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Review Article

ROLE OF CRISPR TO IMPROVE ABIOTIC STRESS TOLERANCE IN CROP PLANTS

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Abstract: *The study for genetic variation in plant genomes for a variety of crops, as well as developments of genome editing techniques, have made it possible to cultivate for about any desired trait. Zinc finger enzymes; have made strides in genome-editing. Molecular biologists can now more specifically target every gene using transcription activator-like effector nucleases and ZFNs. These methods, on the other hand, are expensive and time-consuming because they involve complex procedures. Referring to various genome editing techniques, CRISPR/Cas9 genetic modification is simple to construct and clone and the Cas9 could be used with different guide RNAs controlling different genes. Following solid evidence demonstrations using the main CRISPR-Cas9 unit in field crops, multiple updated Cas9 cassettes are often used in plant species to improve target precision and reduce off target cleavage. Nmcas9, Sacas9, as well as Stcas9 are a few examples. Furthermore, Cas9 enzymes are readily available from a variety of sources. Bacteria that had never been discovered before has found solutions available to improve specificity and efficacy of gene editing techniques. The choices are summarized in this analysis to plant's experiment to develop crops using CRISPR/Cas9 technology; the tolerance of biotic & abiotic stress may be improved. These strategies will lead to the growth of non-genetically engineered crops with the target phenotype, which will further improve yield capacity under biotic & abiotic stress environments.*

Keywords: CRISPR, Crops, Quantitative trait loci, biotic stress, TALEN, abiotic stress, ZFN

Introduction

In the present situation, the most critical problem facing humanity is ensuring food sustainability for an expanding world population. By 2050, the global population may have increased to ten billion people, necessitating a 60–100percent annual rise in global food demand. Extreme weather, limited agricultural land supply, and rising biotic & abiotic pressures, in addition to the raising population rate, are important constraints for food production. The development of technology that can aid in crop enhancement can help to boost output to some point. Physical, biochemical, & biological (T-DNA transposons) metagenomic methods have made significant contributions in understanding the function of genes & transposons. For the determination the biological processes that have been resulted from the improved crop populations during recent decades (Ma *et al*., 2016); the transgenic technologies have been used to learn about basic plant science as well as to improve crops for the past 3 decades. After all, whenever it relates to food crop plants, the implementation of transgenic organisms into target genes are non-specific, occasionally unstable, as well as a source of public consideration (Shimatani *et al*., 2017). Any use of genome editing technology of sequence-specific nucleases (SSNs) has shown accurate gene editing for both animal & plant environments over the last decades. Double stranded breaks in the target genes are caused by these SSNs. The Non-homologous end joining (NHEJ) and homology directed rearrangement pathways are used to repair double-strand breaks (DSBs), resulting in insertion or deletion and replacement mutations throughout the target region (Jinek *et al*., 2012). Genome editing techniques, in comparison to transgenic approaches, which result in spontaneous implantations or in many cases, random phenotypes, yield identified mutations, making them a useful tool in practical genomics as well as crop breeding. Genome modified crops have such a benefit over transgenic crops in that they 'carry' the target trait's edited DNA (Mali *et al*., 2013). When opposed to traditional genetically modified plants, such improved plants can also be used in development programs, as well as the resulting plants can be used immediately with fewer acceptability or consumption problems and fewer regulatory procedures (Waltz, 2018). The benefits and uses of 2nd generation genetic modification technologies like CRISPR/Cas9 as well as its variants over 1st generation genetic modification methods like meganucleases, ZFNs and transcription activator-like effectors nucleases.

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Genome editing throughout the new period of engineered enzymes

A pattern of DNA binding domains has been fused to a non-specific enzyme domain in genetic engineering. The intended gene can also be precisely cleaved by such fused nucleases, as well as the damage can be restored using NHEJ and Homology directed repair (HDR), thus the name "genome editing" (Gaj *et al*., 2013). The genetic engineering techniques such as meganucleases and Transcription activator-like effector nucleases (TALENs) require labour intensive as well as time-consuming techniques to achieve goal precision. Second-generation genetic modification methods, such as CRISPR or Cas9, necessitate simpler design and implementation methodologies, as well as being less expensive and time consuming. For even more than a decade, ZFNs have been extensively used for genetic modification in both animal & plant systems (Govindan and Ramalingam, 2016). Due to poor target accuracy, labour intensive nature, numerous off target cleavages, and a small number of possible active sites, ZFNs have been now less favored (Chen and Gao, 2013; Kim *et al*., 2018; Osakabe, 2015). TALENs are created by altering activator effector domain sequences for desired target tracking, then fusing them with the FokI enzyme to create a TALEN that can be used to change target genomes. Engineered TALENs, like ZFNs, recognize 18–20 base pairs sequences, with a 14–20 base pair spacer required for FokI dimerization (Ueta *et al*., 2017). Because of their duration, TALENs have a higher average target affinity than ZFNs. TALENs, on the other hand, are difficult to plan and assemble due to the necessity of a T base only at the starting point, as well as their large scale and repetitiveness like in tobacco and brachypodium (Zhang *et al*., 2013: Shan *et al*., 2013). TALENs were used to modify the genomes of plants such as *Arabidopsis thaliana* (Cermak *et al*., 2011), rice (Li *et al*., 2015).

CRISPR/Cas9

The CRISPR/Cas9 genetic modification method was first described in mammals in 2012, as well as its effectiveness in genetic modification has changed the world studies in plants and animals biology (Jinek *et al*., 2012). Unlike ZFNs & TALENs, CRISPR genetic engineering is simpler, requiring the development of a reference RNA of around 20 nucleotides that is comparable to target nucleotides in DNA stretch. CRISPR stands for "Clustered Regularly Interspaced Short Palindromic Repeats (Jansen *et al*., 2002). Relates to Random copies flanked with – anti DNA sequences which were first discovered in the *Escherichia coli* Intracisternal A particles (iap) genomes' downstream regions (Ishino *et al*., 1987). External DNA sequences originating from plasmid DNA & phages were shown to be structurally similar to any of these – anti sequencing. Following that, the process of template cleavage was investigated for genetic modification, as well as CRISPR/Cas9 cleavage new technologies 'arrived' as just a successful genome editing method (Malony *et al*., 2005; Li *et al*., 2018).

The CRISPR cleaved process requires a synthetic RNA template of 20 nucleotides that binds to the DNA Template and (ii) the RNA-guided nuclease enzyme, which cleaves three or four bases just after the nucleotide binding motif (Jinek *et al*., 2012). Since its inception, the CRISPR cleavage technique has been widely used in genome editing in a variety of species. Between 2010 well as 2018, approximately 5000 papers documenting the use of CRISPR were written. The measures for executing a CRISPR program are as follows: (i) identifying the PAM segment in the host genome, (ii) analyzing a single gRNA, (iii) copying the sgRNA it into compatible binary vector, (iv) insertion into host species or cell lines transformations, (v) sampling, and (vi) testing of edited segments. Even a limited lab with such a basic genetic manipulation set up will carry out genetic modification trials due to the easy steps used in CRISPR/Cas9 facilitated genome editing. CRISPR/Cas9 methods were used to alter plant sequences more widely in recent years. ZFNs or TALENs are really a reflection of ZFNs or TALENs. It is easy to use. After all, in plants, the majority of the editing has already taken place. *Arabidopsis*, wheat, and other model species have all shown this. Only tobacco or a few crop varieties have been studied using CRISPR technologies (Jiang *et al*., 2013). The development of substantial off-target cleaved areas as a result of complex formation of a gRNA to misaligned comparable target DNA inside the gene is among the large shortcomings of the CRISPR/Cas9 technique. As a result, so many Cas9 enzymatic modifications have also been intended to enhance selectivity and decrease off target cleavage. A further strategy for reducing off-target cleavages is to extend the size of the nucleotide binding motif. Cas9 enzymes from various bacterial species would have unique as well as enlarged protospacer adjacent motif (PAM) segments, which could help increase on-target selectivity. The Nmecas9 CRISPR system, which was extracted from *N. meningitidis*, identifies an 8-mer PAM pattern (fifty-NNNNGATT), which can enhance target specificity as well as start reducing off target cleavage (Lee *et al*., 2016). 2 Cas9 casettes (st1cas9 and st2cas9) were collected from *S. thermophilus*. PRKDC as well as CARD11 are 2 human loci for which the st3cas9 was used to

edit. Compared to the prior years, there were fewer off-target levels for which SpCas9 was established (Pauwels *et al*., 2016; Hu *et al*., 2018) and used 50-NGA PAM to effectively edit the genome. Finally, to increase target accuracy, multiple CRISPR/Cas9 homologues have been discovered (Zetsche *et al*., 2017). CpfI, unlike Cas9, cleaves with such a single RNA directed (crRNA) complex or creates cohesive endings with 4–5 nucleotide fifty-overhangs. The CRISPR/CpfI technique has been extensively used for both animals and plant systems, with few to no off--target effects. In addition to Cpf1, approximately 53 certain CRISPR/Cas9 targets have also been identified, including the C2c2 enzyme by *Leptotrichia shahii*. It has dual enzyme activity and therefore can target single-strand RNA (Zhang *et al*., 2017).

CRISPR for genetic modification in crops

CRISPR has played an important role for genetic modification in crops through using Cas9 as well as gRNA is expressed in plant systems using plant-specific reverse transcriptase 3 promoters [AtU6 (*Arabidopsis*); TaU6 (wheat); OsU6 and OsU3 (rice). Add gene is a non-profit, global recombinant registry that can currently provide more than thirty empty gRNA Back-bones in binary vector2. The plant RNA polymerase III promoters as well as gRNA scaffold on the hollow gRNA back-bones can be used to implant the gRNA of importance.

Use of CRISPR to improve crop production

So far, the CRISPR or Cas9 gene editing system has been used in approximately twenty crop species (Ricroch *et al*., 2017) for a variety of traits such as yield enhancement and biotic as well as abiotic stress control. Several of the published papers are called solid evidence experiments since they explain the use of the CRISPR/Cas9 method to take out particular genes involved in abiotic as well as biotic stress tolerance mechanisms. Microbial communities cause biotic stress, which poses serious challenges throughout the growth of disease-resistant plants, accounting for more than 60% of possible yield reductions and 70-75% of global food output decreases (Ran *et al*., 2015; Ren *et al*., 2016). Crop stress resistance and immunity to large abiotic stresses including drought conditions have also been improved using CRISPR/Cas9-based genetic modifications. The following is a summary and use of CRISPR for genetic modification in multiple crop species.

Monocots (Rice)

Rice (*Oryza sativa* L.) is an important staple food crop for even more than 50 percent of the world's population. It is well-known among the global population and, owing to its limited genome size, it is well-known among scientists. Monocots have been analyzed and used as a sample crop. Several experiments have recently demonstrated the use of CRISPR-based genetic modification in rice, and a few experiments have documented the use of genome editing to increase herbicide resistance for rice crops production. The rice gene has a lot of possible PAM locations (one in ten base pair) (Osakabe and Osakabe, 2015; Xie and Yang, 2013). In the coming years, CRISPR technologies can also be used to explore any feature of concern in the genome sequence (Shan *et al*., 2013) demonstrated sequence-specific. For first time in every crops cell, CRISPR/Cas9 induced genomic alteration of 3 rice proteins, (OsPDS), and (OsBADH2), or mitogen-activated related protein (OsMPK2), and is used to monitor response to different abiotic stress signals also using protoplast & particle deluged rice calli structures. For OsPDS and OsBADH2, edit levels of approximately 9% and 7%, accordingly, were found (Xie and Yang, 2013). There have been developed 2 rice-specific genetic modification vectors, pRGE3 and pRGE6, to illustrate an RNA-guided genetic engineering method. Rice explants were used to assess a negative controller of biotic as well as abiotic stresses that was chosen for genetic manipulation using 3 gRNAs. A much more detailed gRNA architecture methodology led to a low level of the off target.

For a set of genes, include OsDERF1, OsEPSPS, and OsMYB5, the productivity of the CRISPR/Cas9 mechanism in causing targeted mutations and heredity in mutated rice varieties was assessed (Zhang *et al*., 2014).). A broad range of mutation frequencies (21-66%) was observed in M_0 generation different genes of no or one base pairs off-target mutations. In the T_2 generation, homozygosity mutants accounted for up to 11% of all mutations. This activation induced nucleoside deaminase target method allowed for targeted bp editing of a herbicidal gene C287 of rice (Shimatani *et al*., 2017). Base edit without the use for DSBs was achieved using dCas9 fusion with C deaminase (Zong *et al*., 2017). Li *et al*. (2017) used the BE3 bases editor for base edit the rice OsPDS & OsSBEIIb genes. BE3 editor is a new genetic modification technology that mixes nicked and unnicked DNA (Table 1).

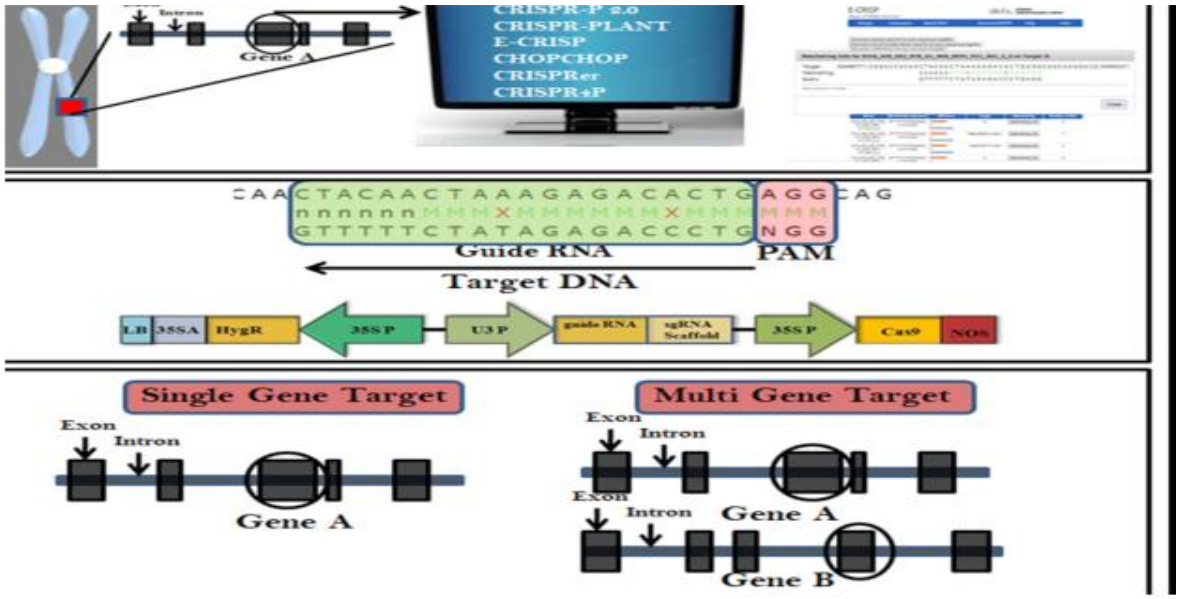

Figure 1 | Since 2005 through 2018, analysis & review papers on TALEN, & CRISPR were written. (A) The search terms ZFN, TALEN, or CRISPR were used also are using web of scientific web page throughout the title. Information was analyzed over two different time periods: 2010–2014, and 2015–2018.

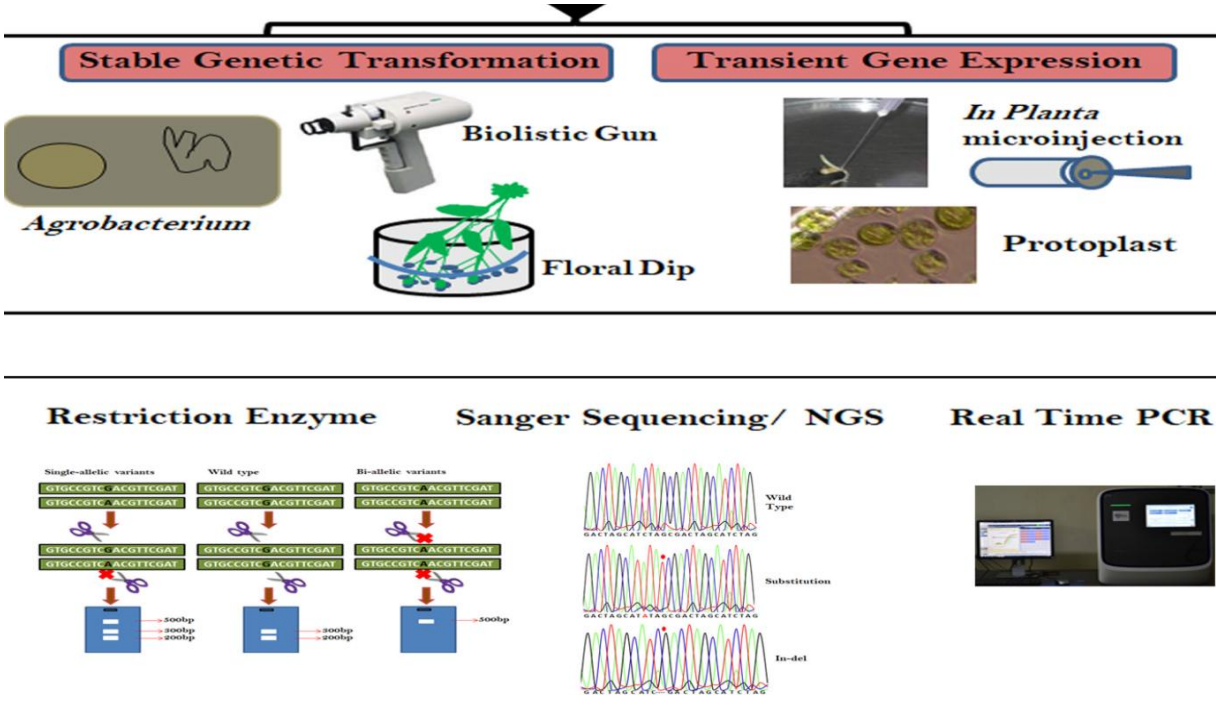

Figure 2 | A flow map depicting the processes required in genome editing using CRISPR/Cas9. **Initial step** covers gene sorting & gRNA design, while **Step 2** covers cloning that gRNA into a binary vector. **Step 3** displays the single & multiplex editing options. **Step 4** illustrates trans-formation method, **Step 5** describes edited crops screening techniques, and **Step 6** shows how to evaluate and pick the desired transgene plant for both the target trait. The efficient application of base pairs edit in rice was illustrated in this research. CRISPR/Cas9 allows for multiplex genetic modification with an almost infinite specific gene (Yang *et al*., 2017).) and was shown in rice and *Arabidopsis* recently (Zhang *et al*., 2018). For each plant modification in rice, single binary vector was used to

accurately edit 8 agronomic genomes. The proteins were ligated with intermediary vectors using isocaudamer procedure. The study found that a cascade of RNAs had no effect on the genetic variation of CRISPR/Cas9, and that a cascade of sgRNAs had no effect on the mutation of CRISPR/Cas9 (Xu *et al*., 2015; Watanabe *et al*., 2018).

Wheat

Wheat is an important cereal grain; grown as a staple food crop all over the world (Shan *et al*., 2014). The CRISPR/TaMLO deletion has also been shown to impart tolerance to *Blumeria graminis*. Tritici downy mildew disorder (Btg).4 of the 7.2 T_0 knock MLO grain homoeolog transgenic lines tested for restriction enzyme utilizing T7 endonuclease was found to have the restriction enzyme sequence edited (Wang *et al*., 2014). The numbers of transgenic lines produced can be improved or increased using build distribution methods. SSNs & gRNA are usually delivered using T-DNA-based guidance systems. However, genome amplicons tend to improve gene targeting efficiency (Gil-Humanes *et al*., 2017) where have used grain dwarf-virus (WDV)-based Genome replicons of transient or straightforward production of Cas9 cassettes, culminating in a twelve-fold rise in ancestral ubiquitin expression of genes in hexaploid wheat. In the future, elevated gene targeting with WDV-based Genome replicons may be a viable approach for complex genome editing (Kim *et al*., 2018).

The CRISPR/Cas9 genetic modification method in wheat explants to edit two biotic stress genes, wheat drought sensitive element protein complex 2 (TaDREB2) & wheat ethylene sensitive factor 3 (WER3). The T7 ribonuclease assay reported that nearly 70% of the total of protoplasts was successfully transduced, while expression of modified genes was confirmed. Transgene incorporation including offtarget mutations were major issues with the use of CMGE of crops (Karkute *et al*., 2017) showed an effective process of genetic modification using the transient expression delivery mechanism of CRISPR/Cas9 ribo-nucleoproteins to solve these problems (RNPs). In wheat varieties, the CRISPR/Cas9 and RNP complex was used to edit two separate genes (TaGW2 & TaGASR7) in 2 distinct varietal contexts. Off-target results are greatly reduced as this complexity was degraded in vivo, but no off-target results were observed in the bread wheat mutated species. An expanded RNP distribution protocol has also been made accessible (Ron *et al*., 2014). Multi-plexed genetic modification using CRISPR/Cas9 was shown to edit many essential agronomic variants simultaneously in model plants (Wang *et al*., 2015).

TABLE 1 | Use of CRISPR-based genetic modification in plants to improve biotic, abiotic, & nutritional traits

Crop Plants	Target Gene	References
Rice	OsERF922 (ethylene-responsive factor)	Wang *et al*., 2016
Rice (IR24)	OsSWEET13	Zhang *et al*., 2017
Tomato	SlMAPK3	Wang *et al*., 2015
Bread wheat	TaMLO-A1, TaMLO-B1, andTaMLOD1	Wang *et al*., 2014
Tomato	Rin	Ito *et al*., 2015
Maize	ARGOS8	Shi *et al*., 2017
Soybean	GmPDS11 & GmPDS18	Du *et al*., 2016
Potato	ALS1	Butler *et al*., 2016
Wheat	TaVIT2	Cai *et al*., 2018

Maize

Maize is an important cereal grain, with phytic acid accounting for more than a quarter of the plant. It is thought to be nutritional and an environmental contaminant since it is not absorbed by animals (Liang *et al*., 2014). In *Z. mays*, researchers have confirmed knocking out genes associated with phytic acid synthesiss (ZmIPK1A and ZmMRP4) (Endo *et al*., 2016) and used the maize U6 snRNA promoters to edit the (PSY1). PSY1 is active in the biosynthetic pathway of carotenoids, and its allele (psy1) causes white seeds or albino seeds. 7 M0 lines were confirmed to bear the psy1 cutoff trait between fifty two M0 benefits by *Agrobacterium*-mediated mutation, but all 7 lines were deep transcribed to recognize the requirement of variance and to assess the mutation quality. No in sites were modified, and healthy psy1 variants were collected (Feng *et al*. (2016) and have demonstrated the efficacy of the CRISPR/Cas9 method in maize by accessing the albino marker-gene using an explants system. The sg-RNA was engineered to reach a region in the eight exons of Zmzb7 and the maize U3 promoters had been used for expression, resulting in albino plants. Two lines have been shown to have 31% mutation efficiency after *Agrobacterium*-mediated

transformation of maize embryo. Other than model plants, the CRISPR/Cas9 genetic modification technique has been used to improve important traits in many other monocot crops (Kapusi *et al*., 2017). The (ENGase) gene was knocked out during barley using CRISPR/Cas9. ENGase was knocked out using particles disruption or *Agrobacterium*-mediated conversion with a set of 5 gRNAs. The mutational output of T0 to T1 mutant barley line was found to be 78%. In functional genes, such knockout crops would be important for analyzing gene expression (Kaur *et al*., 2018). CRISPR/Cas9 modification was shown in banana cultivars for RAS-PDS gene, which plays a significant role in the biosynthesis of carotenoids. The use of CRISPR to knock out RAS-PDS in bananas resulted in thirteen mutants that were tested for carotenoid & chlorophyll content.

Dicots (*Arabidopsis*)

Arabidopsis to show recombinant target genetic modifications for the very first time 3 *Arabidopsis* genes linked to phenology (Feng *et al*. (2013). The genes for (BRI1) AND (JAZ1), & (GAI) were edited then genotyped using the floral dip process. Further sampling validated the genetic recombination efficiency (26–84%) (Pan *et al.,* 2016) and used CRISPR/Cas9 genetic modifications in *Arabidopsis* to modify albinism-related genomes, (CHLI1) & CHLI2, with mutated plants screened using Increased Fragment Length-Polymorphism. They showed how crucial the modern genome editing technology is for gene correction and elimination of huge genomic segments in plant genomes. CRISPR/Cas9 genetic modification was used to investigate the effectiveness, heredity, accuracy, and sequence of transformed genes (Feng *et al.,* 2014)

Cotton

Cotton seeds have substantial oil stocks, making it a good choice for biofuels in comparison to becoming a fiber crop (Fang *et al.,* 2016; Ji *et al*., 2015). The gene encoding of *G. hirsutum* has been published (Li *et al*., 2015). CRISPR tools can now be used to make accurate DNA changes (Janga *et al*., 2017). The CRISPR/Cas9 method was used to perform selective genetic modification in cotton for the first time. Three reference sites throughout the GFP sequence were used as a keeping quality for activatory in green fluorescent protein (GFP) integrated in genetically modified cotton. Seven of around nine M0 plantlets tested for gRNA2 knockouts showed homozygosis changes, while another nine demonstrated biallelic indels markers (Hayut *et al.,* 2017; Janga *et al*., 2017).

Soybean

Soybean other name (*Glycine max* L.) is a major seed oil plant with a higher protein content in the seeds. The seeds also include a number of biologically active compounds (Cai *et al*., 2015). To effectively accomplish recombinant genetic modifications in soybean, the researchers used a simple sgRNA for a gene of interest (bar) or six sgRNAs that targeted separate sites of two nominal soybean genes (GmFEI2 & GmSHR), and then tested the effectiveness of the sgRNAs in such hairy plant roots. Small deletions and insertions resulted from mutagenesis of specific gene locations in soybean chromosome four (DD20 or DD43) (Li *et al*., 2015). At the callus point, specific PCR analysis identified targeted gene compatibility by HDR. In comparison to *Arabidopsis* AtU6 to 26 promoters, the soybean GmU6—16-1 promoters was thought to be highly effective in simultaneously editing several homoeoalleles (Du *et al*., 2016).

Tomato

Because of availability of effective transformation methods, practical genomic analysis, and extensive history on quality assurance, tomato (*Solanum lycopersicum* L.) is an excellent candidate for studying CRISPR/Cas9 genetic modification (Pan *et al*., 2016: Brooks *et al*., 2014; Tang *et al*., 2016). SlAGO7, the tomato ARGONAUTE gene, were discovered to have effective CMGE that can be easily distinguished phenotypically because mutants formed first leaflet without petioles. The SHORT-ROOT gene was knocked out of tomatoes' hairy roots using CRISPR, indicating that gene activity is conserved between *Arabidopsis* & tomato. SHR also controls transcription of the transcriptional gene (SCR) as well as root length (Ron *et al*., 2014). One of most important concepts in the field of fleshy fruits is ripening regulation.

Potato

Potato is a staple food for global food production, although with global warming, it's critical to breed potatoes to adapt and also find breeding materials that could be used to expand the area where they are grown. Through using CMGE to mutate the (GBSS) gene in potato, the waxy genetic diversity was established. Classification of starch in genetic code lines showed only the existence of amylopectin and no amylose, suggesting that all four (GBSS) alleles were knocked out (Andersson *et al*., 2017). ACETOLACTATE SYNTHASE one has also been mutated in potatoes to achieve multiallelic mutants StALS1 (Bertier *et al*., 2018; Butler *et al*., 2016).

Conclusion

Selective breeding technologies require scientists to inject desired traits more accurately and rapidly than traditional breeding. CRISPR/Cas9-based genetic modification is a great technology. In the future, crops enhancement using genome editing techniques

to increase yield, nutrient content, disease tolerance, as well as other traits would be a major focus. It has been used extensively in many different plants for practical research, countering biotic & abiotic stresses, and improving other essential agronomic ally important traits in the last five years. While some changes to this technology are needed to increase on-target performance, the majority of the work done so far is tentative and needs improvement. Nonetheless, CRISPR/Cas9-based genetic engineering will grow in popularity or become a necessary technique for obtaining 'suitably edited' crops that will aid in achieving the zero hunger target and feeding the world's growing population.

Conflict of interest

The authors declared absence of conflict of interest.

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