genes are required for higher rice yields. Deeper understanding of the process underlying yield improvement was made possible by this work, which may pave the way for the development of better rice varieties through molecular breeding. A dwarf type of indica rice crop with higher yield has been created using genome editing technology (Han et al. 2019). In accordance with this, Hu et al. 2019 generated knockout alleles of *sd1*, a semi-dwarf variety of rice known to exist in elite landraces. When the *DEP1* and *GN1A* genes were knocked out using the CRISPR/Cas system, rice yield increased by 40.9 and 21%, respectively, over the control (Huang et al. 2018b).

3.2 Stress Tolerance

Rice strains containing *Xanthomonas* demonstrated a strong resistance to this pathogen using CRISPR-mediated targeted knockout of effector proteins (Oliva et al. 2019). The plant exhibited a reduced viral load of around 43–45% of the cassava brown streak virus when the *nCBP-1* and *nCBP-2* genes were knocked out. According to Gomez et al. (2019), these genes directly interact with the virus's genome-encoded proteins to result in function loss. Due to frameshift mutations in the *TaHRC* gene brought about by CRISPR, the fusarium head blight disease in wheat was reduced by more than 40% (Su et al. 2019). Similar to this, CRISPR-mediated targeted deletions in *Theobroma cacao* resulted in the mutation of *TcNPR3*, a regulator of the pathogen defence response gene, which decreased *Phytophthora tropicalis*-caused leaf lesions by more than 60% (Shi et al. 2013; Fister et al. 2018). It has been discovered that the novel transcription factor known as ABA-induced transcription repressor (*AITR*) functions as a negative regulator under abiotic stress conditions. As a result, knocking out the *AITR* genes has significantly exacerbated the stress caused by salinity and drought in *Arabidopsis thaliana*. Abiotic stressors like salt, drought, and cold can also cause issues, but CRISPR gene editing can help (Mushtaq et al. 2018; Zafar et al. 2020).

3.3 Biofortification

There is an increasing need for nutritious food on a global scale. Through improved nutritional status, the CRISPR/Cas9-based gene-editing method has the potential to increase crop value and food quality. Bioactive substances found in plants include carotenoids, lycopene, isoflavones, vitamins, and several other nutrient-dense materials with significant medicinal potential. Therefore, there is a strong chance that the use of CRISPR/Cas9 editing will enhance the amount of these powerful bio compounds. One effective way to prevent the build up of harmful substances like heavy metals is to knock down the genes that control the intake of poisonous compounds. The effectiveness of cadmium reduction by molecular breeding has been demonstrated; for example, *OsNramp5* knockdown reduced cadmium accumulation in rice by over 97% without affecting yield (Ishikawa et al. 2012; Tang et al. 2017). According to Jiang et al. (2017), knocking out the *FAD2* gene in *Camelina* resulted in a more than 70% reduction in linolenic acid, while knocking out the *CYP79D1* and *CYP79D2* genes also resulted in a 92% decrease in cyanide concentration. Eliminating Granule bound starch synthase (*GBSS1*) in cassava resulted in edited lines with no amylose in the roots (Bull et al. 2018). When cooking cassava, removing the amylose is a desired feature for those who eat it frequently. Cadmium (Cd) is a heavy metal that is poisonous, but rice may grow in contaminated soil because it absorbs Cd. Consuming rice grains that have accumulated lead (Cd) may eventually be dangerous for living things. Recently, Chen et al. (2023) eliminated *OsLCD* in rice that has a high affinity towards Cd in order to diminish the accumulation of Cd in rice. This produced an exceptional variety of

Commented [DV40]: Mention the species and italicize them

Commented [DV41]: Correct spelling as "effector"

Commented [DV42]: Scientific names should be in italics

Commented [DV43]: Scientific names should be in italics

Commented [DV44]: Scientific names should be in italics

rice germplasm with lower accumulation of Cd. These techniques can be used to enhance agricultural features in different crops.

3.4 Quality Enhancement

Crop improvement programs work continuously to improve the general quality of crops for use in industrial or consumer applications. Quality enhancement is another crucial criterion for any food product to increase its market value. To enhance the quality of crops, some work has been done in this direction using CRISPR. The starch content of staple crops like rice, wheat, and maize—which is a blend of amylose and amylopectin—is one of their key characteristics. Similar research using GBBS gene knockout to reduce amylose content has also been done on potatoes (Andersson et al. 2018; Kusano et al. 2018) and rice (Zhang et al. 2018b). Very long-chain fatty acids (VLCFAs), which are typically regarded as harmful for both industrial and food use, are more prevalent in camelina sativa seed oil. Therefore, the fatty acid elongase1-encoding FAE1m gene was knocked out using CRISPR/Cas9 to raise oleic, linoleic, and α -linolenic acid levels and subsequently lower (2%) VLCFA levels in comparison to wild-type (22%). (Ozseyhan et al. 2018). Increasing a product's shelf life is crucial for improving its value on the market, as it directly affects the price of the produce, particularly tomatoes. Ailsa Craig, Mamirio, and Golden Bell tomato cultivars were among the two that had their ripening inhibitor (RIN) gene knocked out using CRISPR/Cas9 gene editing. This led to a delayed ripening process and a decreased lycopene content (Ito et al. 2015; Jung et al. 2018). *Solanum tuberosum*, or potatoes, are one of the main crops consumed worldwide. Steroid glycoalkaloids, or SGAs, are accumulated in most potato tissues and are harmful to a wide range of species in addition to having a bitter taste. Reducing SGA is hence essential for potato breeding. To solve this issue, the St16DOX gene was deleted from potato hairy roots, and the modified hairy roots did not exhibit a discernible level of SGAs (Nakayasu et al. 2018).

4. Drawbacks of the current CRISPR/Cas9 system

The CRISPR/Cas9 framework has for some time been proclaimed as the essential decision for genome altering (GE) in plant species; be that as it may, its widespread application faces imperatives, as delineated in Figure 4. Late exploration has steadily centered around refining this framework to support proficiency and unwavering quality, bringing about the development of novel CRISPR/Cas variations like spCas9-NG, base altering, and xCas9 (Veillet et al. 2020). The accompanying sections outline the main points of interest with CRISPR/Cas9 and feature the worthwhile qualities the variations. One critical test relates to the enormous size of the CRISPR/Cas9 framework, which hampers altering effectiveness as well as blocks bundling viral vectors for physical tissue conveyance. The basis for a more minimal CRISPR/Cas framework becomes clear, particularly for accomplishing productive genome altering in plants (Tanks et al. 2019).

Commented [DV45]: Where is figure 4?

5. Conclusion

The accuracy and flexibility presented by CRISPR/Cas9, worked with by guide RNA and the Cas9 endonuclease, have changed practical genomics and harvest upgrades. The developmental excursion of CRISPR innovation is followed, stressing its different applications in plant breeding and its urgent job in tending to worldwide agrarian difficulties. The adaptability of CRISPR/Cas9 is featured through its different apparatuses, empowering quality knockouts, exact alterations, multiplex genome designing, and quality actuation or constraint. Key areas of the center incorporate upgrading plant nourishment, supporting illness opposition, and creating dry season lenient

yields. The survey investigates the advancement of conveyance strategies, from conventional Cas9-gRNA frameworks to the development of CRISPR ribonucleoproteins (RNPs), offering answers for conquering restrictions related to plasmid-based frameworks. In the midst of the rising worldwide interest in food crops, CRISPR/Cas9 arises as a strong answer for improving yield qualities. Its expense viability and time productivity, outperforming prior quality-altering devices like zinc finger nucleases (ZFNs) and TALENs, position it as a critical innovation for exact genome focusing. Changed Cas9 tapes and chemicals from different bacterial species further upgrade explicitness and effectiveness in quality altering. The audit not only highlights CRISPR/Cas9's true capacity for creating non-hereditarily adjusted crops with wanted attributes yet in addition tends to difficulties related to this innovation. Giving important experiences, the article guides analysts in outfitting CRISPR/Cas9 for crop improvement, plant reproducing, and quality practical examination. As the interest both quality and amount crops rises, CRISPR/Cas9 remains an extraordinary device, offering accuracy, flexibility, and expectation for the eventual fate of horticulture.

Reference

- Abdallah, N. A., Elsharawy, H., Abulela, H. A., Thilmony, R., Abdelhadi, A. A., & Elarabi, N. I. (2022). Multiplex CRISPR/Cas9-mediated genome editing to address drought tolerance in wheat. *GM Crops & Food* 17. <https://doi.org/10.1080/21645698.2022.2120313>.
- Ahmar, S., Saeed, S., Khan, M. H. U., Ullah Khan, S., Mora-Poblete, F., Kamran, M., ... & Jung, K. H. (2020). A revolution toward gene-editing technology and its application to crop improvement. *International Journal of Molecular Sciences*, 21(16), 5665. <https://doi.org/10.3390/ijms21165665>
- Andersson, M., Turesson, H., Nicolai, A., Fält, A. S., Samuelsson, M., & Hofvander, P. (2017). Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISPR-Cas9 expression in protoplasts. *Plant cell reports*, 36, 117-128. <https://doi.org/10.1007/s00299-016-2062-3>
- Andersson, M., Turesson, H., Olsson, N., Fält, A. S., Ohlsson, P., Gonzalez, M. N., ... & Hofvander, P. (2018). Genome editing in potato via CRISPR-Cas9 ribonucleoprotein delivery. *Physiologia Plantarum*, 164(4), 378-384. <https://doi.org/10.1111/ppl.12731>
- Andersson, M., Turesson, H., Olsson, N., Fält, A. S., Ohlsson, P., Gonzalez, M. N., ... & Hofvander, P. (2018). Genome editing in potato via CRISPR-Cas9 ribonucleoprotein delivery. *Physiologia Plantarum*, 164(4), 378-384. <https://doi.org/10.1111/ppl.12731>
- Arslan, E., Agar, G., & Aydin, M. (2021). Humic acid as a bio stimulant in improving drought tolerance in wheat: The expression patterns of drought-related genes. *Plant Molecular Biology Reporter*, 39(3), 508-519. <https://doi.org/10.1007/s11105-021-01322-6>
- Becker, S., & Boch, J. (2021). TALE and TALEN genome editing technologies. *Gene and Genome Editing*, 2, 100007. <https://doi.org/10.1016/j.ggedit.2021.100007>
- Bhuyan, S. J., Kumar, M., Devde, P. R., Rai, A. C., Mishra, A. K., Singh, P. K., & Siddique, K. H. (2023). Progress in gene editing tools, implications and success in plants: a review. *Frontiers in Genome Editing*, 5. <https://doi.org/10.3389/fgeed.2023.1272678>

- Bibikova, M., Golic, M., Golic, K. G., & Carroll, D. (2002). Targeted chromosomal cleavage and mutagenesis in *Drosophila* using zinc-finger nucleases. *Genetics*, *161*(3), 1169-1175. <https://doi.org/10.1093/genetics/161.3.1169>
- Bull, S. E., Seung, D., Chanez, C., Mehta, D., Kuon, J. E., Truernit, E., ... & Vanderschuren, H. (2018). Accelerated ex situ breeding of GBSS-and PTST1-edited cassava for modified starch. *Science advances*, *4*(9), eaat6086. <https://doi.org/10.1126/sciadv.aat6086>
- Chaudhary, J., Alisha, A., Bhatt, V., Chandanshive, S., Kumar, N., Mir, Z., ... & Deshmukh, R. (2019). Mutation breeding in tomato: advances, applicability and challenges. *Plants*, *8*(5), 128. <https://doi.org/10.3390/plants8050128>
- Chen, H., Ye, R., Liang, Y., Zhang, S., Liu, X., Sun, C., ... & Yi, J. (2023). Generation of low-cadmium rice germplasm via knockout of OsLCD using CRISPR/Cas9. *Journal of Environmental Sciences*, *126*, 138-152. <https://doi.org/10.1016/j.jes.2022.05.047>
- Chen, K., Wang, Y., Zhang, R., Zhang, H., & Gao, C. (2019). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annual review of plant biology*, *70*, 667-697. <https://doi.org/10.1146/annurev-arplant-050718-100049>
- Christian, M., Cermak, T., Doyle, E. L., Schmidt, C., Zhang, F., Hummel, A., ... & Voytas, D. F. (2010). Targeting DNA double-strand breaks with TAL effector nucleases. *Genetics*, *186*(2), 757-761. <https://doi.org/10.1534/genetics.110.120717>
- Curtin, S. J., Zhang, F., Sander, J. D., Haun, W. J., Starker, C., Baltes, N. J., ... & Stupar, R. M. (2011). Targeted mutagenesis of duplicated genes in soybean with zinc-finger nucleases. *Plant physiology*, *156*(2), 466-473. <https://doi.org/10.1104/pp.111.172981>
- Davidson, A. R., Lu, W. T., Stanley, S. Y., Wang, J., Mejdani, M., Trost, C. N., ... & Sontheimer, E. J. (2020). Anti-CRISPRs: protein inhibitors of CRISPR-Cas systems. *Annual review of biochemistry*, *89*, 309-332. <https://doi.org/10.1146/annurev-biochem-011420-111224>
- Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, *346*(6213), 1258096. <https://doi.org/10.1126/science.1258096>
- Fan, Yawei, and Yibo Li. "Molecular, cellular and Yin-Yang regulation of grain size and number in rice." *Molecular Breeding* 39.12 (2019): 163.
- Fister, A. S., Landherr, L., Maximova, S. N., & Gultinan, M. J. (2018). Transient expression of CRISPR/Cas9 machinery targeting TcNPR3 enhances defense response in *Theobroma cacao*. *Frontiers in plant science*, *9*, 329023. <https://doi.org/10.3389/fpls.2018.00268>
- Gago, C., Drosou, V., Paschalidis, K., Guerreiro, A., Miguel, G., Antunes, D., & Hilioti, Z. (2017). Targeted gene disruption coupled with metabolic screen approach to uncover the LEAFY COTYLEDON1-LIKE4 (LIL4) function in tomato fruit metabolism. *Plant Cell Reports*, *36*, 1065-1082. <https://doi.org/10.1007/s00299-017-2137-9>
- Gaillochet, C., Develtere, W., & Jacobs, T. B. (2021). CRISPR screens in plants: approaches, guidelines, and future prospects. *The Plant Cell*, *33*(4), 794-813 <https://doi.org/10.1093/plcell/koab099>.
- Gomez, M. A., Lin, Z. D., Moll, T., Chauhan, R. D., Hayden, L., Renninger, K., ... & Bart, R. S. (2019). Simultaneous CRISPR/Cas9-mediated editing of cassava eIF 4E isoforms nCBP-1 and nCBP-2 reduces

- cassava brown streak disease symptom severity and incidence. *Plant biotechnology journal*, 17(2), 421-434. <https://doi.org/10.1111/pbi.12987>
- González, M. N., Massa, G. A., Andersson, M., Turesson, H., Olsson, N., Fält, A. S., ... & Feingold, S. E. (2020). Reduced enzymatic browning in potato tubers by specific editing of a polyphenol oxidase gene via ribonucleoprotein complexes delivery of the CRISPR/Cas9 system. *Frontiers in Plant Science*, 10, 497481. <https://doi.org/10.3389/fpls.2019.01649>
- Gordon-Kamm, W., Barone, P., Svitashv, S., D Sander, J., Kumar, S., & Jones, T. (2021). Strategies for CRISPR/Cas9-mediated genome editing: from delivery to production of modified plants. <https://doi.org/10.19103/AS.2020.0082.12>
- Gostimskaya, I. (2022). CRISPR–Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. *Biochemistry (Moscow)*, 87(8), 777-788. <https://doi.org/10.1134/S0006297922080090>
- Hahn, F., Sanjurjo Loures, L., Sparks, C. A., Kanyuka, K., & Nekrasov, V. (2021). Efficient CRISPR/Cas-mediated targeted mutagenesis in spring and winter wheat varieties. *Plants*, 10(7), 1481. <https://doi.org/10.3390/plants10071481>
- Han, Y., Teng, K., Nawaz, G., Feng, X., Usman, B., Wang, X., ... & Li, R. (2019). Generation of semi-dwarf rice (*Oryza sativa* L.) lines by CRISPR/Cas9-directed mutagenesis of OsGA20ox2 and proteomic analysis of unveiled changes caused by mutations. *3 Biotech*, 9, 1-17. . <https://doi.org/10.1007/s13205-019-1919-x>
- Hu, J., Huang, L., Chen, G., Liu, H., Zhang, Y., Zhang, R., ... & Ding, Y. (2021). The elite alleles of OsSPL4 regulate grain size and increase grain yield in rice. *Rice*, 14, 1-18. <https://doi.org/10.1186/s12284-021-00531-7>
- Huang, J., Li, J., Zhou, J., Wang, L., Yang, S., Hurst, L. D., ... & Tian, D. (2018). 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*, 115(32), E7559-E7567. . <https://doi.org/10.1073/pnas.1806110115>
- Huang, L., Zhang, R., Huang, G., Li, Y., Melaku, G., Zhang, S., ... & Hu, F. (2018). Developing superior alleles of yield genes in rice by artificial mutagenesis using the CRISPR/Cas9 system. *The Crop Journal*, 6(5), 475-481. . <https://doi.org/10.1016/j.cj.2018.05.005>
- Isaacson, W. (2021). The code breaker: Jennifer Doudna, gene editing, and the future of the human race. Simon and Schuster.
- Ishikawa, S., Ishimaru, Y., Igura, M., Kuramata, M., Abe, T., Senoura, T., ... & Nakanishi, H. (2012). Ion-beam irradiation, gene identification, and marker-assisted breeding in the development of low-cadmium rice. *Proceedings of the National Academy of Sciences*, 109(47), 19166-19171. <https://doi.org/10.1073/pnas.1211132109>
- Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., & Nakata, A. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. *Journal of bacteriology*, 169(12), 5429-5433. <https://doi.org/10.3390/ijms21165665>
- Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., & Nakata, A. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of

- the gene product. *Journal of bacteriology*, 169(12), 5429-5433. <https://doi.org/10.1128/jb.169.12.5429-5433.1987>
- Ito, Y., Nishizawa-Yokoi, A., Endo, M., Mikami, M., & Toki, S. (2015). CRISPR/Cas9-mediated mutagenesis of the RIN locus that regulates tomato fruit ripening. *Biochemical and biophysical research communications*, 467(1), 76-82. <https://doi.org/10.1016/j.bbrc.2015.09.117>
- Jiang, W. Z., Henry, I. M., Lynagh, P. G., Comai, L., Cahoon, E. B., & Weeks, D. P. (2017). Significant enhancement of fatty acid composition in seeds of the allohexaploid, *Camelina sativa*, using CRISPR/Cas9 gene editing. *Plant biotechnology journal*, 15(5), 648-657. <https://doi.org/10.1111/pbi.12663>
- Jiang, W., Zhou, H., Bi, H., Fromm, M., Yang, B., & Weeks, D. P. (2013). Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice. *Nucleic acids research*, 41(20), e188-e188. <https://doi.org/10.1093/nar/gkt780>
- Jung, Y. J., Lee, G. J., Bae, S., & Kang, K. K. (2018). Reduced ethylene production in tomato fruits upon CRISPR/Cas9-mediated LeMADS-RIN mutagenesis. *Horticultural Science and Technology*, 36(3), 396-405. <https://doi.org/10.12972/kjst.20180039>
- Komatsu, A., Ohtake, M., Shimatani, Z., & Nishida, K. (2020). Production of herbicide-sensitive strain to prevent volunteer rice infestation using a CRISPR-Cas9 cytidine deaminase fusion. *Frontiers in Plant Science*, 11, 925. <https://doi.org/10.3389/fpls.2020.00925>
- Kozaveva, E., Nielsen, Z. S., Nieto-Domínguez, M., & Nickel, P. I. (2024). The pAblo- pCasso self-curing vector toolset for unconstrained cytidine and adenine base-editing in Gram-negative bacteria. *Nucleic Acids Research*, gkad1236. <https://doi.org/10.1093/nar/gkad1236>
- Kusano, H., Ohnuma, M., Mutsuro-Aoki, H., Asahi, T., Ichinosawa, D., Onodera, H., ... & Shimada, H. (2018). Establishment of a modified CRISPR/Cas9 system with increased mutagenesis frequency using the translational enhancer dMac3 and multiple guide RNAs in potato. *Scientific Reports*, 8(1), 13753. <https://doi.org/10.1038/s41598-018-32049-2>
- Lau, C. H., Tin, C., & Suh, Y. (2020). CRISPR-based strategies for targeted transgene knock-in and gene correction. *Faculty Reviews*, 9. [10.12703/r/9-20](https://doi.org/10.12703/r/9-20)
- Li, B., Rui, H., Li, Y., Wang, Q., Alariqi, M., Qin, L., ... & Jin, S. (2019). Robust CRISPR/Cpf1 (Cas12a)-mediated genome editing in allotetraploid cotton (*Gossypium hirsutum*). *Plant biotechnology journal*, 17(10), 1862. <https://doi.org/10.3390/cells11182902>
- Li, H., Yang, Y., Hong, W., Huang, M., Wu, M., & Zhao, X. (2020). Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. *Signal transduction and targeted therapy*, 5(1), 1. <https://doi.org/10.1038/s41392-019-0089-y>
- Li, J. F., Norville, J. E., Aach, J., McCormack, M., Zhang, D., Bush, J., ... & Sheen, J. (2013). Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. *Nature biotechnology*, 31(8), 688-691. <https://doi.org/10.1038/nbt.2654>
- Liu, Q., Yang, F., Zhang, J., Liu, H., Rahman, S., Islam, S., ... & She, M. (2021). Application of CRISPR/Cas9 in crop quality improvement. *International Journal of Molecular Sciences*, 22(8), 4206. <https://doi.org/10.3390/ijms22084206>

- Lusser, M., Parisi, C., Plan, D., & Rodríguez-Cerezo, E. (2012). Deployment of new biotechnologies in plant breeding. *Nature biotechnology*, *30*(3), 231-239. <https://doi.org/10.1038/nbt.2142>
- Maharajan, T., Krishna, T. A., Rakkammal, K., Ceasar, S. A., & Ramesh, M. (2022). Application of CRISPR/Cas system in cereal improvement for biotic and abiotic stress tolerance. *Planta*, *256*(6), 106. <https://doi.org/10.1007/s00425-022-04023-w>
- Matsuo, K. (2021). CRISPR/Cas9-mediated knockout of the DCL2 and DCL4 genes in *Nicotiana benthamiana* and its productivity of recombinant proteins. *Plant Cell Reports*, 1-11. <https://doi.org/10.1007/s00299-021-02809-y>
- Mohamadi, S., Bostanabad, S. Z., & Mirnejad, R. (2020). CRISPR arrays: A review on its mechanism. *Journal of Applied Biotechnology Reports*, *7*(2), 81-86. <https://doi.org/10.30491/jabr.2020.109380>
- Montecillo, J. A. V., Chu, L. L., & Bae, H. (2020). CRISPR-Cas9 system for plant genome editing: Current approaches and emerging developments. *Agronomy*, *10*(7), 1033. <https://doi.org/10.3390/agronomy10071033>
- Mushtaq, M., Bhat, J. A., Mir, Z. A., Sakina, A., Ali, S., Singh, A. K., ... & Bhat, R. (2018). CRISPR/Cas approach: A new way of looking at plant-abiotic interactions. *Journal of plant physiology*, *224*, 156-162. 2. <https://doi.org/10.1016/j.jplph.2018.04.001>
- Nakayasu, M., Akiyama, R., Lee, H. J., Osakabe, K., Osakabe, Y., Watanabe, B., ... & Mizutani, M. (2018). Generation of α -solanine-free hairy roots of potato by CRISPR/Cas9 mediated genome editing of the St16DOX gene. *Plant Physiology and Biochemistry*, *131*, 70-77. <https://doi.org/10.1016/j.plaphy.2018.04.026>
- Nasir, A., & Caetano-Anollés, G. (2015). A phylogenomic data-driven exploration of viral origins and evolution. *Science advances*, *1*(8), e1500527. <https://doi.org/10.1126/sciadv.1500527>
- Nekrasov, V., Staskawicz, B., Weigel, D., Jones, J. D., & Kamoun, S. (2013). Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. *Nature biotechnology*, *31*(8), 691-693. <https://doi.org/10.1038/nbt.2655>
- Oliva, R., Ji, C., Atienza-Grande, G., Huguet-Tapia, J. C., Perez-Quintero, A., Li, T., ... & Yang, B. (2019). Broad-spectrum resistance to bacterial blight in rice using genome editing. *Nature biotechnology*, *37*(11), 1344-1350. <https://doi.org/10.1038/s41588-019-0425-8>
- Oliva, R., Ji, C., Atienza-Grande, G., Huguet-Tapia, J. C., Perez-Quintero, A., Li, T., ... & Yang, B. (2019). Broad-spectrum resistance to bacterial blight in rice using genome editing. *Nature biotechnology*, *37*(11), 1344-1350. <https://doi.org/10.1038/s41587-019-0267-z>
- Ozseyhan, M. E., Kang, J., Mu, X., & Lu, C. (2018). Mutagenesis of the FAE1 genes significantly changes fatty acid composition in seeds of *Camelina sativa*. *Plant Physiology and Biochemistry*, *123*, 1-7. <https://doi.org/10.1016/j.plaphy.2017.11.021>
- Palacios Araya, D., Palmer, K. L., & Duerkop, B. A. (2021). CRISPR-based antimicrobials to obstruct antibiotic-resistant and pathogenic bacteria. *PLoS Pathogens*, *17*(7), e1009672. <https://doi.org/10.1371/journal.ppat.1009672>
- Parry, M. A., Madgwick, P. J., Bayon, C., Tearall, K., Hernandez-Lopez, A., Baudo, M., ... & Phillips, A. L. (2009). Mutation discovery for crop improvement. *Journal of Experimental Botany*, *60*(10), 2817-2825. <https://doi.org/10.1093/jxb/erp189>

- Rajput, M., Choudhary, K., Kumar, M., Vivekanand, V., Chawade, A., Ortiz, R., & Pareek, N. (2021). RNA interference and CRISPR/Cas gene editing for crop improvement: Paradigm shift towards sustainable agriculture. *Plants*, *10*(9), 1914. <https://doi.org/10.3390/plants10091914>
- Raju, S. K., Bhuvaneswari, V., Prasadji, J. K., & Satyanarayana, P. V. (2020). Present Scenario of Diseases in Rice (*Oryza sativa* L.) and Their Management. *DISEASES OF FIELD CROPS: DIAGNOSIS AND MANAGEMENT*, *1*.
- Ramesh, P., Mallikarjuna, G., Sameena, S., Kumar, A., Gurulakshmi, K., Reddy, B. V., ... & Sekhar, A. C. (2020). Advancements in molecular marker technologies and their applications in diversity studies. *Journal of biosciences*, *45*, 1-15. <https://doi.org/10.1007/s12038-020-00089-4>
- Rato, C., Carvalho, M. F., Azevedo, C., & Oblessuc, P. R. (2021). Genome editing for resistance against plant pests and pathogens. *Transgenic Research*, *30*(4), 427-459. <https://doi.org/10.1007/s11248-021-00262-x>
- Rawashdeh, O., Rawashdeh, R. Y., Kebede, T., Kapp, D., & Ralescu, A. (2022). Bio-informatic analysis of CRISPR protospacer adjacent motifs (PAMs) in T4 genome. *BMC Genomic Data*, *23*(1), 1-10. <https://doi.org/10.1186/s12863-022-01056-8>
- Sabooni, N., & Gharaghani, A. (2022). Induced polyploidy deeply influences reproductive life cycles, related phytochemical features, and phytohormonal activities in blackberry species. *Frontiers in Plant Science*, *13*, 938284. <https://doi.org/10.3389/fpls.2022.938284>
- Saini, P., Saini, P., Kaur, J. J., Francies, R. M., Gani, M., Rajendra, A. A., ... & Chauhan, S. S. (2020). Molecular approaches for harvesting natural diversity for crop improvement. *Rediscovery of genetic and genomic resources for future food security*, 67-169. https://doi.org/10.1007/978-981-15-0156-2_3
- Samson, J. E., Magadan, A. H., & Moineau, S. (2015). The CRISPR-Cas immune system and genetic transfers: reaching an equilibrium. *Microbiology spectrum*, *3*(1), 3-1. <https://doi.org/10.1128/9781555818982.ch12>
- Sauret-Gueto, S., Frangedakis, E., Silvestri, L., Rebmann, M., Tomaselli, M., Markel, K., ... & Haseloff, J. (2020). Systematic tools for reprogramming plant gene expression in a simple model, *Marchantia polymorpha*. *ACS Synthetic Biology*, *9*(4), 864-882. <https://doi.org/10.1021/acssynbio.9b00511>
- Shi, Z., Zhang, Y., Maximova, S. N., & Gultinan, M. J. (2013). TcNPR3 from *Theobroma cacao* functions as a repressor of the pathogen defense response. *BMC Plant Biology*, *13*, 1-12. . <https://doi.org/10.1186/1471-2229-13-204>
- Sinha, D., Maurya, A. K., Abdi, G., Majeed, M., Agarwal, R., Mukherjee, R., ... & Chen, J. T. (2023). Integrated Genomic Selection for Accelerating Breeding Programs of Climate-Smart Cereals. *Genes*, *14*(7), 1484. <https://doi.org/10.3390/genes14071484>
- Slavin, J. L., & Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in nutrition*, *3*(4), 506-516. <https://doi.org/10.3945/an.112.002154>
- Sun, W., Liu, H., Yin, W., Qiao, J., Zhao, X., & Liu, Y. (2022). Strategies for enhancing the homology-directed repair efficiency of CRISPR-cas systems. *The CRISPR journal*, *5*(1), 7-18. <https://doi.org/10.1089/crispr.2021.0039>

- Tang, L., Mao, B., Li, Y., Lv, Q., Zhang, L., Chen, C., ... & Zhao, B. (2017). Knockout of OsNramp5 using the CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield. *Scientific reports*, 7(1), 14438. <https://doi.org/10.1038/s41598-017-14832-9>
- Tang, L., Mao, B., Li, Y., Lv, Q., Zhang, L., Chen, C., ... & Zhao, B. (2017). Knockout of OsNramp5 using the CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield. *Scientific reports*, 7(1), 14438. <https://doi.org/10.1038/s41598-017-14832-9>
- Távora, F. T. P. (2021). Development of blast resistant rice plants using CRISPR/Cas9 system for genome editing.
- Van Vu, T., Das, S., Hensel, G., & Kim, J. Y. (2022). Genome editing and beyond: what does it mean for the future of plant breeding? *Planta*, 255(6), 130. <https://doi.org/10.1007/s00425-022-03906-2>
- Voytas, D. F. (2013). Plant genome engineering with sequence-specific nucleases. *Annual review of plant biology*, 64, 327-350. <https://doi.org/10.1146/annurev-arplant-042811-105552>
- Wang, B., Zhong, Z., Wang, X., Han, X., Yu, D., Wang, C., ... & Zhang, Y. (2020). Knockout of the OsNAC006 transcription factor causes drought and heat sensitivity in rice. *International Journal of Molecular Sciences*, 21(7), 2288. <https://doi.org/10.3390/ijms21072288>
- Wang, W., Wang, W., Pan, Y., Tan, C., Li, H., Chen, Y., ... & Ma, C. (2022). A new gain-of-function OsGS2/GRF4 allele generated by CRISPR/Cas9 genome editing increases rice grain size and yield. *The Crop Journal*, 10(4), 1207-1212. <https://doi.org/10.1016/j.cj.2022.01.004>
- Wenefrida, I., Utomo, H. S., & Linscombe, S. D. (2013). Mutational breeding and genetic engineering in the development of high grain protein content. *Journal of agricultural and food chemistry*, 61(48), 11702-11710. <https://doi.org/10.1016/j.cj.2022.01.004>
- Xu, R., Yang, Y., Qin, R., Li, H., Qiu, C., Li, L., ... & Yang, J. (2016). 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*, 43(8), 529-532. <https://doi.org/10.1016/j.jgg.2016.07.003>
- Yigider, E., Taspınar, M. S., Aydın, M., & Agar, G. (2021). Humic acid effects on retrotransposon polymorphisms caused by zinc and iron in the maize (*Zea mays* L.) genome. *Cereal Research Communications*, 49, 193-198. <https://doi.org/10.1007/s42976-020-00111-3>
- Zafar, S. A., Zaidi, S. S. E. A., Gaba, Y., Singla-Pareek, S. L., Dhankher, O. P., Li, X., ... & Pareek, A. (2020). Engineering abiotic stress tolerance via CRISPR/Cas-mediated genome editing. *Journal of Experimental Botany*, 71(2), 470-479. <https://doi.org/10.1093/jxb/erz476>
- Zafar, S. A., Zaidi, S. S. E. A., Gaba, Y., Singla-Pareek, S. L., Dhankher, O. P., Li, X., ... & Pareek, A. (2020). Engineering abiotic stress tolerance via CRISPR/Cas-mediated genome editing. *Journal of Experimental Botany*, 71(2), 470-479. <https://doi.org/10.1093/jxb/erz476>
- Zaidi, S. S. E. A., Mahas, A., Vanderschuren, H., & Mahfouz, M. M. (2020). Engineering crops of the future: CRISPR approaches to develop climate-resilient and disease-resistant plants. *Genome biology*, 21(1), 1-19. <https://doi.org/10.1186/s13059-020-02204-y>
- Zegeye, W. A., Tsegaw, M., Zhang, Y., & Cao, L. (2022). CRISPR-based genome editing: advancements and opportunities for rice improvement. *International Journal of Molecular Sciences*, 23(8), 4454. <https://doi.org/10.3390/ijms23084454>

- Zhang, H., & Wang, S. (2013). Rice versus *Xanthomonas oryzae* pv. *oryzae*: a unique pathosystem. *Current Opinion in Plant Biology*, 16(2), 188-195. <https://doi.org/10.1016/j.pbi.2013.02.008>
- Zhang, J., Zhang, H., Botella, J. R., & Zhu, J. K. (2018). Generation of new glutinous rice by CRISPR/Cas9-targeted mutagenesis of the Waxy gene in elite rice varieties. *Journal of integrative plant biology*, 60(5), 369. <https://doi.org/10.1111%2Fjipb.12620>
- Zheng, M., Lin, J., Liu, X., Chu, W., Li, J., Gao, Y., ... & Hu, Z. (2021). Histone acetyltransferase TaHAG1 acts as a crucial regulator to strengthen salt tolerance of hexaploid wheat. *Plant Physiology*, 186(4), 1951-1969. <https://doi.org/10.1093/plphys/kiab187>
- Zink, I. A., Wimmer, E., & Schleper, C. (2020). Heavily armed ancestors: CRISPR immunity and applications in archaea with a comparative analysis of CRISPR types in sulfobacterales. *Biomolecules*, 10(11), 1523. <https://doi.org/10.3390/biom10111523>

Commented [DV46]: Add all authors name