Journal of Multidisciplinary Sciences
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CRISPR/Cas9 applications and future prospectus in crop genetic improvement

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Received: 25 September 2020; Accepted: 27 November 2020; Published online: 08 December 2020

Abstract. The demand for food in 2050 is projected to rise as the global population crests about 10 billion people and meet the challenge, and agricultural production should be increased dramatically. Gene editing system has been revolutionized in genetics and crop improvement with the multipurpose technology of CRISPR/Cas9 (clustered regularly interspaced short palindromic repeat). CRISPR/Cas9 system is based on the complementary of the guide RNA (gRNA) to a specific sequence and the Cas9 endonuclease activity that brought a new opportunity in agricultural research to develop innovative plant varieties with the addition or deletion of significant characters. The CRISPR/Cas9 technology of genome editing is variously applied to improve crop quality worldwide. CRISPR technology has shown great potential in manipulating genes in the plant, making the crops' quality and stability. CRISPR/Cas9 is a recent and reliable molecular scissor for genetic engineering, making the advanced revolution in life science. The review is based on the multidimensional application of the CRISPR/Cas9 system in plant nutritional improvement, enhancement of plant disease resistance, and drought-tolerant plant production.

Keywords: *Agriculture, molecular scissor, specific sequence, genetic engineering, multidimensional application.*

Cite this as: Das, B.D. & Paudel, N. (2020). CRISPR/Cas9 applications and future prospectus in crop genetic improvement. J. Multidiscip. Sci. 2(2), 56-66.

1. Introduction

The global human population is anticipated to reach 10 billion by 2050. While the available farmland and water are being reduced, the global demand for food will increase by 25-70% above current production levels. Thus, feeding a rapidly growing population is a massive challenge. Therefore, an urgent need to improve food production and accelerate sustainable agricultural development (Hunter et al., 2017). CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated proteins) genome editing system can facilitate crops' development to meet future demands. However, gene editing is obstructed by many crop species' long life cycles, and targeted genotypes may require multiple generations to achieve. Single-celled microspores are haploid cells that can grow into double haploid plants, which have been generally used as a breeding tool to generate homozygous plants within a generation. CRISPR/Cas9 genome editing is a life-changing technology that will help develop crops to meet future demands (Bhowmik et al., 2018).

Plants are crucial for human life as they provide various plant-based products such as fruits, food grains, vegetables, and medicine. The plant characters can be improved by plant breeding and genetic engineering activities (Molinar, 2012). The CRISPR /Cas9 system was first discovered as an adaptive immune system against invading viruses in many bacteria and most archaea (Deveau et al., 2010). The CRISPR/Cas9 system was first introduced in bacteria and archaea. It can degrade exogenous substrates developed as a gene-editing technology in 2013, which has received broad attention due to its easy manipulation, high efficiency, and complete application in gene mutation and transcriptional regulation in mammals and plants. The process of CRISPR/Cas9 is advanced, and its application has also developed dramatically. Therefore, CRISPR/Cas9 is considered are revolutionary technology in plant biology (Liu et al., 2017).

Various tools such as overexpression, RNA interference, Zinc finger TALEN nuclease, and CRISPR/Cas9 are applied for genetic engineering in plants (Mahfouz et al., 2014; Ain et al., 2015; Andersson et al., 2018; Castel et al., 2019). Among all of them, the CRISPR/Cas9 genome editing tool is derived from the bacterial CRISPR system, which involves the immune system. CRISPR/Cas9 technology has become popular due to its specific and efficient application in editing the genome. Many CRISPR/Cas9 and its modifications have been used for the gene-editing of many desired genes. The CRISPR locus and its associated proteins are found in few bacteria, and it is associated with immunity against phages. The different points of CRISPR locus interpreted and drove to the formation of CRISPR-RNA (crRNA) and trans-activating CRISPR-RNA (tracrRNA). The crRNA, tracrRNA, and Cas9 encounter the phage DNA. The guide RNA (gRNA) is a synthetic gene comprised of crRNA and tracrRNA (Hsu et al., 2014).

Haft et al. (2005) categorized CRISPR/Cas system and characterized 45 CRISPR-associated (Cas) protein families, which are divided into core proteins (Cas1, Cas2, Cas3, Cas4, Cas5, Cas6), 8 CRISPR/Cas subtypes and RAMPs (Repeat Associated Mysterious Proteins) complexes. Class 1 CRISPRs possess multiple subunit effector complexes, whereas class 2 CRISPRs concentrate most of their functions with single protein effectors. For example, the Class 1 CRISPR system contains various nucleases for pre-crRNA processing, spacer sequence loading, and targeted cleavage processing. However, in class 2, a single protein achieves all of these functions. Type IV and type V possess class I and class II systems, respectively. Two V and VI types are also recognized, elaborating the classification to a two-class–six-type–19-subtype system (Shmakov et al., 2015; Table 1). Cas1 and Cas2 genes are typical in all CRISPR/Cas types (Makarova et al., 2011).

The terms genetic engineering, genome editing, and gene editing indicate the conversion (insertions, deletions, substitutions) in the genome of a living organism. The most usually applied access to genome editing nowadays is based on CRISPR/Cas9. CRISPR/Cas9 in prokaryotes is an adaptive immune system that protects the cells from DNA virus infections. CRISPR/Cas9 has been modified to create various gene-editing technologies with a wide diversity of applications in medicine, agriculture, and necessary studies of gene functions. CRISPR/Cas9 is commonly used in many monocot and dicot plant species to improve yield, quality, and nutritional value, to introduce or enhance tolerance to biotic and abiotic stresses, among other applications. This promising technology, with its potential contributions being applied in food production for the benefit of the growing human population (El-Mounadi et al., 2020). This paper reviewed the current situations in the advancements and applications of CRISPR/Cas-based gene-editing tools in crop improvement.

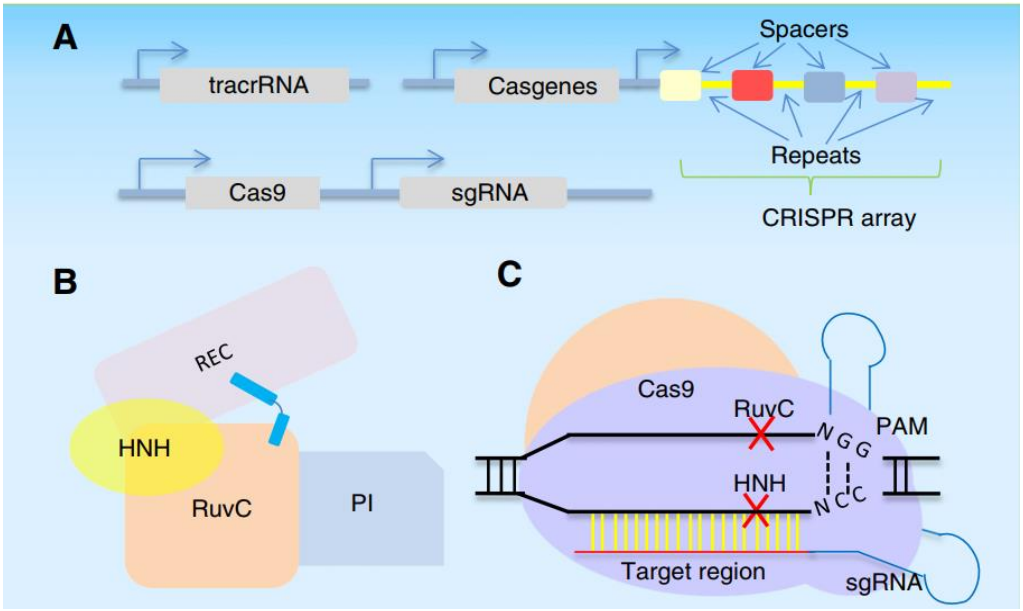

Figure 1. Components of the type II CRISPR/Cas system. A. Genomic structures of the native bacterial CRISPR/Cas system (top) and the engineered CRISPR/Cas9 system (bottom). tracrRNA, trans-activator RNA; sgRNA, single guide RNA; B. A schematic representation of the Cas9 protein structure. Domains include REC (large recognition lobe) and RuvC (a nuclease domain) linked

with an arginine-rich region. HNH is a second nuclease domain. PI, PAM-interacting domain. C. The conformation of Cas9-sgRNA complex in the process of DNA cleavage (Song et al., 2016).

Table 1. Classification of the CRISPR/Cas9 system (Makarova et al. 2011; 2015)

Class	Type	Sub-types	Organism harboring respective types	Signature Cas proteins	Other core proteins
Class 1	I	I-A	*Archaeoglobus fulgidus*	Cas3, Cas8	Cas1, Cas2, Cas5, Cas6, Cas7
		I-B	*Clostridium kluyveri*	Cas3, Cas8	Cas1, Cas2, Cas5, Cas6, Cas7
		I-C	*Bacillus halodurans*	Cas3, Cas8	Cas1, Cas2, Cas5, Cas7
		I-D	*Cyanothece sp.*	Cas3, Cas10	Cas1, Cas2, Cas5, Cas6, Cas7
		I-E	*Escherichia coli*	Cas3, Cas8	Cas1, Cas2, Cas5, Cas7
		I-F	*Yersinia pseudotuberculosis*	Cas3, Cas8	Cas1, Cas2, Cas5, Cas6, Cas7
		I-U	*Geobacter sulfurreducens*	Cas3, Cas8	Cas1, Cas2, Cas5, Cas6, Cas7
	III	III-A	*Staphylococcus epidermidis*	Cas10	Cas1, Cas2, Cas5, Cas6, Cas7
		III-B	*Pyrococcus furiosus*	Cas10	Cas1, Cas2, Cas5, Cas6, Cas7
		III-C	*Methanothermobacter thermaautotrophicus*	Cas10	Cas5, Cas7
		III-D	*Roseiflexus sp.*	Cas10	Cas5, Cas7
	IV	IV	*Acidithiobacillus ferrooxidans*	Csf1	Cas5, Cas7
Class 2	II	II-A	*Streptococcus thermophilus*	Cas9	Cas1, Cas2
		II-B	*Legionella pneumophila*	Cas9	Cas1, Cas2
		II-C	*Neisseria lactamica*	Cas9	Cas1, Cas2
	V	V	*Francisella cf. novicida*	Cpf1	Cas1, Cas2
	VI	VI	*Leptotrichia shahii*	C2c2	Cas1, Cas2

2. Applications of CRISPR/Cas in crop improvements

The increasing world population's current situation is the most critical challenge in front of a fast-growing global population. To meet this challenge, the development of technologies that can contribute to crop improvement and increase agricultural production of the crop to some extent by applying CRISPR/Cas9 technology.

Although CRISPR arrays were identified in the *Escherichia coli* genome in 1987 (Ishino et al., 1987), their biological roles were not known until 2005, when it was shown that the spacers were homologous to viral and plasmid sequences suggesting a role in adaptive immunity (Bolotin et al., 2005; Pourcel et al., 2005). Two years later, CRISPR arrays were confirmed to protect against invading viruses when combined with Cas genes (Barrangou et al., 2007). CRISPR/Cas systems are part of bacteria and archaea's adaptive immune system, protecting them against invading nucleic acids such as viruses by cleaving the foreign DNA in a sequence-dependent manner. The immunity is acquired by integrating short fragments of the invading DNA known as spacers between two adjacent repeats at the proximal end of a CRISPR locus. The CRISPR arrays, including the spacers, are transcribed during subsequent encounters with invasive DNA and are processed into small interfering CRISPR RNAs (crRNAs) approximately 40 nt in length, which combine with the trans-activating CRISPR RNA (tracrRNA) to activate and guide the Cas9 nuclease (Barrangou et al., 2007).

Reports published in 2013 discussing the application of CRISPR/Cas9-based genome editing in plants (Feng et al., 2013; Shan et al., 2013; Xie and Yang, 2013). This first group of studies already demonstrated the immense versatility of the technology in the field of plant biology by embracing the model species *Arabidopsis thaliana* and *Nicotiana benthamiana* as well as crops such as rice, by using a range of transformation platforms (protoplast transfection, agro infiltration and the generation of stable transgenic plants), by targeting both endogenous genes and transgenes and by exploiting both NHEJ and HR to generate small deletions, targeted insertions, and multiplex genome modifications. Subsequent work focused on additional crop species such as sorghum (Jiang et al., 2013), wheat (Upadhyay et al., 2013; Wang et al., 2014), and maize (Liang et al., 2014).

Numerous disease resistance crop plants have been developed using CRISPR/Cas9 technology. Wang et al. (2014) discovered the work of desired genome editing in sweet orange using sgRNA/cas9. Genetic modification of citrus is limited due to its slow growth, pollen incompatibility, polyembryony, and parthenocarpy. Xcc (*Xanthomonas citri* subsp. *citri*) facilitated agroinfiltration was employed to deliver Cas9 and *CsPDS* gene-specific sgRNA into sweet orange. DNA sequencing confirmed the mutated *CsPDS* gene at the target site with a mutation rate of 3.2 to 3.9%. No off-target mutagenesis was reported.

CRISPR/Cas9 technology has been used to modify a wide range of crop plant species, including, rice (Macovei et al., 2018), wheat (*Triticum aestivum*) (Okada et al., 2019), maize (Lee et al., 2019), soybean (*Glycine max*) (Li et al., 2015), sorghum (Che et al.,

2018), cotton (*Gossypium hirsutum* L.) (Li J et al., 2018; Qin et al., 2019), rapeseed (*Brassica napus* L.) (Braatz et al., 2017), barley (*Hordeum vulgare* L.) (Lawrenson et al., 2015), *Nicotiana benthamiana* (Jansing et al., 2018), tomato (*Solanum lycopersicum* L.) (Ortigosa et al., 2019), potato (*Solanum tuberosum*) (Enciso-Rodriguez et al., 2019), sweet orange (*Citrus sinensis* L.) (Peng et al., 2017), cucumber (*Cucumis sativus* L.) (Chandrasekaran et al., 2016), wild cabbage (*Brassica oleracea* L.) (Lawrenson et al., 2015), wild legume (*Lotus japonicus* L.) (Wang et al., 2016), lettuce (*Lactuca sativa* L.) (Woo et al., 2015), *Medicago truncatula* (Michno et al., 2015), tobacco (*Nicotiana tabacum* L.) (Baltes et al., 2014), *Nicotiana attenuate* (Woo et al., 2015), grape (*Vitis vinifera* L.) (Wang X et al., 2018), apple (*Malus pumila*) (Malnoy et al., 2016), tropical staple cassava (*Manihot esculenta*) (Odipio et al., 2017), watermelon (*Citrullus lanatus*) (Tian et al., 2017), and others given in Table 2.

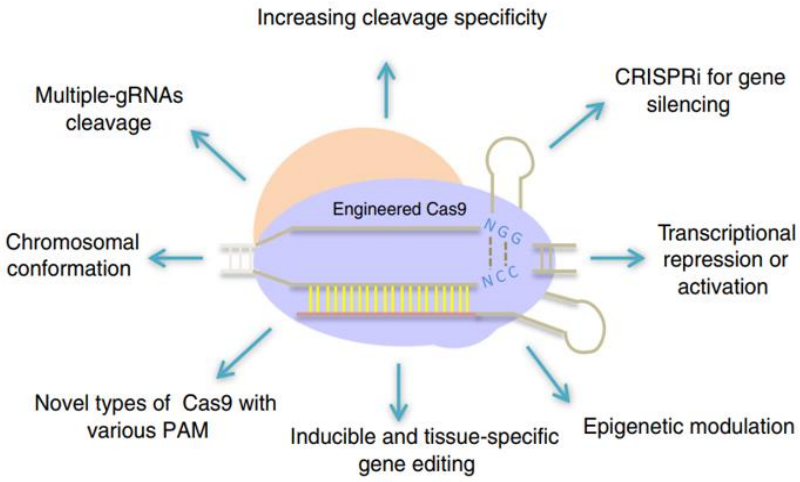

Figure 2. The development of engineered CRISPR/Ca9 systems (Song et al., 2016).

Table 2. Applications of the CRISPR/Cas9 System in some significant crops

Crops	Gene	Reasons for editing	Significance	References
Rice (*Oryza sativa* L.)	*OsSWEET11, OsSWEET14* (rice bacterial blight susceptibility	To resist Bacterial blight of rice against *Xanthomonas oryzae*.	PEG stimulated Cas9/sgRNA gene uptake in rice protoplast (Agrobacterium independent method), Cas9 / sgRNA mutations occur within plant cells, free of bacterial cell involvement	Jiang et al., 2013
Rice (*Oryza sativa* L.)	*TMS5*	To develop commercial 'transgene clean'TMGES rice lines.	The offspring were observed to be healthier and provided a more significant yield compared with the control variety.	Zhou et al., 2016
Rice (*Oryza sativa* L.)	*OsERF922* (ethylene-responsive factor transcription factor)	To fight against Rice blast disease caused by Magnaporthe oryzae	42% T_0 mutant lines; 6 T_2 homozygous mutants showed high blast resistance and had the same agronomic traits	Wang et al., 2014
Rice (*O. Sativa* L.)	*ALS*	To introduce various distinct point mutations in the rice ALS gene. To increase resistance against herbicide.	Knock-in and resistance against sulfonylurea herbicides	Sun et al., 2016
Wheat (*Triticum aestivum* L.)	*TaMLO* homologs	To increase resistance against powdery mildew in wheat.	Knockout and resistance to powdery mildew	Wang et al., 2014
Wheat (*Triticum aestivum* L.)	*TaGW2*	For efficient and specific genome editing	TaGW2 gene plays a vital role in grain weight control.	Liang et al., 2017

Table 2. Applications of the CRISPR/Cas9 System in some significant crops

Crops	Gene	Reasons for editing	Significance	References
Upland cotton (*Gossypium hirsutum* L.)	*GhCLA1* (Chloroplasts alterados 1)	For targeted mutagenesis of the cotton genome	Mutations were detected in the cotton protoplast.	Chen et al., 2017
Upland cotton (*Gossypium hirsutum* L.)	*GhVP* (vacuolar H^+-pyrophosphatase)	For targeted mutagenesis of the cotton genome	Mutations were detected in the cotton protoplast.	Chen et al., 2017
Allotetraploid cotton (*G. hirsutum* L.)	An endogenous gene *GhCLA1* and *DsRed2* (Discosoma red fluorescent protein2)	For targeted mutagenesis of the cotton genome	Disappeared red fluorescence and showed the albino phenotype	Wang P et al., 2018
Cotton (*G. hirsutum* L.)	*GhMYB 25-like*	For efficient and specific genome editing	GhMYB25-like is involved in the development of cotton fiber.	Li C et al., 2017
Maize (*Zea mays* L.)	*ZmAgo18a* and *ZmAgo18b* unless (a1 and a4) (Argonaute 18) and reductase or anthocyanins (a1 and a4)	For mutagenesis frequency and heritability	Involved in the biosynthesis of 24- nt phase RNA in anthers	Char et al., 2017
Soybean (*Glycine max* L. Merr.)	*GmPPD1* and *GmPPD2*	Inheritable site-directed mutagenesis	Trifoliate leaves were observed thicker with deeper green color, longer petioles, and more giant	Kanazashi et al., 2018
Soybean (*Glycine max* L. Merr.)	*GmFT2a*	To induce targeted mutagenesis of GmFT2a	Showed late flowering under both short-day and long-day conditions	Cai. et al., 2018
Sorghum (*Sorghum bicolor* L. Moench)	Whole *k1C* gene family	To create kafirin variants for the improvement of protein digestibility and quality.	Vitreousness and a-kafirin levels were reduced, whereas an increase in the grain protein digestibility and lysine content was observed a-kafirin levels were observed.	Li A et al., 2018
Barley (*Hordeum vulgare* L.)	*HvPM19*	To induce targeted mutagenesis of barley genes.	Dwarf phenotype	Lawrenson et al., 2015
Brassica oleracea	*BolC.GA4.a*	To induce targeted mutagenesis of *B. oleracea* genes.	Dwarf phenotype	Lawrenson et al., 2015
Rapeseed (*Brassica napus* L.)	*RGAs, FULs, DAs*, and *A2.DA2*	To induce targeted genome modifications at multiple loci.	Dwarf phenotype	Yang et al., 2017
Oilseed Rape (*B. napus* L.)	Rapeseed *SPL3* homologous gene copies	To rapidly generate and identify mutagenesis of multiple gene homologs simultaneously.	Showed developmental delay phenotype	Li C et al., 2018
Tropical staple cassava (*Manihot esculenta*)	*MePDS* (phytoene desaturase) gene	To induce targeted mutagenesis in cassava.		Odipio et al., 2017
Watermelon	*ClPDS* (phytoene desaturase)	To effectively create knockout mutations in watermelon.	Apparent or mosaic albino phenotype	Tian et al., 2017
A traditional Chinese medicinal herb (*Salvia miltiorrhiza*)	Diterpene synthase gene (*SmCPS1*)	To precisely knockout the SmCPS1 in *S. miltiorrhiza*	The roots of homozygous mutants were white in appearance, whereas wild-type plants had red-colored roots.	Li B et al., 2017
Lotus japonicas	*SYMRK* (symbiosis receptor-like kinase), *LjLb1, LjLb2, and LjLb3*	Effectively target single and multiple SNF genes.	The triple mutant produced white nodules, whereas control plants formed pink nodules.	Wang et al., 2016
Grape cultivar (*Vitis vinifera* L.)	*MLO-7* (Mildew Locus O)	To increase tolerance against powdery mildew.	MLO genes are conserved, and their loss of function results in resistance against powdery mildew	Malnoy et al., 2016
Apple (*Malus pumila*)	*DIPM-1*, *DIPM-2*, and *DIPM-4*	To increase resistance to fire blight disease.	Physical interaction with the disease-specific gene of Erwinia amylovora	Pessina et al., 2016

Table 2. Applications of the CRISPR/Cas9 System in some significant crops

Crops	Gene	Reasons for editing	Significance	References
Citrus sinensis Osbeck	Gene *CsLOB1* (LATERAL ORGAN BOUNDARIES1) promoter	Exhibited enhanced tolerance against citrus canker as compared with wild type	Mutant plants showed tolerance against citrus canker	Peng et al., 2017
Duncan grapefruit (*Citrus paradise* Macf.)	*CsLOB1* (*C. sinensis* Lateral Organ Boundaries) gene	For generating canker-resistant grapefruit	Exhibited canker symptoms same as wild type	Jia et al., 2016
Banana (*Musa acuminata*)	PDS (phytoene desaturase)	Basic methodology	Slower germination growth on the selection medium.	Kaur et al., 2018
Nicotiana benthamiana	BeYDV (short intergenic region, transacting replication initiation protein)	To fight against Leaf thickening, chlorosis, curling caused by Bean yellow dwarf virus (BeYDV)	87% reduction in targeted viral load. The study proved that IR targeting via sgRNA confer better resistance	Baltes et al., 2014
N. benthamiana	*TYLCV-IR* (intergenic regions), RCA regions	Against Leaf curl disease	Mutants showed delayed and reduced viral DNA accumulation	Ali et al., 2015; 2016
N. benthamiana	*NtPDS* and *NtPDR6*	For targeted mutagenesis and examination of transient genome editing activity	The psd mutant showed etiolated leaves, and the pdr6 mutant displayed more branches	Gao et al., 2015
Tobacco (*N. tabacum* L.)	*XylT* (b(1,2)-xylosyltransferase) fucosyltransferase)	To remove plant type glycans by inactivation of those two enzymes.	Devoid and strong reduction of a(1,3)- fucose and b(1,2)- xylose	Mercx et al., 2017
Tobacco (*N. benthamiana* L.) and Arabidopsis	*EDS1* and *PAD4*	For induction of inheritable chromosomal deletions	EDS1-family genes are essential regulators of plant innate immunity.	Ordon et al., 2017
Tomato (*Solanum lycopersicum* L.)	*SlPDS* (phytoene desaturase) and *SlPIF4*(phytochrome interacting factor)	For targeted mutagenesis in tomato plants	The mutants of SlPDS showed an albino phenotype.	Pan et al., 2016
Tomato (*S. lycopersicum* L.)	*SlIAA9* (auxin-induced 9)	To generate parthenocarpic tomato plants	Morphological changes in seedless fruit and leaf shape	Ueta et al., 2017
Potato (*Solanum tuberosum*)	*GBSS* (granule- bound starch synthase)	To alter the starch quality.	Reduction of amylose content in starch	Andersson et al., 2017
Cucumber (*Cucumis sativus* L.)	*eIF4E* (eukaryotic translation initiation factor4E) gene	To enhance tolerance against the virus in cucumber.	Enhanced tolerance against the infection of Papaya ringspot mosaic virus-W(PRSV-W)	Chandrasekaran et al., 2016
Lettuce (*Lactuca sativa*)	*LsBIN2* (A. thaliana BRASSINOSTEROID INSENSITIVE 2)	For DNA-free genome editing	Calli contained monoallelic and biallelic mutations	Woo et al., 2015

Nishihara et al. (2018) investigate to induce mutations in the torenia flavanone 3-hydroxylase *(F3H*) gene encoding a primary enzyme involved in flavonoid biosynthesis. The CRISPR/Cas9 system effectively produced pale blue (almost white) flowers at a high frequency (ca. 80% of regenerated lines) in transgenic torenia T_0 plants. Sequence analysis of PCR amplicons by Sanger and next-generation sequencing declared the occurrence of mutations such as base substitutions and insertions/deletions in the *F3H* target sequence, thus declaring that the achieved phenotype was induced by the targeted mutagenesis of the endogenous *F3H* gene. This result indicates that flower color modification by genome editing with the CRISPR/Cas9 system is quickly and efficiently achievable. These findings reveal that this system may be helpful for future research on flower pigmentation and functional analyses of new genes in torenia.

Wang P et al. (2018) described four guide RNAs for the VvWRKY52 transcription factor gene for use with the CRISPR/Cas9 system. Furthermore, they obtained transgenic plants via Agrobacterium-mediated transformation, using somatic embryos of the Thompson Seedless cultivar, and concluded that the CRISPR/Cas9 system permits actual genome editing the first generation of grape and produces a valuable tool for gene functional analysis and grape molecular breeding. Bhowmik et al. (2018) combined the CRISPR/Cas9 system with microspore technology. They developed an advanced haploid mutagenesis system to activate genetic modifications in the wheat genome and establish the value of linking microspore technology and CRISPR/Cas9-based gene editing for trait discovery and plant improvement. Agrobacterium-mediated transformation was performed on microspore-derived callus when targeting the *HvPDS* gene, and to albinos with targeted mutations was successfully obtained the commercial purpose (Han et al., 2020). Johansen et al. (2019) significantly improved CRISPR/Cas9 editing efficacy by applying endogenous

potato StU6 promoters for driving the CRISPR component of the CRISPR/Cas system and demonstrate that this optimization has a dramatic effect on editing frequencies at both the protoplast and shoot/ex-plant level. Shu et al. (2020) studied that CRISPR-Cas9 could be used in peanut hairy root transformation systems for peanut functional genomics studies, specifically on the roots' gene function. Using CRISPR/Cas9 targeting peanut *AhNFR* genes in a hairy root transformation system, the role of *AhNFR5* genes in nodule formation in peanut was approved. Out of crop plants, CRISPR/Cas9 technology has also been significantly applied to modify some other plant species, such as *Arabidopsis thaliana* (Schiml et al., 2016; Pyott et al., 2016; Li et al., 2018), *Petunia hybrid* (Zhang et al., 2016; Subburaj et al., 2016).

3. Future prospectus CRISPR/Cas9

CRISPR/Cas9 technology marks the future of skilled genome editing with potent and successful results. The ability of gene editing in crop plants has been completely changed by CRISPR/Cas9 technology. Analyzing the primary biology of plant improvement and stress response will facilitate designing exclusive and excellent crops. The CRISPR/Cas9 influences a very bright future in making designer plants by taking only the gene of interest from a wild-type species, and the gene is then directly admitted at a definite location, which in turn opens many channels for plant breeders for making designer plants. Recently, various tools have been explored for editing complex and straightforward genomes. The clustered regularly interspaced short palindromic repeats CRISPR/Cas9 system has widely been used in GE due to its high efficiency, ease of use, and accuracy. It can be used to add desirable and remove undesirable alleles simultaneously in a single event.

4. Conclusion

CRISPR/Cas9 system is considered the most powerful tool for gene editing in various essential crops due to its high efficiency, relatively low cost, and ease of use compared with other genome engineering techniques, such as ZFNs and TALENs. This system has been revolutionized and achieved significant progress to increase its efficiency and target specificity; more work remains to be done to improve it. Various approaches will design plants in such a manner, which could combat all possible bitter challenges. The newly emerged CRISPR/Cas9 RNP system evaded the need to rely on target cell potential for Cas9 translation and its plausible meeting with gRNA. The plant products, which are achieved through the CRISPR/Cas9 system, have no exogenous DNA and can be accepted by all the rules and regulations accommodating to combat against genetically modified cases and get accessible approval by the department of agriculture. Finally, CRISPR/Cas9 technology will boast of a promising future in making the desired mutation in plants because it has transformed and metamorphosed our potential to modify and regulate prokaryotic and eukaryotic genomes. The extensive use of this technology will accelerate its rate.

Conflicts of interest. There are no conflicts of interest.

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