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A Comprehensive Review on Recent Advances in CRISPR/Cas9 Technology for Precision Gene Editing in Plants

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Abstract

The clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system has been discovered to be revolutionary in plant genome editing regarding opportunities in agricultural advancements. This review captures the existing literature concerning the CRISPR/Cas9 system; it describes the components and biochemical processes including RNA-guided DNA cleavage. Advanced applications are looked into: base editing, prime editing, and multiplex editing for their role in its broad-spectrum achievements. CRISPR/Cas9 application in crop plants are presented to emphasize its use in yield increase programs, nutritional enhancement, disease resistance, and tolerance to abiotic stresses. Successful case studies of major crop species regarding food security and sustainable agriculture underline the importance of the results. The review also, identifies the drawbacks and potential problems of the applied technology – off-target effects, inefficiency of delivery systems, and regulatory hurdles. Possible ways of dealing with such problems and recommendations concerning the appropriate application of gene-edited crops are mentioned. Also, the comparison of the CRISPR/Cas9 with other gene-editing tools including the Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) highlights the simplicity, cost-effectiveness, and flexibility of the tool was brought to fore. Emerging trends are discussed, including new areas of targeted epigenome editing or RNA editing with Cas13. From this review, the conclusion stresses the need for an integration of different disciplinary approaches, stakeholders' involvement, and further research to fully harness the utilization of CRISPR/Cas9 in dealing with complex issues in agriculture. The review also emphasizes the need to adopt CRISPR/Cas9 and other biotechnologies toward proper innovations in plant genetic engineering for efficient advancement of the agriculture industry.

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Introduction

CRISPR/Cas9 is the most revolutionary genome editing machine that has paved the way for remarkable progress in plant breeding. Derived from the adaptive immune system of a bacterial, it operates as a swift and accurate tool for the genetic modification of crops (Jinek *et al*., 2012). CRISPR/Cas 9 (clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9) system comprises of a Cas9 nuclease enzyme that can be guided by the small RNA molecule to cut DNA at the desired target position. Genome editing technology makes it possible to disrupt existing genes, to correct or to add new genes (Yin *et al*., 2017). One of the significant uses of CRISPR in plants is the enhancement of agronomic traits, such as high yield, drought tolerance, disease resistance, and enhanced nutritional value. Researchers have manipulated it through many genes that control traits such as plant structure, time of flowering, and nutrient use efficiency (Zeng *et al.*, 2020). Through CRISPR technology, we now have ways to domesticate orphan crops and breed new varieties that are climate-resistant (Lemmon *et al.*, 2018). As a result, it has created conditions for fast production of model plants for research (Michno *et al.*, 2015). Beyond the improvement of traits, CRISPR provides the ability to determine gene functions through studies of mutagenesis. By knocking out genes one by one, the functions of genetic information like growth, development, and stress response are explored. CRISPR also enables high precision of advanced plant synthetic biology and metabolism engineering applications for the production of valuable

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chemicals, biofuels, and biomaterials. (Malzahn *et al.*, 2019; Xu *et al.*, 2020). No matter what the restrictions around CRISPR-modified crops are in countries, a few of the CRISPR-engineered products received approvals, and commercialization has been initiated (Lassoued *et al.*, 2021). Over time more proactive enhancement of the technology through the step of the more precise way of delivery methods and further development of targeting ranges will unfold more potential for the application (Manghwar *et al.*, 2017). Engagement in societal discussions should be included to create systems that sustain the ethical application of technology.

However, CRISPR/Cas9 has its safety concerns that, nevertheless, can be possible as the most powerful crop necessities for 21 Century. Concerning this development, sophisticated CRISPR methods offer cutting-edge features such as specificity, multifaceted applications, and eco-friendly technology, which enable the development of new areas of basic scientific research and applied biotechnology to achieve sustainable agriculture (Camerlengo *et al.*, 2022). Remarkable as it was, CRISPR first allowed researchers to make gene knockouts and small changes, whereas better methods are slowly being developed. Moreover, base editing is conducted with even more precision than those mentioned above and does not cause double-strand breaks and, accordingly, fewer off-target effects (Chen *et al.*, 2019). This technique is called prime editing and involves using a reverse transcriptase enzyme to write over the DNA sequence directly and this also allows for the insertions of larger stretches of genes (Anzalone *et al.*, 2019). These new CRISPR systems are being harnessed to modify multiple genes simultaneously through multiple editing methods. This makes it possible to pyramid many of these trait-improving alleles into superior plant varieties (Shen *et al.*, 2017). CRISPR is integrated with other biotechniques such as RNA interference (RNAi) to reach high-order engineering of metabolic pathways' (Mei *et al.*, 2019). Looking ahead, CRISPR is very promising for solving the key issues of agriculture production, including yield, nutrition quality, and abiotic and biotic stresses, drought, heat, salinity, and pests. It is also noteworthy that for next-generation crop enhancement, researchers are employing CRISPR to modify the specific genes, that are engaged in photosynthesis, nitrogen fixation, and abiotic stress tolerance (Ricroch *et al.*, 2017; Zafar *et al.*, 2020).

As the knowledge of crop genomes and the complex traits associated with them increases with the help of functional genomics, such applications will rely heavily on CRISPR. This approach's effectiveness lies in its capability to make desired modifications to gene families, regulatory regions, and even chromosomal domains, which cannot be matched by other methods (Wang and Cheng, 2022). When used in combination with other future developments such as speed breeding and AI designing, CRISPR shall lead to a new generation of faster breeding and bio-based innovations. Also, a promising area of application is the application of CRISPR technologies to manipulate plant-microbe interactions and promote plant performance. Thus, for the genetic modification and manipulation of plants, the engineers and researchers in plant science reserve the right to post-transformation and post-amendment of plant genes involved in immune responses to microbes and microbial colonization (Feng *et al.*, 2018). CRISPR also has been employed to unveil the way of molecular communication between plants and beneficial microbes such as rhizobacteria and mycorrhizal fungi (Kamel et al., 2017). Moreover, the extension of CRISPR has made plants an important form of research in which it is forming the bridge between basic research in plant science and its application. Like editing the formerly undomesticated crops to have valuable chemical constituents, a wealth of newly developed pharmaceuticals, biomaterials, and specialty chemicals have become available from other plant species (Reed *et al.*, 2001). The ability to generate edited hairy root cultures is speeding the bio-manufacturing industry (Wilson *et al.*, 2019). New sophisticated ways of delivering the specific CRISPR machinery into the plant cell are making more things possible. Others are nanoparticle carriers and viral vectors as a potentially viable alternative to tissue culture and Agrobacterium transformation (Demirer *et al.*, 2019; Ellison *et al.*, 2020). These could enable achieving plant editing and even the manipulation of the mature plants. Despite its immense positives, current CRISPR/Cas9 has some demerits. Among the limitations are, off-target effect, linkage drag, somatic effect, and low efficiency in editing polyploid crops (Karadotchev *et al.*, 2022). It is also an issue that remains the centre of a legal debate between jurisdictions that ban all CRISPR-edited crops as Genetically Modified Organisms (GMOs) and those that have excluded specific CRISPR products (Lassoued *et al.*, 2021).

In general, it is possible to say that the role of the CRISPR technique cannot be overstated – it exposed the task of manipulating the genomes with a rather large set of plant species to numerous researchers. The further advancement of this method alongside other similar technologies will improve our possibility to

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explore and manipulate plant biology for food production and effective bio-production.

This review aims to provide an overview and critical analysis of the current trends in the use of CRISPR/Cas9 technology for targeted editing of plant genes. Our primary goal here will be to explain perspectives on the current state of development of CRISPR/Cas9 technology and its modern improvements that increase the accuracy, speed, and adaptability of the method for different plant species. The review will also focus on describing developments in the delivery of proposed action to minimize the off-target impact, and demonstrating how the technology may be used to advance crop improvement. Moreover, we will look at biosafety and regulatory issues on CRISPR-edited plants, therefore giving information on the policies of the development and application of plants globally. In this review, an attempt was made to synthesize the information available from the molecular biology, agriculture, and policy perspectives to present a broad overview of the topic, which prompted the application of CRISPR-Cas9 in approaching modern challenges in plant biotechnology and define potential directions for additional research that could potentially provide support to sustainable agriculture and worldwide food security.

Importance of precision gene editing in plants for agricultural advancements.

Technologies like CRISPR/Cas9 for gene editing are fundamental in the innovation process and the overcoming of issues that affect agriculture. According to the United Nations estimates, the people count of the entire world is expected to be about 7 billion. It is projected to reach 9 billion by 2050 (United Nations, 2019); thus, it becomes even more imperative to uplift sustainable food production. Gene editing enables the enhancement of crop qualities such as high output, biofortification, tolerance to pests and diseases, and climate change among others (Mushtaq *et al*., 2021). This can enhance yields at the same time lessen inputs such as water, fertilizers, and chemicals such as pesticides. In addition, gene editing allows for the realization of the utilization of underutilized crops and expanding the undomesticated plant species to be used for food and feed in addition to providing fuel and industrial employment (Lemmon *et al.,* 2018). It also offers an effective way of realizing the genetic potential of wild and semi-wild plants. It is advancing the production of such sophisticated crops suitable for new markets including; plant proteins, functional foods, industrial materials, and sustainable bioenergy (Qi *et al.,* 2016).

From a generally fundamental investigative point of view, such tools as CRISPR are exceptionally accommodating for making functional genomics tests to understand gene functions and regulatory networks underlying complex agronomic traits (Liu *et al.,* 2022). This information can at that point be utilized with the CRISPR-Cas9 quality-altering apparatus to move forward on these characteristics in an eco-friendly way but without the joining of foreign transgenes (Zhang *et al.,* 2016). It would be outlandish to hone atomic breeding and to develop the cause of biofortification without quality editing Gene editing is thus a key enabler of molecular breeding and biofortification strategies. Importantly, gene editing overcomes some drawbacks of conventional transgenic approaches. Precisely edited crops have fewer regulatory hurdles in many countries and higher public acceptance (Lassoued *et al*., 2021). Products with simple gene knockouts or native allele swaps may not be classified as GMOs. This facilitates bringing improved varieties to market rapidly at lower costs (Van de Wiel *et al.,* 2017).

From the above-mentioned points of interest, it seems to be concluded that accuracy quality altering offers numerous benefits over more seasoned mutagenesis methods, counting the amazing opportunity to specifically alter plant qualities without any hurt to the genome, or connecting undesirable qualities to the desired locus (Wang and Cheng, 2022). It permits the pyramiding of numerous favorable alleles from different germplasm into prevalent assortments. In this way, when coordinated with other biotechnologies such as genomic selection, as well as phenotyping, quality altering can significantly diminish breeding time (de Lange *et al*., 2022).

Fundamentals of CRISPR/Cas9 Technology

CRISPR/Cas9 originated from the bacterial adaptive immune system and is a novel technique that has enabled genome editing across different species; plants inclusive. At its core, the system comprises two key molecular components: the Cas9 nuclease protein along with a single guide RNA or sgRNA (Jinek *et al*., 2012). Cas9 is an RNA-directed DNA endonuclease and nuclease, that introduces a double-strand break at the site specified by the sgRNA. The sgRNA is a synthetic, engineered RNA molecule consisting of two critical domains; thus, it incorporates a target-specific 20 nucleotide RNA sequence at the 5' end through base pairing with the target site of the DNA: The second strand derived from the trans-activating crRNA (tracr RNA) which will help in the binding of Cas 9 endonuclease. (Nishimasu *et al.,* 2014). Once the Cas9-sgRNA complex comes in contact with the target

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DNA sequence it then cuts the DNA three nucleotides before a target protospacer adjacent motif (PAM) which is necessary for Cas9 function (Sander & Joung, 2014). This targeted double-strand break (DSB), triggers the cell's inherent DNA repair equipment which is mainly the Non-Homologous End Joining (NHEJ) or the Homology Directed Repair (HDR) pathways.

The NHEJ pathway is itself mutagenic since it results in the creation of insertions or deletions (indel) at the break site and can thus knockout or inactivate the desired gene. However, in the HDR pathway, the new DNA sequence or the specific nucleotide alteration can be introduced into the chromosome when a repair template is provided for gene targeting or gene replacement (Puchta, 2005). From this, the following could be deduced the use of the CRISPR/Cas9 system is made easier through the fact that new sgRNAs can be designed and can be synthesized whenever there are changes made on the guide sequence of 20 nucleotides. This has led to higher throughput and multiplex genome editing where two or more genes target or two or more edits can be carried out on a single test (Wang *et al.*, 2018). However, the original Cas9 protein originating from *Streptococcus pyogenes* has been genetically modified to be implemented, but other Cas proteins have been identified in bacteria which are different with their orthologs and features such as PAM specificities, enzyme characteristics, and targets in the genome have been studied (Ran *et al.*, 2015). In addition, the base editors, prime editors, and epigenetic editors are some of the embodiments of the CRISPR toolkit that has given more sophisticated approaches to work with the genome (Anzalone *et al.*, 2020; Komor *et al.*, 2016).

The CRISPR/Cas9 is one of the most important molecular tools in plant research because of its use in functional genomics, genetic modification for enhancing crop yield, and creating another new form of applications in plant synthetic biology and metabolic engineering as commented by (Michno *et al.*, 2015; Zsögön *et al.*, 2018). The bigger picture for using the CRISPR machinery is that as more knowledge is gained about the tools and with new and improved delivery systems and optimization methodologies appearing as time passes, we will see constant growth in not only the application of this novel technology but also in the fields of sustainable agriculture and bio-based industries.

Figure 1: Basic Mechanism of the CRISPR-Cas9 Genome Editing System; (Sources: Jinek *et al.*, (2012), Ran *et al.*, (2013), and Doudna *et al.*, (2014))

Recent Advances in CRISPR-Cas9 Applications in Plant Genome Editing

The technique that has recently come into focus for plant genome editing is CRISPR/Cas9 which has opened new horizons of precision and efficacy in the process of genetic manipulation. This revolutionary technology is applied to other model plants and economically important crop species for the improvement of crop plants and fundamental research. For instance, in *Nicotiana benthamiana* and *Arabidopsis thaliana*, functional genomics and exploration of gene function using CRISPR/Cas9. For example, scientists have used CRISPR/Cas9 to create knockout mutants of the plant regulatory gene, and its functions concerning development, growth, and stress responses are studied (Kapoor *et al.*, 2020; Wan *et al.*,

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2020). Also, CRISPR/Cas9 has allowed for the high-order mutant faster and multiple gene interference, thus providing a way to study more complex genetic networks and architectures concerning traits (Zhang *et al.*, 2021). These model plant studies have providentially created a platform for the extension of CRISPR/Cas9 applications to agricultural value crops. In rice and wheat, cereal crops, CRISPR/Cas9 has been applied to develop various agronomic traits and to increase tolerance to both biotic and abiotic stresses. For example, using CRISPR/Cas9, researchers have enhanced genes related to plant structure, yield increase, and disease tolerance for better rice varieties (Srivastava *et al.*, 2020; Zhang *et al.*, 2021). CRISPR/Cas9 has been used to improve grain quality, drought tolerance as well as resistance to fungal diseases in wheat (Dong and Ronald, 2019; Elsharawy *et al.*, 2023).

Another added advantage of biolistic-based CRISPR/Cas9 is that it is capable of producing plants without the transgene and with the target modification through null segregants, wherein, through selfing in the next generations, the transgenic CRISPR components are eliminated from the plants (Ellison *et al.*, 2020). It has been used in tomato, maize, and soybean to advance carrying out the generation of enhanced varieties without invoking the concept of gene transfer of another organism (Rodríguez-Leal *et al.*, 2017; Bräutigam et al., 2020). The generation of efficient null segregants is also crucial for the approval and acceptance of genome-edited crops in particular countries since they have different allowable standards in comparison with old GMOs this was noted by Lassoued *et al*. This can help increase the application of CRISPR-edited crops with better characteristics that enhance the efficiency of agriculture as well as food security. With the emergence of new variants like base editors and prime editors and, improvement in the delivery methods, it is highly expected that with future advancements, the use of CRISPR/Cas9 technology in plant genome editing will continue to grow by being able to perform more complex forms of genetic manipulation (Anzalone *et al.*, 2020; Li *et al.*, 2021). Cooperation between authors, breeders, and other regulatory authorities will play a vital role in the future development of this unprecedented technology to prevailing difficulties for crop improvement in agriculture around the world.

CRISPR/Cas9 technology has shown immense potential in addressing agricultural challenges. For instance, rice, a staple food for billions, has been a focal point for CRISPR-based improvements. The development of drought-resistant rice varieties using this technology holds significant promise for enhancing food security in regions grappling with water scarcity. Similarly, maize, another crucial crop, has benefited from CRISPR-mediated resistance to pests and diseases, leading to potential reductions in pesticide usage and increased crop yields.

In the realm of human health and dietary needs, CRISPR has demonstrated its utility in wheat engineering. By modifying gluten content, researchers aim to provide safe and nutritious wheat options for individuals with celiac disease. Furthermore, the agricultural landscape could be transformed by CRISPR-enhanced soybean varieties. Higher oil content in soybeans can potentially boost biofuel production, contributing to a sustainable energy future and economic growth.

Table 1: Examples of Successful Applications of CRISPR-Cas9 in Plant Engineering

Plant Species	Trait Edited	Outcome	Reference
Rice (*Oryza sativa*)	Herbicide resistance	Development of rice varieties resistant to glufosinate, a broad-spectrum herbicide (Li et al., 2012). This allows for more targeted weed control and potentially reduces herbicide use.	(Li *et al.*, 2012
Tomato (*Solanum lycopersicum*)	Increased disease resistance	Introduction of resistance to Tomato yellow leaf curl virus (TYLCV) through editing susceptibility genes (Tashkandi et al., 2018). This provides a valuable tool for combating a devastating tomato disease.	Tashkandi *et al.*, 2018
Soybean (*Glycine max*)	Improved oil quality	Modification of fatty acid biosynthesis genes to increase the content of desirable oils (Do et al., 2019). This contributes to the development of healthier and more nutritionally valuable soybean products.	Do *et al.*, 2019
Potato (*Solanum tuberosum*)	Reduced tuber bruising	Editing genes involved in starch biosynthesis to create potatoes with firmer flesh and reduced bruising after harvest (Anderson et al., 2017). This can lead to	Andersson *et al.*, 2017)

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		significant economic benefits by minimizing post-harvest losses.	
Wheat (*Triticum aestivum*)	Enhanced drought tolerance	Introduction of genes associated with drought tolerance from wild wheat relatives (Placido et al., 2013). This shows the promise to improve wheat yields in drought-prone regions.	Placido *et al.*, 2013

Advancements in delivery vectors for CRISPR components and Targeted manipulation of plant genomes

It is well understood that CRISPR/Cas9 genome-editing efficiency in plants is partially dependent on levels of CRISPR components carrying out capable plant cell and tissue delivery. Various delivery methods have been designed following an efficient technique to enhance plant biotechnology using CRISPRs. Among the early methods of gene delivery, plasmid vectors were used where Agrobacterium-mediated transformation or biolistic (gene gun) methods were applied to transfer CRISPR/Cas9 expression to the plant genome (Mao *et al*., 2019). However, these approaches lead to the insertion of foreign DNA sequences which are a biosafety issue and regulatory problem in the commercialization of edited crops. To overcome this problem, ribonucleoprotein (RNP), a purified Cas9 protein linked with guide RNA, has been applied to deliver the CRISPR components directly to the plant cell (Liang *et al*., 2018). RNPs can be targeted to weak gene Editing and do not require the integration of DNA thus can be ionized to develop a plant with no transgene. Some of the delivery techniques used and developed for the effective delivery of RNP are protoplast transformation, biolistic delivery, and nanoparticle-mediated delivery in various plant species (Demirer *et al.*, 2019; Mitter *et al*., 2017). Also, plant virus-based vectors have been used as promising nanocarriers for the CRISPR components. These vectors leverage the natural ability of plant viruses to multiply and circulate within plant tissues, to achieve systemic delivery of CRISPR equipment (Ellison *et al*., 2020). This approach has been previously shown in several crop species such as tomato, wheat, and rice, and is suited to make heritable and transgene-free genome modifications (Jiang *et al*., 2019; Kis *et al.*, 2019).

Novel advancements in delivery techniques have enhanced the use of CRISPR/Cas9-targeted genome editing in plants to alter characteristics such as stress tolerance, metabolism, and qualities that affect the yield. For instance, CRISPR/Cas9 has been applied to edit the genes responsible for drought, salt tolerance, and disease resistance in plants like rice, wheat, and tomato (Manghwar *et al*., 2019; Wang *et al.*, 2019). Such efforts have come up with new crop varieties from which farmers get better yields, especially under unfavourable environmental conditions. Similarly, metabolic engineering has also been addressed using CRISPR/Cas9 technology for synthesis of the specific biomolecules like pharmaceuticals, nutraceuticals, and biofuels in plants (Liu *et al*., 2019; Scheben and Edwards, 2018). It is now possible to turn 'on and off' some of these genes that control the rate of metabolism besides enhancing the synthesis of preferred products. Thus, in the process to yield improvement, CRISPR/Cas9 has been used for genome editing of genes controlling plant morphology, nutrient acquisition, and yield attributes in rice, maize, and wheat (Che *et al.*, 2021; Zheng *et al*., 2020). They have contributed to the stewardship of the enhanced varieties in terms of improving the desirable characteristics for higher crop yields. With the improvement of the CRISPR/Cas9 toolkit especially for base editors and prime editors as well as with efficient delivery methods, the possibility of targeting genome in plants will keep rising. Cooperation between the plant biotechnologists, plant breeders, and regulating bodies will be the key to using these developments for sustainable agriculture, food safety, and the biobased economy.

Applications of CRISPR/Cas9 in Plant Improvement

The genetics and functional genomics of plants have benefitted immensely from the existing tool known as the CRISPR/Cas9 gene-editing technique, which makes it easier to edit specific genes in a plant's genome and understand the complexities of certain characteristics or actions. This versatile technology has found one of its most extensive uses in targeted mutagenesis for gene function analysis and has offered the best possible look into the roles of new genes in the formation of plant phenotypes. CRISPR/Cas9 is adaptable for targeted mutagenesis through the introduction of mutations at predefined genomic resolution resulting in insertions, deletions, or base substitutions (Wolter & Puchta, 2019). This is done by using the Cas9 nuclease to create double-strand breaks (DSBs) following the targeting of a specific DNA sequence by a programmable single-guided RNA (sgRNA). The consequent DSBs are then processed by the cell's intrinsic repair machinery, mainly the non-

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homologous end joining (NHEJ) or the homology-directed repair (HDR) pathways. In essence, the error-prone NHEJ pathway induces small insertions or deletions (indels) which are capable of knocking out or disrupting genes while the HDR pathway enables the introduction of wanted sequences or editing of genes. Targeted mutagenesis through CRISPR/Cas9 is one of the most potent tools for making knockout mutants for a particular gene or an entire genome. By manipulating the target genes' coding sequence or regulatory region, functional analysis of genes in multiple aspects of growth, development, stress, and metabolism, can be investigated (Zhang *et al.*, 2018). It has been most useful where there are functionally equivalent gene products – this has been an issue where most traditional mutational analyses of gene function have been a problem.

For instance, CRISPR/Cas9 has been quite usefully applied for knockout mutants of transcription factors and regulatory genes in the process of development and stress response in the main model plants like *Arabidopsis thaliana* and *Nicotiana benthamiana* (Kapoor *et al*., 2020; Wan *et al.*, 2020). Such kinds of tasks have provided tangible insights regarding various aspects of genes focusing on; flowering time, development of leaves, stress from drought, and others. In addition, the recent development of CRISPR/Cas9 system-based targeted mutagenesis has assisted in estimating the role of such genes in establishing the significant agronomic traits in crop species. For instance, CRISPR/Cas9 was used to create knockouts of genes of interest that were discovered using genome-wide association studies (GWAS) or quantitative trait locus (QTL) mapping concerning functional annotation of the genes' contribution to yield, grain quality, and stress tolerance traits (Zafar *et al.*, 2020; Zhang *et al*., 2021). In rice, the germplasm architecture of genes with respect to grain size and resistance to diseases, has been made differentiable by CRISPR/Cas9 (Huang *et al*., 2018; Lu *et al*., 2017). Similarly, in wheat supply Özgen *et al.*, (2020), the role of genes regarding some aspects like grain constitution, water-deficient tolerance, and diseases has been studied through mutagenesis (Jouanin *et al*., 2021; Singh *et al.*, 2018). Since the knowledge of the genomes of plants and the functions of genes is increasing, targeted mutagenesis through the CRISPR/Cas9 system will play a significant part in the learning of the genetic control of complex phenotypes along with crop improvement. The potential of producing and screening numerous knockout mutants for the candidate genes will also strengthen the practical use of genomic data in plants and further sustainable agriculture.

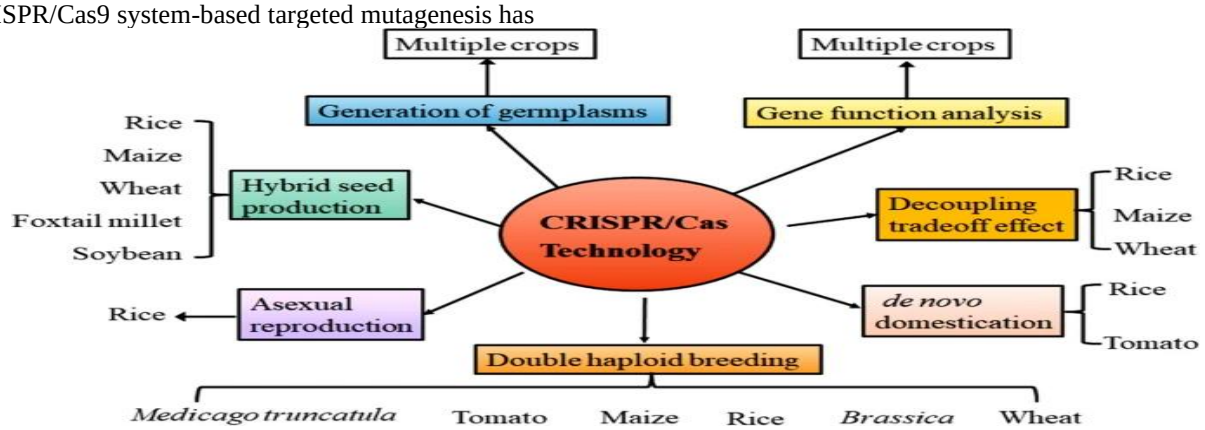

Fig. 2: Applications of CRISPR/Cas Technology in Crop Improvement; (Source: media.springernature.com)

Advancements in CRISPR-based plant genome editing techniques

CRISPR/Cas9 can easily be used to edit plants' genes as may be seen from the research that has been done on different types of organisms. While the first applications were concerned with the generation of double-strand breaks (DSBs) and the use of endogenous cellular repair machinery for genomic alterations, there has been spatial development of the base editing systems that emerged to enable single nucleotide alteration in target genomes without intervening in the generation of DSBs. These innovations have influenced the growth of CRISPR and expanded the toolbox adding unique accuracies and flexibilities that added to the plants' genome editing. Of various types of tools, some of the early developments in this area are cytosine base editors (CBEs) and adenine base editors (ABEs). These systems include a non-catalytic Cas9 fused with CBEs or ABEs to change the target site's cytosine to thymine

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or adenine respectively within the window of the target site (Komor *et al*., 2016, Gaudelli *et al*., 2017). However, in comparison to any eukaryotic gene-editing systems, base editors do not form DSBs which lowers the possibility of insertions, deletions, or large genomic rearrangements in the process of targeted base substitutions is much more precise and effective.

Base editing has been used in several crop plants such as rice, wheat, tomato, and maize for introducing particular nucleotide exchange for change of trait or functional genomics wanted (Li *et al.,* (2018) and Zong *et al.,* (2017)). For example, there is an application of base editors in modifying the agronomically relevant genes relating to the plant architecture, grain quality, and disease resistance, leading to the development of improved crop varieties (Ren *et al.,* 2021; Kang *et al*., 2018). That was the base editing concept; however, there is a newer and more complex technique that is called prime editing. Thus, prime editing combines Cas9 nickase and reverse transcriptase enzyme, and the prime editing guide RNA (pegRNA), which targets the site and encodes the change (Anzalone *et al.,* 2019). This system directly writes new genetic information into the target locus, enabling precise insertions, deletions, and all possible base-to-base conversions without the need for exogenous repair templates or DSBs.

Prime editing has been performed and validated in several plant species; including rice, wheat, and Arabidopsis for making gene-specific changes and introducing favorable genetic traits (Lin *et al.,* 2020; Xu *et al.,* 2020). For instance, Xu *et al.,* (2020) have used prime editing to add herbicide and disease-resistance genes into high-quality crop lines, this creates the basis for breeding improved cultivars that feature stress tolerances.

However, prime editing has been demonstrated to overcome the issues related to the use of polyploid crops since the conventional approaches of CRISPR/Cas9 are always accompanied by the problem of incomplete or mosaic patterns of editing within multiple genome copies (Wang *et al.,* 2019). Since prime editing allows for the editing of specific genes, it may offer the possibility to increase yield, nutritional value, and resistance to diseases in economically important polymodal crops such as wheat, cotton, and potato. Thus, these base editing technologies provide high accuracy and flexibility, although constant work is being carried out to improve their effectiveness, targeting, and compatibility with various plant organisms. Moreover, the emergence of more efficient delivery methods and tissue-specific regulation will also be a potential future work to avail these tools for plant biotechnology (Li *et al.,* 2021). As new genome editing is added to the CRISPR kit including base editing, prime editing, and others that are in development, the limits of plant genome editing are sure to be opened up to more possibilities in crop enhancement, fundamental plant biology, and agriculture.

Crop Improvement Strategies and Emerging Challenges in CRISPR/Cas9 Technology

While CRISPR/Cas9 technology has changed the approaches to plant genome editing and has revealed numerous opportunities for crop enhancement, some issues remain crucial and should be solved to advance the opportunities of its application. One of the major issues is the plant tissues, genetic loci, and species-specific differences in editing effectiveness (Lowe *et al.,* 2016). Striped and off-target effects depend on various parameters like the delivery efficiency of the CRISPR components, the status of chromatin, and the constrain or the limitation of targets (Manghwar *et al.,* 2019). Still, editing multiple copies within polyploid crops such as wheat, cotton, and potato is still cumbersome and often rather incomplete (Wang *et al*., 2019). Thus, the presence of edited and non-edited alleles making a genotype 'mosaic' makes phenotype expression incomplete, and complicates the breeding process (Son & Park, 2022).

Off-target effects, in which other genomic regions with homologous sequences to the intended site may be modified, is another important factor (Zhang *et al.,* 2018). However, tools for managing this risk through bioinformatics and strategies for experimental validation have been established and Wolt *et al.,* 2016 suggest that since plant genomes are large and there may be off-target effects on regulatory or coding regions the assessment and optimization of it should be thorough. Besides, there is evidence of off-target effects or issues particularly large deletions and complex genomic rearrangements that can affect one or several neighboring genes or the nearby regulatory regions (Lee *et al.,* 2019). Thus, these adverse effects emphasize the suggestions regarding full characterization of edited plant lines to determine genetic purity and phenotypic stability (Kawall, 2019). But the following strategies have been employed to improve on the CRISPR/Cas9 system for crop improvement. The first one is to touch on the use of tissue-specific or inducible promoters on the regulation of the CRISPR components which may increase the rate of the editing and may reduce the off-target effects (Cermák *et al*., 2017). Moreover, the generation of particular novel advanced CRISPR techniques such as base editors and prime editors

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improves the efficiency of genome editing in the plant genome (Anzalone *et al.,* 2020, Ren *et al.,* 2021).
Multiplexed editing approaches, where multiple target sites are edited simultaneously, have proved the advancement in stacking desired traits needed for the functional genomics (Shen *et al*., 2017). However, such a strategy should take into account possible genetic relations of the outputs and possible side effects (Mehravar *et al*., 2019). Moreover, when integrated with other biotechnologies such as rapid cycling breeding techniques, high through-put phenotyping, and genomic selection, the act of using the CRISPR/Cas9 technology can prove beneficial in increasing the development of improved crop varieties (Hickey *et al*., 2019). The solutions and sound paradigms of the safe deployment of crops that have been genetically edited by the new toolset of CRISPR will be attained through multi-disciplinary alliances between bio-scientists and plant biotechnologists, plant breeders and regulatory agencies. Delivery, either viral or Agrobacterium-mediated and tissue specificity still remain as two of the most challenging factors that define the optimal application of CRISPR/Cas9 for plants. This means low trans delivery and expression of CRISPR components, plus problems with tissue or cell-specific targeting and with trying to deliver the CRISPR tools exactly where they are needed (Hahn & Nekrasov, 2019). Even though Agrobacterium-mediated transformation and biolistic delivery are popular, they give low editing expression and low mosaicism frequency. In order to solve these problems new technologies in delivery systems and tissue targeting are being created which includes nanoparticle-mediated delivery, viral vectors, and plant cell-penetrating peptides (Demirer *et al*., 2019; Ellison *et al.,* 2020). Genetic redundancy and pleiotropy, which are the key characteristics of crop plants, are the difficult factors when using CRISPR/Cas9 for crop improvement. All the known agronomic traits with few exceptions are polygenic which means they are determined by several genes and regulatory elements (Scheben and Edwards 2018). The issue of genetic redundancy, where often there are multiple genes with related functions; pleiotropy, when one gene influences several characteristics; complicates the work. Multiplexed editing approaches, combined with thorough phenotypic characterization, may be required to overcome these challenges (Wang *et al.,* 2018). Stable edited lines are what is needed to translate the genetics edited by means of the CRISPR system into desirable cultivars. The purpose is to create edited lines that are productive, and innoxious and which do not have complex transgenic distortions (Lassoued *et al*; 2021). Some ways to get there include the following; having editing strategies with mixed components of CRISPR or turning to temporary delivery systems. These approaches are used to obtain non-transgenic edited lines though they may need back crossing screening of progeny (Schiml *et al.,* 2016).
There are legal requirements and reception of CRISPR/Cas9 edited crops varies globally. Some areas classify these crops to be GMOs while in other areas they have relatively liberal legal policies (Lassoued et al., 2021). This endless discourse continues to make off-target effects, side effects, and risks to the environment as some of the critical factors affecting biosafety and regulation. In order to explore these issues, promotion of communication, clear risk assessment and public participation is vital for the approval and demand for understanding the methods of governance (Hartley et al., 2016). Biosafety has surfaced as the CRISPR/Cas9 systems are used more often. However, off-target effects are still a major concern, which the current paper asserts despite the high target guidance efficiency of guide RNAs (gRNAs) (Entine *et al.,* 2021). The cleavage efficiency and selectivity in on-target and the off-target sites of CRISPR nucleases defines the genome modifications as a result. Certain genomic loci can only be identified using the high throughput assay methods, and thus the high cleavage efficiency and specificity is desirable. This in turn increases the need for proper designing of guide RNA and better definition of cleavage patterns (Heidar *et al.,* 2021). Intentional and timely deposition and controlled production of CRISPR/cas9 are cardinal for accurate functional genomic analysis. The nuclease must be expressed in the right developmental stage, tissues, and subcellular location added new problems to create delivery vectors and regulatory elements that would allow strict control over the spatial-temporal and dosage patterns of the nuclease (Iyer *et al*., 2022). Stress, physiological burdens, and conditions may alter the function and integrity of CRISPR/Cas9 effectors. They may affect the size and the structure of the target protein or modify the features of the prototype protein or the presence of extra components on it or the location of the protein in the cell. Influence on binding selectivity and target enzymatic cleavage are also critical concerns in the experimental planning in order to obtain the most accurate results (Wiedenhoft *et al.,* 2016). Although CRISPR nucleases are accurate and fast in identifying and cutting target DNAs, such as comprehensive biological environments and multiple applications, there may be some issues that theoretical CRISPR could not accommodate (Doudna, 2020a). It will also be helpful to understand further the research

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capabilities of these nucleases, their total activity profiles, physiological needs, and potential physical restraints for more massive nucleic acid scanning or industrial synthesis in the future with careful observance of the welfare of people (Cribbs and Perera, 2017). Nonetheless, the advancement in CRISPR/Cas9 technology have exciting opportunities of increasing yields in farming and agriculture. The harmony with other technologies, such as high-throughput phenotyping, genomic selection, and complex breeding technologies shows that researchers can build new crop varieties that are resilient to climatic changes, have high yield, and enhanced nutritional value (Gao, 2021; Hickey *et al.*, 2019). Further enhancement, cross disciplinary works, mutual adjustment and the moral governance of this remarkable tool are crucial to supplementing in enhanced yield in agriculture and hence food security. Therefore, despite the highly promising applications of making use of CRISPR technology to modify the entire biological domain, there are significant risks and frailties that every researcher utilizing the framework should take into account regarding the locations and specific features related to these pioneering and innovative experiments.

Table 2: Comparison of CRISPR-Cas9 with Other Gene Editing Tools

Feature	CRISPR-Cas9	Zinc Finger Nucleases (ZFNs)	Transcription Activator-Like Effector Nucleases (TALENs)	References
Mechanism	RNA-guided DNA cleavage by Cas9 nuclease	Engineered proteins with zinc finger domains for DNA recognition and FokI nuclease domain for cleavage	Engineered proteins with TALE repeats for DNA recognition and FokI nuclease domain for cleavage	Jinek *et al.*, 2012; Urnov *et al.*, 2010; Christian *et al.*, 2010
Design	Easy and flexible sgRNA design	Complex and time-consuming protein engineering	Complex and time-consuming protein engineering	Ran et al., 2013; Ramirez *et al.*, 2008; Gaj et at., 2013
Target Specificity	High, but potential for off-target effects	High, but potential for off-target effects	High, but potential for off-target effects	Fu *et al.*, (2013); Pattanayak *et al.*, (2011); Mussolino *et al.*, 2011)
Efficiency	High editing efficiency	Moderate editing efficiency	Moderate editing efficiency	Cong *et al.*, (2013); Porteus *et al.*, (2003); Christian *et al.*, (2010)
Cost	Relatively inexpensive	High cost due to protein engineering	High cost due to protein engineering	Ledford (2015); Ramirez *et al.*, (2008); Pennis (2008)
Delivery	Simple delivery methods (e.g., plasmids, viral vectors)	Complex delivery methods	Complex delivery methods	Ran *et al.*, (2015); Liang *et al.*, (2015); Gaj *et al.*, 2012); Holkers *et al.*, (2013)
Multiplexing	Easy to target multiple genes simultaneously	Difficult to multiplex	Difficult to multiplex	Cong *et al.*, 2013); Wang *et al.*, (2013); Ramirez *et al.*, (2008); Sanjana *et al.*, (2012)
Advantages	Easy to design, efficient, cost-effective, flexible	High specificity	High specificity	Ran *et al.*, (2013); Ledford, (2015); Urnov *et al.*, (2005); Gabriel *et al.*, (2011); Mussolino *et al*, (2011)

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Thus, the option based on CRISPR/Cas9 DNA can be considered a more favorable one in many ways. It also employs RNA-guided DNA cleavage and thus CRISPR/Cas9 is easier to design than ZFNs and TALENs because it entails less in the way of protein engineering. CRISPR/Cas9 also demonstrates a high level of editing capacity, together with lesser costs in comparison with other methods, and easy means of delivery. In addition, it stands out in multiplexing – the task of amplifying multiple genes at once, which is a huge advantage.

Although, all of the mentioned methods are highly specific and have off-target effects as the drawback, the main benefits of using CRISPR/Cas9 are its simplicity, low cost, and flexibility. On the other hand, ZFNs and TALENs though being more specific in cleavage are more difficult and time-consuming to design, comparatively expensive, and less efficient in multiplexing.

Emerging Trends and future directions in CRISPR-Cas9 for Plant Biotechnology

CRISPR/Cas9 system of gene editing which was initially used in bacteria and is now widely used in plants for genetic engineering, is on the rise. It offers excellent opportunities for crop improvement that would enable one to fine-tune the crop genes. However, some trends and prospects that can be observed with relative advancement of the work have already started to shape the future of CRISPR/Cas9 in plants. The specific trend of doing multiple tweaking at once is called multiplex genome editing by utilizing the CRISPR/Cas9 system. This method deals with the capacity to alter two or more genes in one attempt or to introduce two or more desirable traits in the same plant species (Shen *et al*., 2017). This technology is one of the massive opportunities that can be used to improve composite characters that are determined by many genes.

Moreover, it is proposed to use the CRISPR/Cas9 system not only for plant genome editing but for its functioning regulation as well. Researchers are developing strategies to ask for specific desires of genes and employ transcription activators or repressors to attend to concentrating on certain genomic locations (Piatek *et al.,* 2015). This approach reveals new opportunities to study the functions of genes, the fine-tuning of metabolic pathways, and enhancement of the needed characters without changing the DNA code. CRISPR/Cas9 system is being adapted to target epigenetic signatures or epigenetic modifiable marks such as DNA methylation and histones posttranslational modifications (Gallego-Bartolomé, 2020). This new area of research is named as epigenome editing and has the potential of placing a rein on gene expression patterns that has the possibility of opening new areas of crop improvement by manipulating epigenetic standings. It has been incorporated into precision breeding strategies for specific improvement of the desirable traits from the wild type germplasms to high yielding improved varieties (Scheben *et al.,* 2017). That strategy strives to accelerate generation of climate smart, high yielding and nutritious crops varieties and at the same time does not compromise on the useful gene sources. CRISPR/Cas9 is also being used in Large-scale genome- wide screening and functional genomics in plants (Jacobs *et al.,* 2015). This method of scientific research allows learners to investigate gene functions with measures, plan new targets to increase yields in crops, and examine associations of various components. It also enables the researchers to seek information on the target gene function, new gene source for crop improvement and fundamental understanding of the biological processes. Different research workers are optimizing and exclusively developing novel CRISPR methods that could be used for plant applications. These are better delivery systems, tissue specific gene expression and how to tame destructive off target effects (Yin *et al.,* 2017). The advancements of such nature aspire to enhance and diversify the application of CRISPR/Cas9 in plant molecular biotechnology both for the purpose of specificity enhancement and expanded effective uses. CRISPR/Cas9 is used for engineering plants for production of high added value biomolecules such as bio-pharmaceuticals, industrial enzymes, and special chemicals (Shukla *et al.,* 2021). They attempt to enhance the plant's ability to put sugar to diverse utilization, enlarge the formation of products in addition to synthesizing valued small molecules for sale whilst maintaining environmental compatibility.

The efficiency of CRISPR/Cas9 is deemed to make a possibility for biofortification of staple crops' nutritionally significant qualities (Gomez *et al.,* 2019). Thus, by focusing on certain pathways, the quantity of beneficial vitamins, minerals, and other bioactive molecules can be increased, and the nutritional profile of the population as a whole can be enhanced to combat the world's malnutrition issue. As can be seen Climate change is progressive, CRISPR/Cas9 is used to edit several crop kinds improving their resistance to several abiotic stresses, which include among others drought, salinity, heat or cold-stress, and toxic soil (Langner *et al.,* 2018). It is applied here to raise the yield and the ability of the crops to cope with

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unfavourable condition of the environment. CRISPR/Cas9 is also applied widely in enhancement of plant security by means of gene editing to render resistance to diseases, pathogens and pests (Hu *et al.*, 2019). The susceptibility genes or the genes that control the plant immune system are conditions areas that the scientific world is trying to transform so that they can produce crops that are not easily attacked by diseases. Regarding CRISPR/Cas9, it includes the process of the re-domestication of wild genetically similar plant species with preferred characteristics that are allowable to be targeted by the breeders to bring enhancement in the yield, nutritional quality, stress tolerance, and several other characteristics of crop plants (Lemmon *et al.*, 2018). It could, therefore, expand the germplasm base of other cultivated crops by bringing in new characteristics from low-valued plant varieties. Researchers are trying to understand how to overcome some of the challenges associated with manipulation of the genome in polyploid organisms of plants which have multiple copies of the genome (Shen *et al.*, 2021). The purpose of this research plan is to improve the practical suitability of polyploid crops such as wheat, cotton, and sugarcane for predestined genetic manipulations. Hence, cooperative work of various disciplines, proper organizational frameworks, and an active involvement of the public will be crucial for the future progression of CRISPR/Cas9 in attaining goals concerning food security, sustainable agriculture, and conservation of germplasm resources. Genome editing using CRISPR/Cas9 has emerged as the best technique in plants due to its accuracy and efficacy. Subsequent advancements have only added to its functions and use It has also evolved with newer enhancements.

One of the primary concerns surrounding CRISPR/Cas9 is the potential for off-target effects. These occur when the gene-editing tool inadvertently modifies unintended genes, leading to unforeseen consequences. To mitigate this risk, researchers emphasize the meticulous design of guide RNA molecules, which direct the CRISPR system to the target gene. Moreover, advancements in editing techniques have been instrumental in reducing the likelihood of off-target effects. Another hurdle in the widespread application of CRISPR/Cas9 is the efficient delivery of its components into plant cells. Current methods often face challenges in achieving high rates of successful gene editing. To overcome this limitation, scientists are developing innovative delivery systems, such as nanoparticle-based approaches, to enhance the efficiency and precision of CRISPR technology in plants.

Potential Applications of Cas13 Protein for RNA Genome Editing in Plants

This overview looks at the new generation of systems, namely the CRISPR/Cas9 for editing plant genomes and further, the prospects of the Cas13 protein in RNA plant genomes editing. More CRISPR/Cas9 systems have been developed and hence, some of the challenges from the previous systems. The base editing system incorporating cytosine base editors that allow targeted changes without using double-strand breaks have been enhanced to work in plants. In rice and wheat, Zong *et al.* (2017) applied cytosine base editors (CBEs) and obtained high editing efficiency with low frequency of unwanted insertions and deletions. Likewise, adenine base editors have trailed in rice and wheat and the applications have been extended (Li *et al.*, 2018). Another revolutionary technique is prime editing in which parts of the target gene may be directly modified including marking out small elements, and adding in base insertions, deletions and substitutions without the need for double strand breaks or donor DNA. Lin *et al.* (2020) successfully introduced the prime editing on the rice and wheat, and with high efficiency of the desired modification and low level of off-target effects. It has a great potential on the Generation of Complex Genetic Changes in Crop Plants. Engineering of the Cas9 nuclease has also improved the selectivity and potentialities of genome targeting in plants. For instance, SpCas9-HF1 variant with high fidelity of the endonuclease developed for mammalian systems has been used in rice studies with fewer off target activities while enhancing on target activities (Zhang et al., 2019). Various modifications in multiplex genome have been made whereby multiple targets can be inserted simultaneously. Thus, Lowder et al. (2015) established a CRISPR/Cas9 system to edit, and modulate gene expression in *Arabidopsis thaliana* genetic model plant with high alterability in a single round. This has gradually become fine-tuned and used in Numerous crop species making it easier to manipulate complex traits and metabolic pathways.

While CRISPR/Cas9 is targeted to DNA, Cas13 continues to be regarded to open the advancements of RNA editing in plants. Cas13 has RNA-guided RNA-targeting CRISPR effector that has been used for RNA knockdown, RNA editing as well as detection in mammalian systems. (Abudayyeh *et al.*, 2017). Although its application in plants is still in its infancy, several potential uses have been proposed. Viruses represent another opportune area where Cas13 could be used in plants. Subsequently, Aman et al. 2018 have shown that Cas13a could be adapted to target the RNA genome of turnip mosaic virus in *Nicotiana*

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benthamiana and thereby prematurely introduce an undesired stop codon that impedes viral replication as a new strategy to develop virus-resistant crop plants. This strategy could be especially useful in fighting RNA viruses which are causes of crop losses all over the world. RNA editing using Cas13 also has a possible capability of climate change by changing gene expression without changing the DNA code in plants. It could be particularly useful for research on gene function since it is possible to make the change reversible and cell type-specific. Besides, it might provide an opportunity to develop the essential characteristics in crops that may not necessarily generate the reaction of some jurisdictions when they are associated with DNA modifications. It also indicates that the Cas13 system can be used for RNA-guided transcript detection in plants thus allowing the detection of pathogens or monitoring of gene expression. The potential of this application may be monumental in disease diagnostics especially for plants and functional genomics. Nevertheless, several issues must be solved before Cas13 can be successfully used in plant systems. These are; the delivery methods, achieving specificity, and reducing possible negative impacts on the plant's transcriptome. Further, the stability and the activity of Cas13 has to be properly evaluated in various plant species and plant tissues. All in all, novel CRISPR/Cas9 systems that have been developed in recent years have enriched the methods of plant genome modifications in terms of accuracy, speed, and flexibility. The above discussed possibilities of RNA editing using Cas13 in plants opens up various opportunities in crop enhancement, disease control, and functional genetics in plants. The further development of these technologies will help enhance the process of plant breeding and assist in mitigating current worldwide issues regarding agriculture and nourishment.

Targeted epigenome editing, using CRISPR/dCas9 (a nuclease-deactivated version of Cas9) fused with epigenetic modifiers, allows for the precise regulation of gene expression without altering the DNA sequence. This has significant potential in agriculture for modulating traits like stress tolerance and crop yield by controlling gene expression patterns in response to environmental changes. Similarly, RNA editing with Cas13, which targets RNA molecules rather than DNA, offers a transient and reversible approach to gene regulation. This is particularly beneficial for controlling gene expression in response to seasonal or developmental cues without permanent genomic alterations. Applications of Cas13 in agriculture could include the temporary silencing of genes involved in pathogen resistance or the regulation of flowering time to optimize crop yield.

Conclusion

CRISPR/Cas 9 has proved to be a powerful tool in the gene editing process and has greatly affected the future of plant biotechnology. It is to summarize here that, in the above review, an attempt has been made to cover all the recent developments and prospects of using the CRISPR/Cas9 system in precise editing and targeted modification of crop genomes for better yields. From a comprehensive survey of the databases and literature, the status of the various facets of plant science research as offered by CRISPR/Cas9 has come to be well understood in our study.

The review has also highlighted the unique versatility of the CRISPR/Cas9 system includes such tactics as multiplex genome editing (Shen *et al.,* 2017), targeted gene regulation (Piatek *al*., 2015), targeted epigenetic modifications (Tirado *et al*, 2017), and selected breeding techniques (Scheben *et al*., 201 These advancements have provided opportunities to tackle some of the multifaceted problems in the agriculture such as increasing productivity and quality of food crops, managing diseases and abiotic stresses, and improving the nutritional value of the crops. These case studies include some different crop species such as rice by Li *et al*., (2016), tomato by Tashkandi, *et al* (2018), soybean by Do et al.(2019), and wheat by Placido, *et al*.(2013) among others have shown enormous potential in harnessing the power of CRISPR/Cas9 technology for the development of climate-resistant, higher yielding, and nutrition Also, the review has made a comparison of the efficiency of CRISPR/Cas9 with other gene-editing tools, in term of; design; how efficient the tool is; how cheap the tool is; and the flexibility of the tool (Ledford, 2015; Ran *et al.*, 2013). However, it is important to discuss both the opportunities and the risks of the CRISPR/Cas9 system which are considered in the review; these are off-target effects (Fu *et al.*, 2013), aspects concerning delivery constraints (Yin *et al*., 2017) as well as regulatory issues (Andersson *et al.*, 2017). Possible solutions to these challenges have also been predicted, which include enhancing specificity, creating specific delivery procedures, and setting up adequate governance frameworks. Moving forward, more inter-disciplinary collaborations of plant Biologists geneticists, bioengineers, and computational biologists will be essential for further developing the CRISPR/Cas9 for plant uses. Besides, there is a need to involve the public, take ethical factors, and encourage further investigation to develop and apply gene-edited crops as proper solutions to global food

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shortage and various issues in agriculture. In all, this review has presented a systemic view of the topic in the current state and further development of CRISPR/Cas9 in plant biotechnology. With further developments in this ground-breaking technology, CRISPR has tremendous potential to revolutionize crop breeding and, consequently, the idea of sustainable agriculture and feeding the world's growing population in a world of climatic variability.

Addressing complex agricultural issues requires a multifaceted approach that integrates diverse disciplines and involves a wide range of stakeholders. Key strategies include systems thinking, which takes a holistic view of agricultural challenges, and participatory research methods that engage farmers, local communities, and other stakeholders throughout the problem-solving process. Multi-stakeholder platforms and transdisciplinary research teams facilitate collaboration and knowledge sharing among experts, practitioners, and policymakers. These approaches are often complemented by efforts to integrate traditional and scientific knowledge, strengthening the policy-science interface, and adopting a comprehensive value chain perspective.

To navigate the complexities of modern agriculture, practitioners increasingly rely on scenario planning and foresight analysis to prepare for various potential futures. Citizen science initiatives and cross-sector partnerships expand the scope of agricultural research and innovation, while adaptive management approaches allow for continuous learning and adjustment. These interdisciplinary strategies recognize that sustainable solutions to agricultural challenges require collaboration across disciplines and sectors, as well as the active involvement of those directly affected by agricultural practices and policies. By integrating diverse perspectives, knowledge systems, and stakeholder interests, these approaches aim to develop more effective, equitable, and sustainable solutions to the complex issues facing agriculture today.

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