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Role of CRISPR/CAS in Agriculture: A brief review

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Abstract

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) with Cas9 systems have proven to be an effective molecular tool for editing the genome in a variety of organisms, including plants. This technology facilitates to introduce desired mutations at precise location within genomes. In addition, this system also eliminates the usage of transgene and vector free editing with desirable modifications. This tool is being successfully used for the development of disease resistant and climate resilient plant genotypes. This technology not only improves the crop production, but also help in understanding the functions of genes linked with different traits. Hence, CRISPR-Cas9 can be a potential source of second green revolution in the field of agriculture. This review mainly focused on different types and sub-types of CRISPR-Cas system, its classifications and successful application in the field of agriculture to develop disease resistant, stress tolerant and to improve nutritional quality in plants.

Keywords: CRISPR; Crops; Trait Improvement; Biotic; Abiotic Stresses; challenges

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Introduction

The CRISPR-Cas9 (clustered regularly spaced short palindromic repeat/CRISPR-associated protein) technology is essentially about a pair of "micro-scissors" that cut DNA more specifically and accurately than conventional techniques. Geneticists and medical researchers can edit particular areas of the genome by deleting, adding or changing certain DNA sequences using the cutting-edge CRISPR-Cas9 method. To adjust the genome as needed, scientists rely on cells' natural DNA repair mechanisms when DNA is cut.

For humans to survive on earth, food is essential. However, utilizing conventional methods of crop improvement, it is extremely challenging to meet the world's growing food needs due to a population that is expanding quickly. In order to increase metabolic flux and improve the nutritional value of plant species, scientists have long used a variety of genomic techniques to introduce variation at the genetic level (Moore et al., 2014). Unfortunately, these methods of plant breeding are laborious and time-consuming, making them unsuitable for the current needs of a rapidly growing population. Genome engineering (GE) presents desired qualities into plants like tolerance to biotic and abiotic stress, improved yields, feeding the growing populations and fighting human malnutrition by increasing the nutritional (metabolite) value of plant (Tyagi et al., 2020). The bacterial adaptive immune system (*Streptococcus pyogenes*) is the source of the third generation of genome editing technology, which is highly effective, takes less time, is highly precise, quick, and easy compared to conventional gene editing tools like ZFN (zinc finger nucleases) and TALEN (translation activator-like effector nucleases). It is among the greatest techniques for genome engineering (Upadhyay et al., 2013: Wong et al., 2015). With greater accuracy and the ability to introduce heritable changes in the target location, CRISPR-Cas gene editing technology can create plants devoid of transgenes. The utility of this approach was underlined by reports of CRISPR-Cas9 based genome editing in plants in 2013 (Feng et al., 2013; Shan et al., 2013). Following these reports, CRISPR technology was used in various different agricultural applications. Some of these included higher sugar content, enhanced tomato flavor, wheat resilience to powdery mildew, higher yield from mushrooms with less melanin, and maize resistance to drought (Chaudhary et al., 2022). From developing tuberculosis-resistant cows (Gao et al., 2017) which require less antibiotic use, to mushrooms resistant to browning (Waltz, 2016) that lead to less spoilage, CRISPR is changing the landscape of agriculture and food. It can be used to develop high-yielding crops while reducing the need for water, fertilizers and chemical crop protection. As a result, the CRISPR-Cas9 technology has emerged as an essential tool for genome editing in crops and plants (Rao and Wang, 2021). Food security and plant fitness are problems that can be solved through plant breeding innovations. CRISPR-Cas9 technology is one of the innovations that could significantly contribute to solutions in the not-too-distant future. The goal of this paper is to provide an overview of CRISPR/Cas types, the function of the CRISPR-Cas9 system in agriculture, talk about the worldwide CRISPR-Cas9 scenario, obstacles, and potential future applications.

1. Types of CRISPR-Cas

The top three CRISPR-Cas system categories are listed in the classification hierarchy. Between the three categories, there are three different signature genes. Cas9, Cas3, and Cas10 genes are present in the type I, type II, and type III of systems, respectively. Type

IV is a unique type; however, it is less frequent. Each type has further subtypes as shown in figure 1.

Typical CRISPR-Cas system classification and organization for each CRISPR-Cas subtype. A representative genome is provided for each CRISPR-Cas subtype, along with the names of the corresponding gene locus tags. The color-coding and family name used to identify homologous genes. Following the category is a list of names. "Legacy names" are in standard font, and suggested systematic names are in bold. LS large subunit, which is composed of the Cas10, Cas8, Cmr2, Cmr5, and Cse subfamilies, and SS small subunit are abbreviations. Crosses stand for genes that produce large inactive subunits. The genes and domain components of effector complexes are highlighted using various backgrounds. The signature protein family's capacity to recognize and classify subtypes using the appropriate profile can be described as strong or weak. Strong means that it is a reliable signature with a high level of specificity and selectivity, whereas weak means that it is the best signature currently available for a given subtype despite producing a lot of false positives or false due to technological challenges with the sensitivity and selectivity of Cas protein family profiles, uncertainties around Cas1 phylogeny, and known CRISPR-Cas subtype classification, it is currently not possible to fully automate the identification of CRISPR-Cas subtypes in general. The most effective method for ensuring accurate classification is to combine different sources of information, such as Cas1 phylogeny, the identification and annotation of as many Cas proteins as possible in the target locus, and, for type II systems, the identification of the trans-activating crRNA (tracrRNA) genes as shown in figure 2a, 2b and 2c (Rao and Wang 2021).

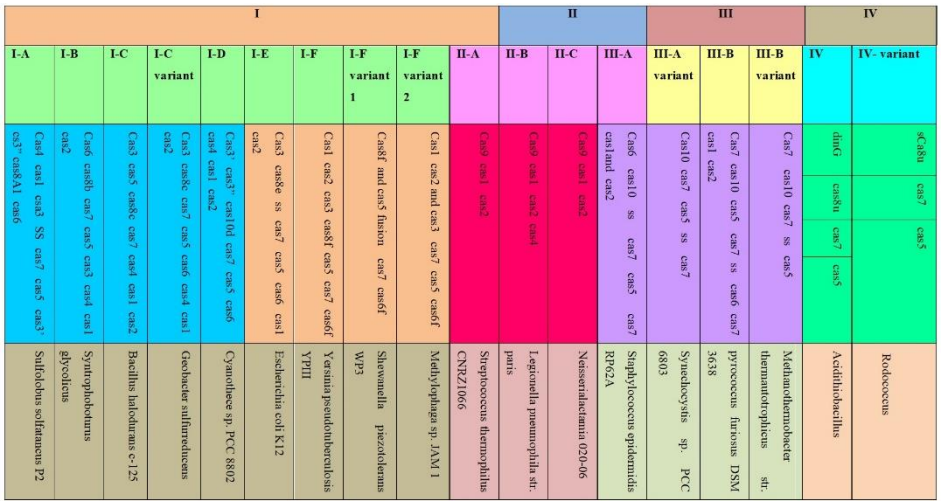

Figure 1. Classification hierarchy of CRISPR-Cas system

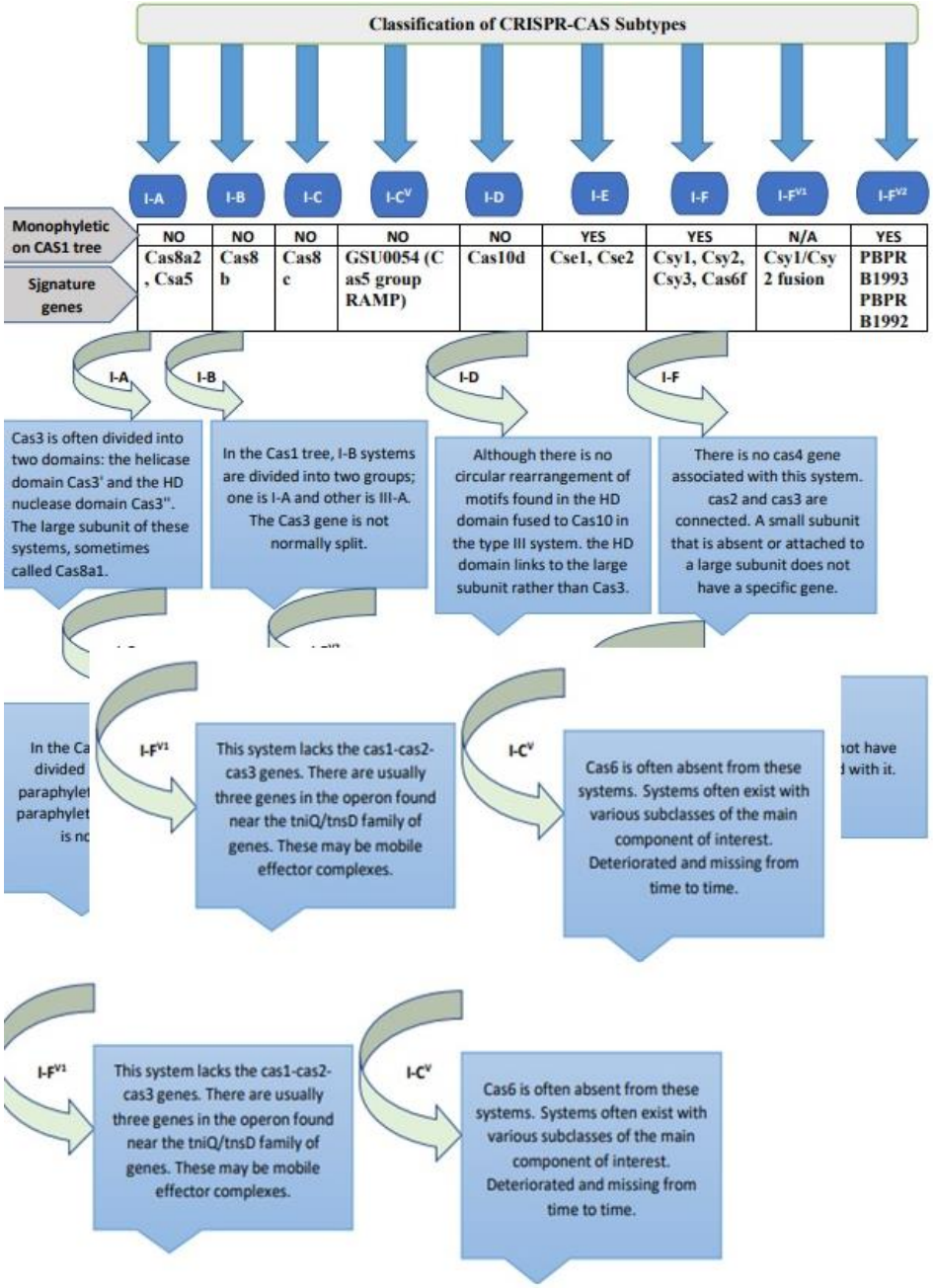

Figure 2a**.** Classification of CRISPR-Cas subtype

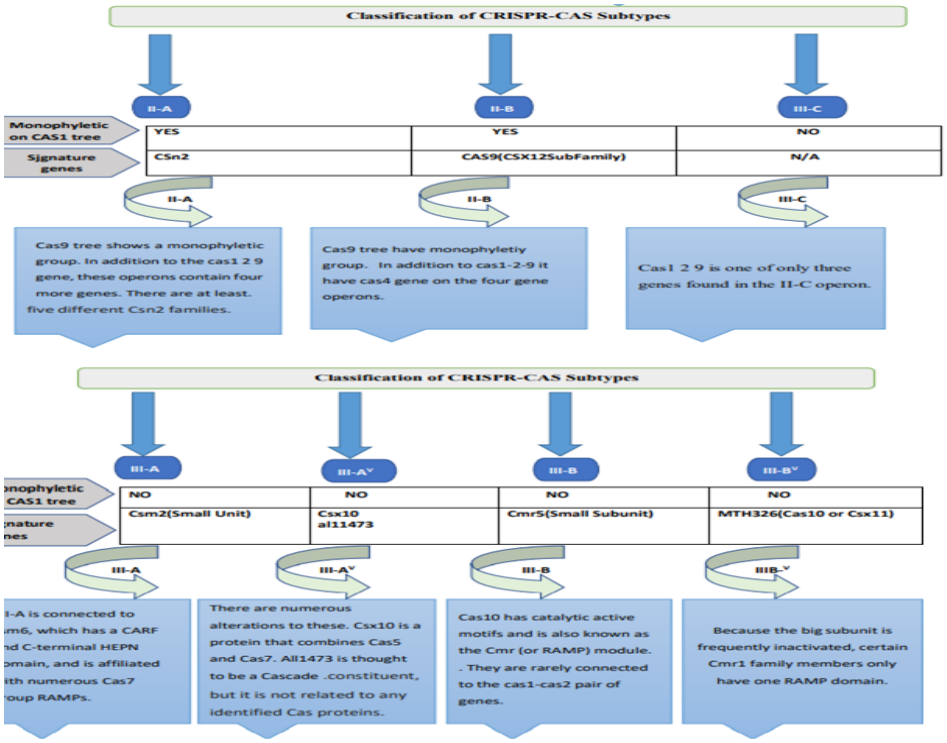

Figure 2b. Classification of CRISPR-Cas subtype

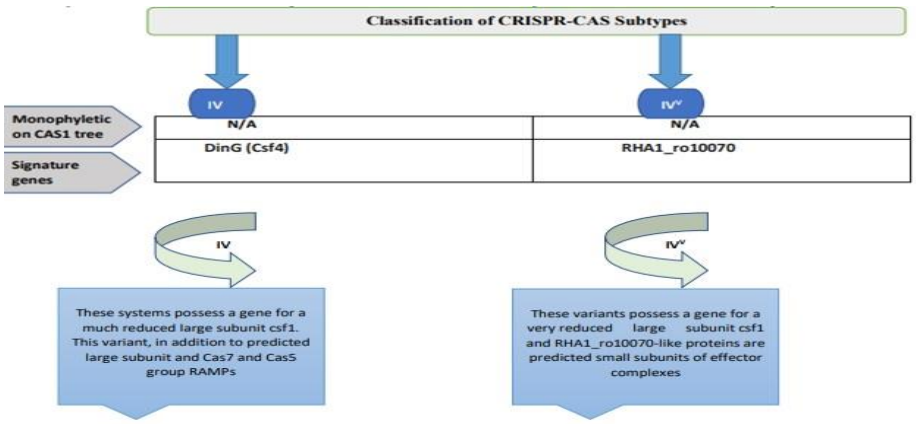

Figure 2c. Classification of CRISPR-Cas subtype

2. Role of CRISPR-Cas9 in agriculture

CRISPR-Cas9 technology has presented a high prospect to boost crops, as well as serve as a helpful research tool for more fast analysis of desired genes involved in growth and development, biofortification, disease resistance, and improved tolerance to a variety of biotic and abiotic stresses, might stimulate agricultural productivity even more by implanting it with conventional breeding techniques. Lately, CRISPR-Cas9 has been used in a broad range of creative breeding methods that manage reproduction-related hereditary characteristic (Das et al., 2022). It can also be used for the genetic modification of plants, which has been neglected so far. The potential this has for breeding crop and sustainable agriculture development is immense (Pineda et al., 2019). Genome editing technologies have demonstrated their enormous potential in the development of improved crop varieties with key agronomic traits (Abdallah et al., 2015). One of the necessary parameters of quality advancement in plants is gene versatility. Unknown plant types can be developed by manipulating the gene pool (Sikora et al., 2011).

2.1 Knockout mediated crop trait improvement

To get a crop variety that can withstand both biotic and abiotic stress and produces more, unfavorable features that have unfavorable effects on the crop must be eliminated. Numerous features, including quality, yield, disease resistance, biotic and abiotic stress tolerance, can be improved using CRISPR-Cas9. By using the knockout mechanism of gene editing, hybrid breeding methods and other crucial areas of crop breeding have been enhanced (Adhikari and Poudel, 2020).

2.1.1. Disease resistant

A growing threat to global food security comes from plant diseases brought on by fungi, bacteria, oomycetes, viruses, and other microbes. The worldwide challenge to food security is becoming more understood to include emerging crop diseases. Diseases in significant food crops cause pre-harvest production losses of up to 15% (Fisher et al. 2012). Technology using CRISPR-Cas9 for genome editing has created new possibilities to rapidly develop disease-resistant plant cultivars by stacking disease resistance (R) genes or disrupting/deleting susceptibility (S) genes as shown in table 1.

Table 1. CRISPR based application for Disease resistance in various crops.

Crop	Target gene	Trait	Resulting Trait	Reference
Bread wheat	TaMLO-A1, TaMLO-B1, and TaMLO-D1	Powdery mildew Resistance.	No apparent growth of fungus was observed on edited plant	(Wang et al., 2014)
Cotton	Two sgRNAs (GhMYB25-like-sgRNA1 and sgRNA2)	Resistance to Verticillium wilt (wilt disease).	Plants with resistance to Verticillium wilt were successfully created through this investigation.	(Li et al., 2017)
Tomato	SIDMR6-1	Deletion of SIDMR6-1 gene	resistance to significant infections such *Xanthomonas* species, *P. capsici*, and *P. syringae*.	(Paula et al., 2016)
Maize	ZmPRms	*Aspergillus flavus*	genotypes of maize that are less contaminated with aflatoxin because they are *A. flavus* resistant.	(Majumdar et al., 2017)

Rice	OsSWEET11, OsSWEET14	Resistance to Rice Bacterial Blight	Promoter of blight susceptible gene was disrupted	(Ma et al., 2018)
Tomato	Fmk1, Hog1, and Pbs2	fungal resistance	Significantly less pathogenesis was seen in tomato after gene silencing.	(Pareek and Rajam, 2017)
Rice blast	two sgRNAs targeting the OsERF922 gene	Enhanced rice blast resistance	Cas9/gRNA can be used to knock off individual and multiple genes.	(Wang et al., 2016)
Cassava	elF4E isoforms nCBP-1 & nCBP-2	Gene Disruption	increased resistance to the cassava brown streak disease.	(Bart and Taylor, 2017)
Cotton	Gh14-3-3d	Verticillium wilt resistance	Knock-in	(Zhang et al., 2018)

2.1.2. Viral resistant

In natural ecosystems, one of the main factors contributing to the loss of valuable crops is viral infections. By causing noticeable symptoms in plants, these diseases significantly impair production and strain the economy. Genetic engineering has shown to be a successful method for boosting plant tolerance to many viral diseases. There are restrictions on how thoroughly or entirely viral infection in crops may be eliminated by previously discovered procedures. Utilizing CRISPR technologies can help overcome these restrictions given that viral infections of crops diminish worldwide yields by 10% to 15% as shown in table (Van Regenmortel and Mahy, 2009).

Table 2. CRISPR based application for Viral resistant in various crops.

Crop	**Target gene**	**Trait**	**Resulting Trait**	**Reference**
Arabidopsis thaliana	host factor, eIF(iso)4E	resistance against Potyvirus	eIF(iso)4E, host factor is deleted, which is essential for survival of virus.	(Pyott et al. 2013)
Tomato	coat protein (CP) or replicase (Rep)	Enhanced resistance against leaf curl virus	Knock out	(Tashkandi et al. 2018)
Soybean	GmF3H1 GmF3H2 GmFNSII-1	Soybean mosaic virus (SMV) resistance	SMV resistance and increased isoflavone levels in soy beans	(Rajput et al. 2021)
Rice	eIF4G	Rice tungro spherical virus	Knock out	(Macovei et al. 2018)

		(RTSV) resistance		
Wheat and barley	CP Rep/Rep4	Wheat dwarf virus WDV) resistance	inhibit replication	(Kis et al. 2016)
Peanut	CP	resistance to Tobacco streak virus (TSV)	Prevent the PSND (Peanut stem necrosis disease)	(Senthilraja et al. 2018)
Nicotiana benthamiana	gBM3+ gBRBS+ gBM1- gB9nt	resistance to Bean yellow dwarf virus (BeYDV)	Destroying the BeYDV genome	(Baltes et al. 2015)
Arabidopsis thaliana and ***Nicotiana benthamiana***	The BSCTV genome contains 43 potential locations in either coding or non-coding regions.	Beet severe curly top virus (BSCTV) tolerance.	gene knockout with Cas9/gRNA	(Ji et al. 2015)

2.1.3. Increasing biotic and abiotic stress tolerance

Abiotic stress and biotic stress are important aspects affecting crop production and quality. The yield loss brought on by disease-causing viruses and other abiotic stresses is far larger, with an estimated yield loss due to plant infections of up to 16% (Ficke et al. 2018). Through genome editing with CRISPR-Cas9 knockouts, a variety of crops have been improved in their resistance to biological stress. By interfering with the OsSWEET13 promoter and knocking off the OsERF922 gene, resistance to crop-damaging diseases, such as the fungal disease known as rice blast, was produced. As a result, rice developed a resistance to bacterial blight as shown in table 3 (Zhou et al., 2015).

Table 3. CRISPR based application for stress tolerance in various crops.

Crop	Target gene	Trait	Resulting Trait	Reference
Cotton	GhPIN1-3, GhPIN2	Abiotic resistance	Drought Resistance	(Dass et al., 2017)
Sugarcane	*ScNsLTP*	Resistance to Abiotic stresses	Dryness and Freezing Resistance	(Chen et al., 2017)
Cotton	GhRDL1	Abiotic resistance	Drought Resistance	(He et al., 2017)
Tomato	SlMAPK3, SlMAPK6	Abiotic resistance	Resistance to wilt, Drought Resistance	(Wang et al., 2017)
Rice	TIFY1b transcription factor	biotic resistance	cold tolerant	(Huang et al., 2107)
Soy bean	GmSnRK1.1	biotic resistance	Phytophthora sojae	(Wang et al., 2019)
Tomato	SlARF4 gene	Knocking out the Auxin Response Factor 4 (ARF4)	Enhances salt and osmotic tolerance.	(Bouzroud et al., 2020)
Rice	OsPRX2	Abiotic resistance	Potassium deficiency tolerance	(Mao et al., 2018)

2.1.4. Yield Improvement

Crop yields are becoming more and more at risk due to unfavorable climate change, diminishing air quality, and deteriorating soil health. Scientists are attempting to develop crops that can resist severe and unpredictable settings while producing more in order to increase food yields in a changing climate (Rodriguez-Leal et al., 2017). In crop species, CRISPR-Cas9 creates revolutionary changes. CRISPR-Cas9 is revolutionizing crop species. That potential has led to several laboratories around the world using this powerful technology (Wang et al., 2021). Rice with less amylose and better eating and cooking quality was generated by using CRISPR-Cas9 to knock out Waxy as shown in table 4 (Zhang et al., 2018).

2.1.5. Quality and nutrition Improvement

With the use of CRISPR-Cas9, yields of crops produced in tropical regions have increased while also improving in quality. Quality qualities change depending on the unique breeding needs. Crop odour, nutritional value, starch content, and storage quality have all been impacted by quality improvement by genome editing thus far (Chen et al., 2019). Malnutrition affects roughly 2 billion people worldwide and has a considerable detrimental effect on society, economics, and health, according to the World Health Organisation (WHO, Geneva, Switzerland). This is especially true in the least developed and developing nations (Camerlengo et al., 2022). Scientists are seeking to design plants that can withstand

challenging and unpredictably occurring conditions while producing more in order to boost food production in a changing climate. Research exploring the potential of CRISPR-Cas9 to increase crop yields is escalating to meet these goals as shown in table 5 (Chakravorty et al., 2022).

Table 4. CRISPR based application for Yield Improvement in various crops.

Crop	Target gene	Trait	Resulting Trait	Reference
Maize	CLE	Grain yield	Enhancing grain-yield trait.	(Liu et al. 2021)
Soybean	By silencing GmFT2a and GmFT5a	High yields.	better yields because it can be grown in warmer areas.	(Cai et al., 2020)
Rice	OsSPL16	Grain yield	Modifying the expression of Pyruvate Enzymes improves grain yield	(Usman et al., 2020)
Rapeseed	BnaMAXI	Enhance yield	Knockout of two *BnaMAX1* homologs increases yield	(Zheng et al., 2020)
Wheat	TaGW2 (negative regulator of seed size).	Increase the seed size.	Knockout the function of all homologs of TaGW2.	(Wang et al., 2018)
Rice	PYL1, PYL4 and PYL6	Enhance yield	Improve growth and increase grain yield	(Mio et al., 2018)
Rice	IPA1, DEP1, Gn1a , and GS3	manipulate multiple regulators of yield-related traits	enhanced grain number, greater grain size, and thicker erect panicles	(Li et al., 2016)
Wheat	GW2, GW7, and GASR7	knock-out of genes	Increased seed size and seed weight as a result.	(Wang et al., 2018)
Maize	CLV-WUS	inflorescence meristem	improve meristematic activity and grain yield	(Rodríguez-Leal et al., 2017)

Table 5. Quality and nutritional features of different crops using CRISPR technology.

Crop	Target gene	Trait	Resulting Trait	Reference
Wheat	TaSBEIIa	High amylose content	Modification of starch composition of wheat	(Miao et al. 2018)
Rice	OsAAP10	Good cooking quality	rice quality improvement	(Wang et al. 2020)
Tomato	lncRNA1459	Prolong shelf life	Alteration in tomato fruit ripening	(Li et al. 2018)
Maize	PRL	nutritional Improvement	Reduction in zein content	(Qi et al. 2016)
Wheat	α-Amylase/ Trypsin Inhibitor Genes	Quality and nutrition Improvement	Reduce Allergen Proteins	(Camerlengo et al. 2020)
potatoes	GBSS gene	Enhance amylose content.	Higher yields of potatoes in less time.	Andersson et al. 2017)
Sorghum	k1C	Disrupted genes in N-terminal ER signal peptide region.	Protein digestibility is increased and High Lysine content.	(Li et al. 2018)

3. *Global scenario of CRISPR-Cas in agriculture*

The CRISPR Cas also help us in the field of horticulture. It used for target gene and change the expression of gene. Different targeted gene and traits were improved with CRISPR Cas shown in table 6, quality improvement in table 7, tolerances to abiotic stresses in table 8 and resistances to biotic stress in table 9.

Table 6. Quality and nutritional features of horticultural crops using CRISPR technology.

Crop species	**Target genes**	**Target crops**
Kiwi fruit	CEN4, CEN	A compact plant's creation with a quick terminal flower and fruit development
Ground cherry	SP, SP5G, CLV	the inclusion of factors related to morphology, flowering, and fruit size
cucumber	WIP1, SP,	gynoecious plant generation
Tomato	SP5G	creation of tomato plants that are sensitive to day-length changes

Tomato	SP, OVATE, MULT, FAS, CycB	introduction of characteristics related to morphology, flowers size and number of tomatoes, as well as the production of lycopene
Tomato	SP, SP5G, CLV3, WUS, GGP1	introduction of morphological characteristics, flower and fruit production, and ascorbic acid synthesis characteristics
Tomato	BOP1, BOP2, BOP3	simple inflorescence and early blossoming
Tomato	GAI	Generation of dwarf tomato plants
Tomato	MBP21	Generation of fruit stems with "jointless" joints
Tomato	MBP21	Generation of “jointless” fruit stem
Tomato	ARF7	making parthenocarpic fruit
Tomato	IAA9	creation of parthenocarpic fruit

Table 7. Quality improvement of horticultural crops using CRISPR technology.

Crop species	**Target genes**	**Target crops**
Tomato	AGL6	Production of parthenocarpic fruit
Tomato	ALMT9	Decrease in malate content
Tomato	SGR1, LCY-E, Blc, LCY-B1, LCY-B2	Increase in lycopene content
Tomato	GAD2, GAD3	GABA content has increased
Tomato	ANT2 (gene insertion)	a rise in the amount of anthocyanins
Tomato	MPK20	genes that regulate sugar metabolism are suppressed
Tomato	ALC	Tomato that keeps well.
Tomato	PL	tomato with a long shelf life
Tomato	ANT2 (gene insertion)	Purple-colored tomato
Tomato	MYB12	tomato with a pink hue Purple-colored
Tomato	PSY1	Yellow-colored tomato

Table 8. Resistance to abiotic stresses in crops using CRISPR technology.

Crop species	Target genes	Target crops
Watermelon	ALS	Resistance against herbicide
Tomato	MAPK3	lowered tolerance to drought stress
Tomato	CBF1	reduction in the ability to withstand cold stress
Tomato	BZR1	lower heat stress tolerance

Table 9. Resistance to biotic stresses in crops using CRISPR technology

Crop species	Target genes	Target crops
Apple	DIPM1, 2, 4	Resistance against fire blight disease
Citrus	LOB1 promoter	Resistance against citrus canker
Papaya	alEPIC8	Protection from Phytophthora palmivora
Cacao	NPR3	Ability to fend off Phytophthora tropicalis
Grape	WRKY52	Resistance against gray mold disease
Grape	MLO7	the ability to resist powdery mildew
Cucumber	eIF4E	Resistance to the viruses that cause cucumber vein yellowing, zucchini yellow mosaic, and papaya ring spot mosaic
Banana	ORF region of virus	Virus resistance against banana streak
Tomato	JAZ2	Resistance to the bacterial illness speck
Tomato	MAPK3	Susceptibility to gray mold disease
Tomato	MAPK3	Susceptibility to gray mold disease
Tomato	Solyc08g075770	Fusarium wilt disease susceptibility
Tomato	PMR4	the ability to resist powdery mildew
Tomato	MLO1	resistance against powdery mildew
Tomato	DMR6	Defeat of downy mildew resistance

4. Challenges in application of CRISPR-Cas9

Despite CRISPR-Cas9's enormous promise for genome editing, a number of key problems still need to be resolved, including off-target mutations, PAM dependency, the synthesis of gRNA, and the methods used to modify CRISPR-Cas9 constructs (Zhang et al., 2014). Although specifically designed Cas9 nickases and mutants that reduce non-specific DNA binding have been created to address this problem, they are still an inadequate solution. Our understanding of sgRNA binding, mismatch tolerance, and the resulting sgRNA design tools has advanced significantly, but it is still rather limited when it comes to off-target effect (Lino et al., 2018).

Applying genome editing to many fruit species is complicated by heterozygosity and polyploidy of the genome since many more copies of the genes must be modified to get the desired phenotype. Due to somatic mosaics of CRISPR-Cas9-induced mutations and limited editing efficiency, finding heritable mutations may be difficult. It is challenging to test new ideas and advance genome editing since many fruit trees have a protracted juvenile stage. Public hostility to GMOs suggests that transgene-free genome editing methods must be developed forcefully for fruit crops (Zhou et al., 2020).

Recombinant viruses with Cas9 endonuclease resistance could start to appear suddenly. These recombinant viruses can avoid CRISPR/Cas9 targeting because of the limited number of InDels targets for the sgRNA sites. The targeted genomic region containing the Cas9 endonuclease cannot be recognized by the sgRNA sites due to these alterations, despite the fact that they do not stop viral replication. Transgenic crops made with these viruses begin to lose their resistance to them as a result of unique viral variants produced by CRISPR-Cas9's genome-editing byproducts, which hasten viral development (Shahriar et al., 2021).

5. Future prospects of CRISPR-Cas9 in agriculture

The CRISPR-Cas9 system can be used to create multiplex gene knockouts, including as double, triple, and quadruple mutants, in some perennial species, including Populus and Eucalyptus. Furthermore, because perennial plants have a lengthy juvenile phase, eradicating the CRISPR construct by backcrossing requires time. Recently, two site-specific recombination methods were developed to eliminate T-DNA from the trans-gene locus in rice. This might do away with the need for backcrossing. Using protoplast transfection, a DNA-free genome editing method based on preassembled CRISPR-Cas9ribonucleoproteins was more recently established in Arabidopsis, tobacco, lettuce, and rice. This DNA-free genome editing method could be advantageous for perennial species of citrus and apples that have well-developed protoplast transfection and regeneration systems (Liu et al., 2016).

The CRISPR-Cas9-mediated genome editing technology has created a fresh potential for the quick generation of disease-resistant agricultural varieties by stacking disease-resistant (R) gene(s) or disrupting/deleting susceptibility (S) gene(s). Such agricultural plants should be regarded as non-GMO, nonetheless, in order for this technology to be promptly implemented and acknowledged at the field level. We think that the use of CRISPR-Cas9 technology to a range of crops will transform agriculture and usher in a second green revolution to ensure the food and nutritional security of the rapidly expanding populations of tropical countries (Haque et al., 2018).

In order to generate fruit and vegetable crops that are resistant to herbicides, biotic stress, and abiotic stress, CRISPR-Cas9 has been widely used. Two examples of new cold- and

drought-tolerant germplasms that can be produced using gene editing are CBF1 (C-repeat binding factor 1), which regulates cold tolerance in plants, and MAPK3 (MAPK3-dependent protein kinase 3), which participates in the drought stress response to safeguard tomato plant cell membranes from oxidative damage (Wan et al., 2021).

Conclusion

In the CRISPR Technology, CRISPR-Cas plays important role in the field of agriculture. It improves the crop production and help in understanding the functions of genes linked with traits. CRISPR-Cas9 system can cause of second green revolution in the field of agriculture.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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