

# CRISPR/CAS9: AN APPROACH TO MEET GLOBAL FOOD DEMAND

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## ABSTRACT

The world's population is increasing exponentially and will reach 9.7 billion by 2050. There is a dire need to increase food production to feed this fast-growing population. Conventional breeding techniques have enormously contributed to improving agricultural yield, climate change, biotic and abiotic stresses still pose a significant threat to crop productivity. New techniques are required to increase agricultural production to meet the rising global food demand. CRISPR (clustered regularly interspaced short palindromic repeat)-Cas9 as a genome-editing tool has shown great potential to address emerging issues in agriculture. This approach can be utilized to alter any genetic sequence to get desirable characteristics in plants. CRISPR/Cas9 is more cost-effective and user-friendly than other current biotechnological techniques like zinc-finger nucleases (ZFNs) and transcription activator-like effector nuclease (TALENs). This technology is also promising to produce non-transgenic gene-edited plants to cope with the adverse effects of changing climate and assure global food demand. CRISPR/Cas9 has improved almost every major crop, including wheat, rice, and barley. However, significant success stories have not yet been reported on many other vital crops that contribute much to the world's food production, such as maize, sorghum, cassava, and potato. This review discusses the potential of CRISPR/Cas9 in crop improvement with updated information. Furthermore, it encourages applying this approach to the above-mentioned crops to meet global food security.

**Keywords:** CRISPR/Cas9; genome editing; climate change; global food security; crop improvement; plants.

## INTRODUCTION

Population growth, climate change, and food shortage are currently some of the most prevalent issues on this planet. The world's population is increasing and is expected to reach 9.7 billion by 2050 [1]. The aptitude to feed this fast-growing population will soon become a significant issue for our community to solve. This situation will become more vulnerable due to adverse climatic change, poor soil and air quality, biotic and abiotic stresses which significantly reduce crop productivity. Climate change impacts agricultural productivity by changing weather patterns and increasing environmental stresses such as drought, soil salinity, new disease outbreaks, and insect pests. According to the International Rice Research Institute (IRRI), one hectare of cultivable land is lost every 7.7 seconds, and this rate of loss may accelerate as global temperatures rise [2]. It is estimated that to feed the fast-growing population, food production must be increased by 50% by 2030 and 70 to 100% by 2050 [3]. This predicted situation demands the development of high-yielding crop varieties with better adaptation to changing climate and resistance to biotic and abiotic stresses on an urgent basis. Conventional breeding techniques have made a tremendous contribution to improving agricultural yield in recent decades. However, the actual crop production appears to be reaching a plateau [1]. One possible method to overcome the constraints of conventional breeding approaches is using biotechnological tools in crop improvement.

In recent times, the applications of site-specific genome editing technologies have shown remarkable ability in precise gene editing of plants [4]. CRISPR/Cas9 has been the most promising technology to address emerging issues in agriculture. Some recent reviews discussed and demonstrated the details of CRISPR/Cas9 technology in plant species [5–8]. In plants, this approach can be utilized to alter nearly every genetic sequence to produce desired characteristics. CRISPR/Cas9 technology is more precise, cost-effective, and highly efficient even at multiplex genome editing as compared to current biotechnological techniques like zinc-finger nucleases (ZFNs) and transcription activator-like

effector nuclease (TALENs) [9]. CRISPR/Cas9 applications for plant genome editing are one of the fast-growing bioscience technologies. More significantly, CRISPR/Cas9 is evolving into a user-friendly approach to produce non-transgenic genome-edited plants to deal with climate change and assure future food security. This review provided current information on the potential of CRISPR/Cas9 in crop enhancement. It also encourages employing this approach to other important crops such as maize, sorghum, cassava, and potato to meet global food security.

## APPLICATIONS OF CRISPR/CAS9 IN CROP IMPROVEMENT

Since the CRISPR/Cas system has been acknowledged as a cutting-edge genome editing approach, both researchers and industries have been utilizing it and achieving promising results. Many site-specific genome editing technologies such as specific nucleases have made tremendous achievements in agriculture to fulfill global food demand. However, CRISPR/Cas9 provides an opportunity for biotechnologists to build a sustainable and resilient agriculture system for improving plant growth and development and response to different environmental stresses (Fig. 1).

### CRISPR/Cas for Improving Plant Yield and Development

Agricultural yield is becoming vulnerable due to adverse climatic changes, poor air, and soil conditions. In order to boost food production in the changing climate, scientists seek strategies to develop plants that can survive and produce more from harsh and unpredictable circumstances. Improving agricultural production through conventional breeding and transgenic technologies has always been a long-term objective. As the yield characteristic is a complex trait controlled by numerous genes, breeders and researchers have not improved significantly through the transgenic. Even though it is tough to discover a single gene that mainly controls the yield, several studies have shown that the expression of some genes has a detrimental impact on crop production. These sorts of genes can enhance crop production directly if they have been suppressed and even

deleted. For this purpose, CRISPR/Cas9 can be utilized to knock out genes that negatively control yield production. Rodríguez-Leal and coworkers applied CRISPR/Cas9 to target the promotor sequence of the quantitative genes in tomatoes. Their results demonstrated that making minute changes in the genes of tomato plants improved fruit shape, size, and plant architecture [10]. Li and coworkers employed CRISPR/Cas9 in rice to target the GS3, DEP1, and Gn1a genes, which negatively regulate grain yield. The results showed that eliminating these genes significantly improved yield-related traits, including the number of grains, grain size, and dense erect panicles [11]. Xu and coworkers used this approach in rice to eliminate GW2, GW5, TGW6 genes involved in lowering grain weight. Their results showed that gene editing with this tool significantly enhanced grain weight [12]. Shen and colleagues [13] utilized CRISPR/Cas9 to identify the grain yield QTLs in rice, which regulates grain number and size. In 2018, Wang and colleagues [14] employed this technology to enhance the seed size in wheat, disrupting the function of TaGW2, a gene that negatively controls seed size. CRISPR/Cas9-edited GASR7 mutants in wheat have enhanced grain weight [15].

In addition, to enhance agricultural yield, CRISPR/Cas9 system has also been employed to improve plant growth and development. Arginase is a key enzyme that regulates root growth by inhibiting nitric oxide synthase. Arginase overexpression hindered lateral root development [16]. In 2017, Wang and coworkers applied CRISPR/Cas9 and eliminated the ARG gene's function in cotton. Their results illustrated that genome-edited cotton lines significantly increased by 25 % lateral roots and by 52% total root surface area [17]. Paul and coworkers also used this technology and demonstrated that MADS-box transcription factor genes MADS78 and MADS79 play an essential regulatory role in endosperm cellularization and early seed development in rice. The MADS78 or MADS79 single knockout mutant demonstrated precocious endosperm cellularization, while double mutants hindered the formation of seeds and could not generate viable rice seeds [17]. Ma and colleagues employed CRISPR/Cas9 in rice to investigate the role of OsSWEET11, a gene responsible for sugar

transport. Their results demonstrated that knockout of the OsSWEET11 gene significantly reduced sugar concentration, grain weight, and seed setting percentage in the mutants. This study suggested that OsSWEET11 plays a vital role in rice plant development [18]. Likewise, MPK1 and MPK6 are essential genes for rice development [19]. CRISPR/Cas9 was used to produce male sterile rice lines by altering the hexokinase hmk5 gene [20]. Similarly, this technology created photo-sensitive Japonica male generic sterile rice lines by targeting carbon starved anther [65]. Early maturing rice varieties were also developed by targeting Hd2, Hd4, and Hd5 genes with CRISPR/Cas9 [66]. In Arabidopsis, male sterility with altered pollen development was achieved by eliminating *sarl1b* and *sarl1c* genes using CRISPR/Cas9 [21]. CRISPR/Cas9 has also shown a great potential to improve plant growth in Brassica. Sriboon and colleagues applied this approach in *Brassica napus* L. to eliminate the terminal flowering (*tfl1*) gene that negatively regulates flowering time. Their findings illustrated that mutants displayed early flowering phenotype [22].

All these triumphs indicate that CRISPR/Cas9 is a valuable tool to improve plant growth and development. Further details of CRISPR applications in improving plant growth and development are documented in (Table 1). In the future, it could be used as the primary tool for scientists to modify crops to meet global food demand.

### **CRISPR in Bio-fortification and Secondary Metabolites**

CRISPR/Cas technology has also been used to explore the possibilities of modifying secondary metabolism in plants to improve the quality of crops (Table 1). The main functional elements of food are carbohydrates, proteins, oil, and bioactive chemicals, and their content determines the quality of an agricultural product. Lycopene is a key bioactive functional component in treating several human illnesses such as cardiovascular disorders and prostate cancer. In recent times, Li and coworkers enhanced lycopene biosynthesis in tomato fruits using this technology. They edited five genes, SGR1, LCY-E, LCY-B1, LCY-B2,

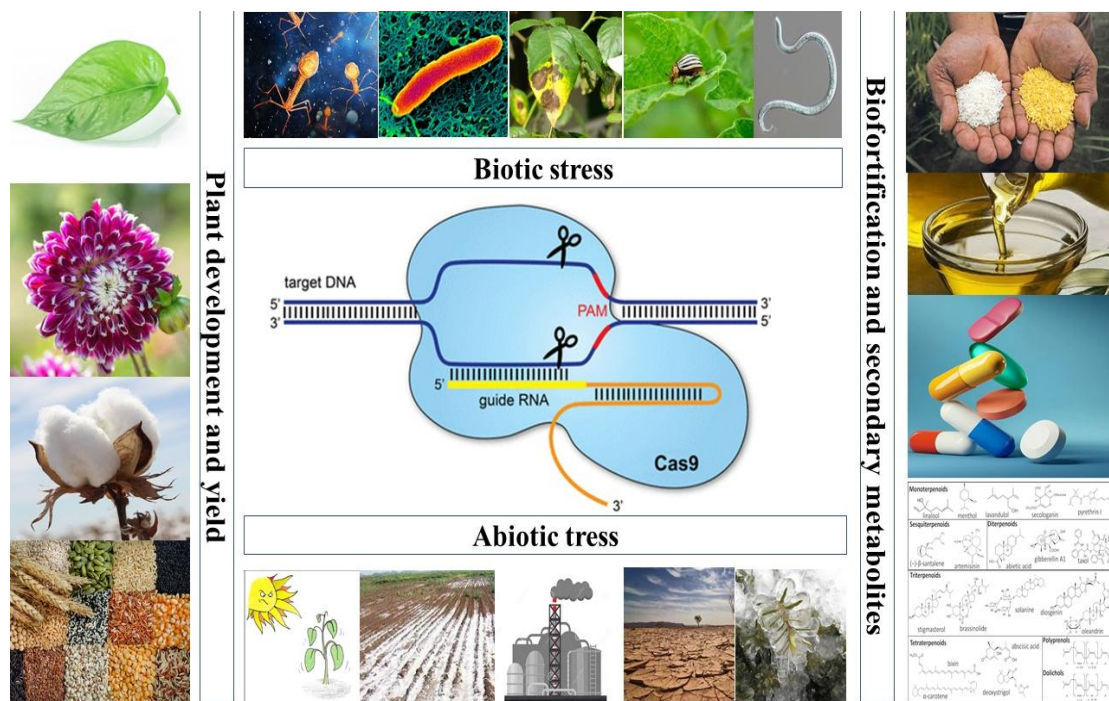
and Blc, associated with the carotenoids metabolic pathway [11].

Protein is an essential crop nutrient. However, the quality of the protein mixture is determined by the protein composition. About 1–2 percent of the human population is sensitive to gluten, found in wheat and other food stuffs. CRISPR/Cas9 was used to develop low-gluten and transgene-free wheat [23]. The d-hordein gene was knocked out in barley using a similar approach, which resulted in an enhanced protein matrix surrounding the starch granules [24].

This approach is also being employed to modify the production and composition of carbohydrates in crops. Grains with high levels of amylose and resistant starch have numerous health benefits. Sun and coworkers [25] employed this approach to disrupt the function of SBEIIb, a starch branching enzyme gene, and developed high

amylose genome-edited rice. CRISPR/Cas9 based deletion of *pat2/5* genes significantly enhanced starch accumulation in the mature seed of allotetraploid *Brassica napus* L. [15]. This technology was also used to achieve altered starch quality with complete deletion of GBSS gene function in potatoes [26].

Oil is yet another important characteristic that researchers aim to enhance utilizing the genome editing approach. Karunarathna and coworkers used CRISPR/Cas9 in rapeseed to eliminate the function of BnSFAR4 and BnSFAR5 genes that negatively regulate seed oil content. The result showed that seed oil content was enhanced without affecting seed germination, vigor, and oil mobilization [27]. Similarly, CRISPR/Cas9-edited BnTT8 mutants in *B. napus* L. have enhanced seed oil and protein content with altered fatty acid composition and have no adverse effects on yield-related traits [28].



**Fig. 1. CRISPR/Cas9 is a vital genome editing tool and has tremendous potential for crop development. It can be employed to introduce or alter the expression of a gene responsible for crop growth and development and biotic and abiotic resistance. In addition, it is also valuable for modifying secondary metabolism to regulate the production of oil, protein, functional component, and carbohydrates**

## **CRISPR/Cas9 Genome Editing Improves Plant Tolerance to Biotic Stresses**

Plant diseases caused by pests and pathogens limit the yield significantly, which is a severe threat to food security. These factors may result in complete yield loss in some instances. Newly developed plant diseases are considered a significant threat to food security. Plant diseases in major crops are responsible for more than 15% yield losses despite having modern agriculture practices. CRISPR/Cas 9 has turned out to be a vital genome editing technology to produce plant-resistant varieties against biotic stresses (Table 1).

Powdery mildew, caused by a fungal pathogen, severely impacts many agriculturally important plant species, including wheat, barley, rice, and tomatoes [15]. Past studies have demonstrated that barley's mildew-resistance locus (MLO) genes might be responsible for powdery mildew infection. These studies also showed that inhibition of these genes repressed powdery mildew disease [29]. The CRISPR/Cas9 was used to knock out MLO genes to confer resistance to powdery mildew in hexaploid wheat [30]. These MLO genes in tomatoes were also eliminated using CRISPR/Cas9, and tomato plants showed significant resistance against powdery mildew [31].

Rice is the second most important cereal crop and consumed worldwide. Rice suffers from many serious diseases which significantly reduce its yield, such as rice blast. Rice blast is a lethal fungal disease and threatens global food security by destroying 30% of rice crops annually [32]. Blast resistance in rice was achieved by disrupting the function of the OsERF922 gene using CRISPR/Cas9 [33]. The CRISPR/Cas9 was also applied in rice to eliminate the function of the OsSEC3A gene and successfully enhanced defense response and resistance against blast [18].

Fusarium wilt is one of the deadliest soil-borne fungal diseases. *Fusarium oxysporum* f. sp. *Niveum* (FON) may reduce 30-80% yield in watermelon. In recent times, Zhang and coworkers used CRISPR/Cas9 to eliminate the Clpsk1 gene, which encodes PSK precursors. Their results demonstrated that watermelon without the Clpsk1 gene has significant resistance against FON [34].

CRISPR/Cas9 has also shown a remarkable ability in producing plant-resistant varieties against viruses. Plant viruses are one of the significant constraints in agriculture production globally. Pyott and his coworkers have successfully developed mutagenized Arabidopsis lines possessing enhanced Turnip mosaic virus (TuMV) resistance. They applied CRISPR/Cas9 technology to target and eliminate eIF(iso)4E gene in the model plant [35]. CRISPR/Cas9 was used to intrude the function of the eIF4E gene in cucumber. The results illustrated that by disrupting the function of the eIF4E gene, cucumber showed significant resistance to Papaya ringspot mosaic virus W, Ipomovirus, and Zucchini yellow mosaic virus [36].

Plant species are also subjected to various bacterial diseases such as bacterial blight and citrus canker. Bacterial blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the severe diseases that routinely affect the yield. It may cause 30-50% yield losses or more in some cases [37]. Kim and coworkers applied CRISPR/Cas9 to knock out the Os8n3 gene, a susceptible host gene for bacterial blight in rice. Their results demonstrated that genome-edited rice plants enhanced significant resistance against Xoo infection [38]. In 2017, Peng and coworkers utilized CRISPR/Cas9 in citrus to eliminate the CsLOB1 gene, a susceptible gene for citrus canker. Their findings illustrated that citrus plants without the CsLOB1 gene have significantly improved citrus canker resistance [39]. In tomatoes, Ortigosa and coworkers [40] used CRISPR/Cas9 to silence the SiJaz2 gene and determined that genome-edited tomato plants improved bacterial speck resistance. These findings indicated that CRISPR/Cas9 has a remarkable ability to change plant attributes in biotic stress resistance.

## **CRISPR/Cas9 Genome Editing Improves Plant Tolerance to Abiotic Stresses**

The global population is quickly expanding. However, agricultural productivity is not improving at the required rate to meet rising food demand. Many abiotic factors such as drought, environmental pollution, salinity, and extreme temperature are partially behind this. Each has a significant impact on plant development, as well

as biomass, productivity, and quality. Since CRISPR/Cas9 was used as a genome editing tool, it has been employed to create plant-resistant varieties against abiotic stresses (Table 1).

Drought stress is one of the important constraints that significantly affect crop production globally. In recent times, Shi and coworkers employed CRISPR/Cas9 in maize to target the ARGOS8 gene, which negatively regulates ethylene responses. Their findings demonstrated that the genome-edited ARGOS8 gene has successfully improved drought resistance [41]. Chen and colleagues [9] applied CRISPR/Cas9 to disrupt the transcription repressors DPA4 and SOD7 in *Arabidopsis* and enhanced drought resistance.

Heat stress resulting from rising temperatures is an issue for agriculture in many parts of the world. Plants undergo various physiological, morpho-anatomical, and biochemical changes due to intense heat, which influence plant growth and may lead to a substantial reduction in economic yield [42]. Adverse effects of heat stress can be minimized by applying the CRISPR/Cas9 technique to generate crop plants with higher

thermo-tolerance. In 2017, Klap and colleagues used CRISPR/Cas9 in tomatoes to target SIAGAMOUS-LIKE 6 (SIAGL6) gene. Their results explained that genome-edited tomato plants under heat stress have significantly improved growth and fruit setting [43].

Salinity is a worldwide issue that affects roughly 20% of irrigated land and drastically decreases crop output. Salinity has adverse effects on plant vigor and growth [44]. In rice, a gene (ppa6) was knocked out using CRISPR/Cas9, and genome-edited rice plants successfully enhanced tolerance to alkaline stress [45]. Duan and coworkers have successfully developed mutagenized lines of rice possessing salt stress tolerance. Furthermore, they applied CRISPR/Cas9 to regulate the function of the GT-1 element (A key regulator) in the salt induction of the OsRAV2 gene [46]. Cui and colleagues also used CRISPR/Cas9 in rice to produce mutants of heterotrimeric G protein. Their findings presented that mutants *gs3* and *dep1* enhanced abiotic tolerance, especially salt tolerance [47]. In tomatoes, tolerance to salinity and osmotic stress was achieved by disrupting the function of the ARF4 gene [48].

**Table 1. Applications of CRISPR/Cas9 in crop improvement**

Plant Specie	Target gene/sequence	Acquired character	References
<b>Yield, plant growth and development</b>			
<i>Arabidopsis thaliana</i>	<i>sar1b</i> and <i>sar1c</i>	Development of male sterile lines with altered pollen development	[49]
Rapeseed	Terminal flowering gene ( <i>tfl1</i> )	Early flowering phenotype	[50]
	<i>Bnsp13</i>	Delay development phenotype	[51]
Cotton	<i>GhARG</i>	Improvement in lateral root formation	[33]
Rice	<i>GS3</i> , <i>DEP1</i> , and <i>Gn1a</i> genes	Improvement in number of grains, grain size, and dense erect panicles	[52]
	<i>GW2</i> , <i>GW5</i> , <i>TGW6</i> genes	Improvement in grain weight	[12]
	hexokinase <i>hvk5</i> gene	Development of male sterile lines	[20]
	Carbon starved anther	Development of photo-sensitive male generic sterile lines	[52]
	<i>Hd2</i> , <i>Hd4</i> , and <i>Hd5</i> genes	Development of early maturing varieties	[52]
Tomato	Promotor sequence of quantitative genes	Improvement in fruit shape, size, and plant architecture	[10]
Wheat	<i>TaGW2</i>	Improvement in grain size	[30]
	<i>GASR7</i>	Improvement in grain weight	[53]
<b>Biofortification and secondary metabolites</b>			
Barley	d-hordein gene	Enhancement in protein matrix surrounding the starch granules	[54]
Rapeseed	<i>pat2/5</i> genes	Improvement in starch	[55]

	BnSFAR4 and BnSFAR5 BnTT8	accumulation Improvement seed oil content Enhanced seed oil and protein content with altered fatty acid composition	[27] [28]
Rice	Starch branching enzyme gene (SBEIIb)	Development of high amylose rice lines	[47]
<b>Tolerance to biotic stresses</b>			
Arabidopsis	eIF(iso)4E	Turnip mosaic virus resistance	[35]
Cucumber	eIF4E	Papaya ringspot mosaic virus W, Ipomovirus, and Zucchini yellow mosaic virus resistance	[36]
Citrus	CsLOB1	Citrus canker resistance	[56]
Rice	Os8n3	Bacterial blight resistance	[57]
	OsERF922	Blast resistance	[58]
	OsSEC3A	Blast resistance	[18]
Tomato	SiMlo	Powdery mildew resistance	[59]
	SiJaz2	Bacterial speck resistance	[60]
Wheat	Mlo	Powdery mildew resistance	[61]
Watermelon	Clpsk1	Fusarium wilt resistance	[62]
<b>Tolerance to abiotic stresses</b>			
Arabidopsis thaliana	Transcription repressors DPA4 and SOD7	Drought resistance	[9]
Maize	ARGOS8 gene	Drought resistance	[41]
Tomato	SIAGAMOUS-LIKE 6 (SIAGL6) gene	Improved growth and fruit setting under heat stress	[43]
	Heterotrimeric G protein ARF4 gene	Salt stress tolerance	[47]
		Tolerance to salinity and osmotic stress	[48]
Rice	ppa6 gene	Tolerance to alkaline stress	[45]

## FUTURE PERSPECTIVE

CRISPR/Cas has become the most powerful tool in plant biotechnology research since its discovery. The rapid development of CRISPR/Cas-assisted genome editing has brought tremendous hope to applied and fundamental research. It is developing to be a powerful biotechnology tool to solve critical agricultural and biological problems. CRISPR/Cas-assisted genome editing will be used in crop improvement. In the last eight years, technologies like CRISPR/Cas have never been developed and utilized so rapidly in biomedical and biological research. Though, to use this powerful system more widely, many problems need to be solved.

### Development of a PAM-Independent CRISPR/Cas System Can Improve its Prospective Applications

A particular PAM sequence is required for the currently available CRISPR/Cas system. That

sequence instructs the editing site for a single event and provides specificity. Though, PAM requirements limit the genes which can use CRISPR/Cas for effective editing. The development of a PAM-independent CRISPR/Cas system can increase its application in fundamental and applied research such as precision breeding and gene function research. Scientists are trying to discover additional Cas proteins and modify existing Cas enzymes to expand PAM variants. However, the DNA sequence CRISPR/Cas can focus on is minor, which means more study is needed. Studying new Cas resources can be the most straightforward and highly cost-effective method to expand a PAM variant. In the last eight years, many PAM expansions have aided in the search for new Cas resources, with discovering that CRISPR/Cas can be combined with RNA sequence for RNA editing [63]. There are thousands of archaea and bacteria, but the number currently being explored is limited. There is no doubt that with the timing and ongoing research in

the future, more Cas resources are likely to be found in various variants of PAM. It is wise to keep changing the existing Cas variants, especially the frequently utilized Cas9, Cas12, and Cas13. Numerous experiments have shown that modification of the Cas enzyme can alleviate the need for PAM and establish new tools for studying gene function. Disabled Cas has more applications, such as epigenome targeting, imaging, and gene regulation.

### **Development of an Efficient Delivery Approach for CRISPR/Cas System is Necessary**

Regardless of the objective, CRISPR /Cas-assisted genome editing is a single-cell editing event that involves converting single cells into whole plants for future research and applications. Therefore, even some plants transforming need to regenerate from a single cell. Today, plant regeneration and transformation systems remain the bottleneck of genome-edited plants. Consequently, it is essential to develop an effective plant delivery along with a regeneration system. The successful transformation/editing of the genome primarily depends on the recipient cells' ability to regenerate the plant. It was difficult for various plant species to obtain regenerated plants, for example, rice, wheat, and corn, in the early days. Then, scientists tried to plant immature seeds or embryos that might grow into whole plants and quickly develop influential tissue culture and regeneration systems for plant species. In the future, we need to pay attention to more immature seeds and seeds used for plant genome editing and transformation [64]. The delivery approach of the CRISPR/Cas system is very crucial. It does affect the efficiency of the transformation and the off-target effects, and the subsequent regulatory purposes. Traditional genome editing and TDNA-mediated transgenic usually insert the T-DNA/target gene into the plant genome. These genes will continue to be expressed and passed on to future generations. The genome editing event occurs without the transgene when delivering the CRISPR/Cas system RNAs or proteins to plant cells because the CRISPR/Cas system is unlikely to be inherited from the plant. It will avoid regulatory issues that typically take years to get safety testing with government authorization.

### **Finding Gene Targets is Important for CRISPR/Cas-based Genetic Improvement**

The rapid advancement of CRISPR/Cas-based genome editing poses new opportunities to improve crops. The quality of crop yield belongs to a complex genetic background. However, it is vital to find the specific genes to precisely improve such traits. Those genes that control several traits can be divided into regulatory and structural types. Structural genes typically encode proteins and directly regulate crop traits. Searching for structural genes is the more secure option to improve the traits influenced by these genes.

On the other hand, several plant characteristics are controlled by regulatory genes. There are many regulatory genes in plants that are not limited to non-coding RNA and transcription factors. A few transcription factors respond particularly to specific biological processes, for example, stress-sensitive transcription factors. Therefore, targeting transcription factor genes can influence numerous structural genes to present downstream for a particularly desirable trait. That is why the present genome editing study focuses on transcription factor genes to improve plant resistance to biotic and abiotic stress. In the upcoming time, the researchers will need to discover key genes that directly or indirectly control the traits that plants need, and these genes will be targets for genome editing. Since regulatory elements and multiple structural genes usually regulate plant traits, multi-gene editing through CRISPR/Cas9 could be an alternate method to improve crops. Currently, there are several successful studies on the simultaneous editing of multiple genes using CRISPR/Cas9 technology. However, it is best to ensure that the large fragments that significantly affect other related characteristics are not removed from the chromosome when utilizing this technique.

### **The Off-target Effect is a Big Concern for CRISPR/Cas Applications**

Fewer off-target effects are observed in the plant than animals but are often reported and disseminated during genome editing events. Off-target is a significant problem in human clinical



trials, and CRISPR /Cas gene therapy should be eliminated [62]. Although CRISPR/Cas in plants is less severe, off-target effects can lead to undesirable consequences. Therefore, genome editing is required to eliminate off-target effects. The CRISPR/Cas delivery system influences the off-target of genome editing. Direct delivery of the RNAs and Cas enzymes protein and sgRNAs, (for example, RNP transformation) can reduce off-target effects. The reason is that these functional molecules have not been added to the plant genome. After the function, it can be swiftly degraded. It has a very short lifespan in plant cells, making it less likely to be targeted elsewhere [34].

### Developing Research Plan to Improve Other Crops

Since discovering the CRISPR/Cas system, it has improved several major crops such as wheat, rice, barley, and cotton. There is no way to deny the role of CRISPR/Cas9 in crop improvement and ensuring global food security. However, many other vital crops such as maize, sorghum, potato, and cassava significantly contribute to the world's food production and face many biotic and abiotic stresses. Unfortunately, many success stories on such crops have not been reported yet. To cope with the rapidly growing population and ensure global food demand, there is a dire need to develop research plans to improve mentioned above crops.

### CONCLUSION

CRISPR/Cas9 is a promising and naturally occurring genome editing technology that can be applied to alter any genetic sequence to achieve desirable traits, including plant growth and development and resistance to several biotic and abiotic stresses. In many plant species establishing a more proficient CRISPR/Cas system with efficient delivery method and minimal off-target effects remains a challenge. Currently available omics technologies such as whole genomic sequence, RNA-seq, and small RNA-seq, will aid in the discovery of new candidate genes for CRISPR/Cas9 assisted crop improvement. In addition, the development of research plans on the improvement of mentioned earlier crops will facilitate us to tackle the rising global food

demand. We anticipate using the CRISPR/Cas system in several crops will transform agriculture into a second green revolution to ensure global food security.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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