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ADVANCEMENT AND APPLICATIONS OF GENE EDITING TOOLS FOR CROP IMPROVEMENTS: The Contemporary

[*1,2]Zakari, S. A., [3] Nuhu, A. A., [4]Aliyu, K. T. and [5] Tadda, S. A.

[1]Institute of Crop Sciences, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China.
[2]Department of Crop Production, Audu Bako College of Agriculture, Dambatta, Kano, Nigeria
[3]Department of Biological Sciences, Faculty of Sciences, Yusuf Maitama Sule University, Kano,
[4]International Institute for Tropical Agriculture (IITA), Kano, Nigeria
[5]Department of Crop Production and Protection, Federal University Dutsin-Ma, Katsina, Nigeria

*Corresponding Authors' email: shamsuado@zju.edu.cn, +2348034265669

ABSTRACT
Advancements in gene editing tools have transformed the fields of the agricultural system and crop improvement; thus, editing tools have been adopted rapidly in the research community. The deletion "Insertion" of DNA may be described, more precisely, as gene editing. Zinc-finger nuclease (ZFN) was the first technique used to cut DNA, later, ZFN+TALEN (transcription activator-like effectors nucleases) uses to target specific DNA, but the practical problem became an issue with this technology. A new advanced tool, "clustered regularly interspaced short palindromic repeats" (CRISPR) solved that problem, with the requirement of nuclease called CAS9 and a piece of ribonucleic acid (RNA). Crop production is facing many challenges such as climate change, salinity, drought, low grain quality, yield, post-harvest loss, low nutrient use efficiency, pest and diseases among others, hence achieving sustainable and secure crop products became a complex network requiring a multi-faceted solution. Recognised means to address these issues is to develop tolerant and high yield varieties using conventional and modern breeding techniques through genetic engineering by introducing desirable traits in crops. Despite many attempts using different strategies for crop improvements, the achievements so far are quite modest. Gene editing tools can be used to enhance crop improvement by allowing the prologue of precise and predictable changes directly in an elite milieu and the CRISPR system is constructive for multiple traits and simultaneous modification. This review paper aims to discuss the most recent advance of gene editing technique and its application in crop improvement as well as challenges that hinder its practical relevance.

Keywords: Advancement: CRISPR: Crop improvement: Gene editing technology: ZFN+TALEN.

INTRODUCTION

In today's world, global food security is one of the major problems that draws the attention of the scientific community, as it concerns climate change, a rapid increase in population, and environmental adversities. Almost one billion people suffer from hunger and malnutrition, while at the same time, the loss of biodiversity is disturbing the agricultural systems. By 2050 the global population will exceed nine (9) billion; present-day agriculture is requiring crops with higher yields and of improved quality with fewer inputs and utilisation of available resources (Foley *et al.*, 2011). Improving agricultural productivity is essential for safeguarding food adequacy, consequently developing new cultivars with superior yield attributes: disease resistant, drought and salinity tolerance, high nutrients use efficiency, high nutritional properties, yield and quality are crucial to boost crop productivity. In the past years, conventional breeding such as hybridisation and transformation means are the most widely used approaches in crop improvement and has played a significant role in improving and increasing crop productivity (Wei *et al.*, 2017). However, it is labor demanding and generally takes several years to progress from the early stages of screening genotypes and phenotypes to the first crosses. Additionally, the systematic declination in natural genetic diversity in crop plants has tremendously affected crop production (Tilman *et al.*, 2011; Govindaraj *et al.*, 2015). In the last era, the use of genetically modified (GM) or transgenic crops from molecular breeding technology has upheld great assurance in overcoming the problems of conventional breeding approach and has reinforced the agricultural productivity (Moose *et al.*, 2008; Mishra and Zhao,

2018). Nevertheless, it is still time-consuming and labour-intensive even in advanced countries, unrelatedly to developing countries. Furthermore, GM crops dilemma arose and became a great challenge to researchers as well as commercial crop producers due to social, religious, environmental, and health issues owing to public concerns (Zhang *et al.,* 2018; Lusser *et al.,* 2012).

In recent times, a novel technology "genome editing" has transpired and make usage of sequence-specific nucleases (SSNs) to insert targeted mutations in crops with high precision and efficiency (Mishra and Zhao, 2018). Genome editing has been successful in sweeping away the limitations and constraints of the conventional breeding approach (Georges and Ray 2017; Sovová *et al.,* 2017). Zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) are the preciously engineered sequence-specific nucleases that have proven to be highly efficient in targeted mutagenesis in a wide range of model crop plants (Zhou *et al.,* 2015; Steinert *et al.,* 2016; Kannan *et al.,* 2018). The artificially engineered SSNs can generate a double-stranded break (DSBs) within the target region of the DNA which is successively repaired by cell's homologous recombination (HR) as natural repair mechanism or by non-homologous end joining (NHEJ). In HR pathway, the repair mechanism is more defined where a donor DNA enclosing sequences homologous to those flanking the DSBs site leading to foreign gene cassette knock-in or gene replacement as intended but in NHEJ pathway, it is basically error-prone as it makes random mutations leading to frameshifts and target gene knockout (Baltes *et al.,* 2015; Brookhouser *et al.,* 2017).

For understanding gene functions, gene loss-of-function and knock-out mutations play indispensable roles in crop improvement, but their applications are still limited as many of the critical traits are conferred by the random point mutation or insertion/indels of a new gene (Lawrenson *et al.,* 2015; Steinert *et al.,* 2016). Even with recent development in gene technology, the use of gene targeting or gene replacement via HR is very minimal in comparison to NHEJ because the latter is less precise than earlier, and the frequency of illegitimate recombination is higher in NHEJ in comparison to HR. However, a new tool, C RISPR from Prevotella and Francisella1 (CRISPR Cpf1) is identified for efficient genome editing, with higher specificity, efficiency and potentially broader applications than CRISPR–Cas9 (Zaidi *et al.,* 2017). Recently, base editing has emerged as a cutting-edge approach that allows conversion of G–C base pairs to A–C base pairs without the need of a DSB/donor template (Komor *et al.,* 2016; Shimatani *et al.,* 2017). These advances in the research genome editing tool can aid in creating novel traits with high efficiency and precision; thus, enhances crop improvement. Several articles on gene editing tools and their applications in biotechnology, medical, and agricultural-related fields have been reviews (Carroll, 2016; Song *et al.,* 2016; Arora and Narula, 2017). In this review, we discussed a brief overview of the advances in the application of gene editing tools for crop improvement including the most recent studies and also addressed the challenges and future prospects of the gene-editing tools concerning crop plant improvement.

Antiquity of gene editing

Theoretically, a gene is the basic physical and functional unit of heredity in all living organisms and it is composed of deoxyribonucleic acid (DNA). To conserve the integrity of the genetic information, DNA must be duplicated with high accuracy and reduce errors that introduce changes to the DNA sequence. A genome contains the full complement of DNA within a cell and is organized into smaller, discrete units called genes that are arranged on chromosomes and plasmids. Any desired DNA sequence can be made in a laboratory using a computer and other research tools. Creating a DNA sequence in a laboratory is uncomplicated while replacing the unwanted DNA and inserting it into a cell is an entirely different system (Hsu *et al.,* 2014). The deletion "Insertion" of DNA may be described more precisely as gene editing. The major problem with gene editing is taking a long period and challenging to practice; the best practices have involved designing proteins in the laboratory that can only edit one segment of DNA at a time. Twenty-five years ago, a technique called zinc-finger nuclease (ZFN) was developed, whereby a specific portion of DNA would then be edited by targeted protein nuclease in the laboratory (Ng *et al.,* 2017). However, construction of new nuclease whenever the need to investigate a different portion of DNA became an expensive process, time-consuming and only

suitable for one genetic modification at a time, making it challenging to study trait that has more than one genetic marker (Carroll, 2011). Eleven years later, the new nuclease technique 'transcription activator-like effectors nucleases' (TALEN) that were easier than ZFNs was developed (Gaj *et al.,* 2013). These nucleases are more accessible, designed for a specific DNA target but their colossal size presented practical problems in the laboratory. Due to this, it became difficult to study any models involving more than one genetic marker at a single instant.

Three years later, scientists showed that a method used in bacteria to inoculate themselves from viruses could also be used as a gene-editing technique in humans using clustered regularly interspaced short palindromic repeats (CRISPR) (Brookhouser, Raman, Potts, & Brafman, 2017). With CRISPR, many models are easier to use and can be used for more than one portion of DNA at a time (Gaj *et al.,* 2013)**.** To determine the actual function of the gene, the gene should br study and observed the changes occur in the organism; generally, it takes decades to adequately remove or "silence" a gene but using the CRISPR method, a gene can be silenced within weeks.

Additionally, CRISPR enables guidance on where the DNA is inserted, instead of just inserting it randomly into the cell. CRISPR technology requires a nuclease called CAS9 and a piece of ribonucleic acid (RNA) that is similar to DNA (Hill *et al.,* 2018). In contrast to prior methods for gene editing where the same nuclease can be used to control any targeted DNA for CRISPR/CAS9 system, the RNA segment tells where to edit the DNA. Apart from DNA deletion, it can also guide the cell's DNA repair mechanisms to the precise location for inserting the edited DNA. ZFNs and TALENs also use the cell's repair mechanisms to guide DNA but CRISPR is much easier to work with, and importantly, several genes can be deleted, and insertion is a matter of weeks rather than years.

The early research with CRISPR centered on Cas9 (protein 9), which is the RNA-guided DNA endonuclease originally segregated from *Streptococcus pyogenes,* that target the GC fertile regions of a genome (Yin *et al.,* 2017). Although this was an essential component in the whole genome editing technology, it had a specific share of limitations such as the ability to target the AT-rich regions of the genome and having adjacent protospacer motif (PAM) limitations. With intense efforts in research, a new CRISPR effector: named CRISPR from Prevotella and Francisella 1 (Cpf1) was isolated for the simplicity of editing the genome (Zetsche *et al.,* 2017). The Cpf1 is the standard prime of nuclease in genome editing because it has an AT-rich PAM which can target the AT-rich regions of the genome, and it does not have an HNH domain but preferably three RuvC domains. These original features distinguished it from Cas9. Besides, Cpf1 can process its crRNAs while multiple proteins are required during the formation of crRNAs in other tools, which makes it adaptable and flexible. Furthermore, Cas9 requires a tracrRNA to pair with the crRNA to make it functional, but a single crRNA is sufficient to make Cpf1 functional. Hence, cloning or synthesizing this set up is the prospect of a shorter scaffold for designing the single guide RNA. Also, Cpf1 creates 4-5 nucleotide long, sticky ends while Cas9 introduces a blunt-ended double-stranded break. This is highly valuable for precise insertion for fragments of nucleotides during the DNA repair mechanisms of Non-Homologous End Joining (NHEJ), or Homology Directed Repair. To this end, with Cpf1 new edits or cuts can be made during every round of interaction, whereas in Cas9, the DSB cur most be near the PAM region. Expansion of the "enhanced specificity" SpCas9 (eSpCas9) via structure-guided protein engineering which has revealed a vivid decrease in off-target indel (insertions-deletions) formation thus, contributed towards a substantial advancement over the *Streptococcus pyogenes* Cas9 (SpCas9) enzyme (Tuhin *et al.,* 2017).

Insight Genome Editing with Site-Specific Nucleases

Attaining different genome modifications depends on the repair pathway and the availability of a repair template (Ainley *et al.,* 2017). Dual -strand breaks induced by a nuclease at a specific site can be edited either by homologous recombination (HR) or non-homologous end-joining (NHEJ). From fig. 1 (1A) gene-edited by NHEJ typically results in the deletion (red) or insertion (green) of random base pairs, instigating gene knockout via disruption. (1B) Showed donor DNA (when available) simultaneously cut by the same nuclease leaving compatible overhangs and gene insertion by NHEJ triumph. (Point C and D) demonstrates a donor DNA template subjugated to modify a gene by introducing precise nucleotide substitutions with HR to achieve gene insertion.

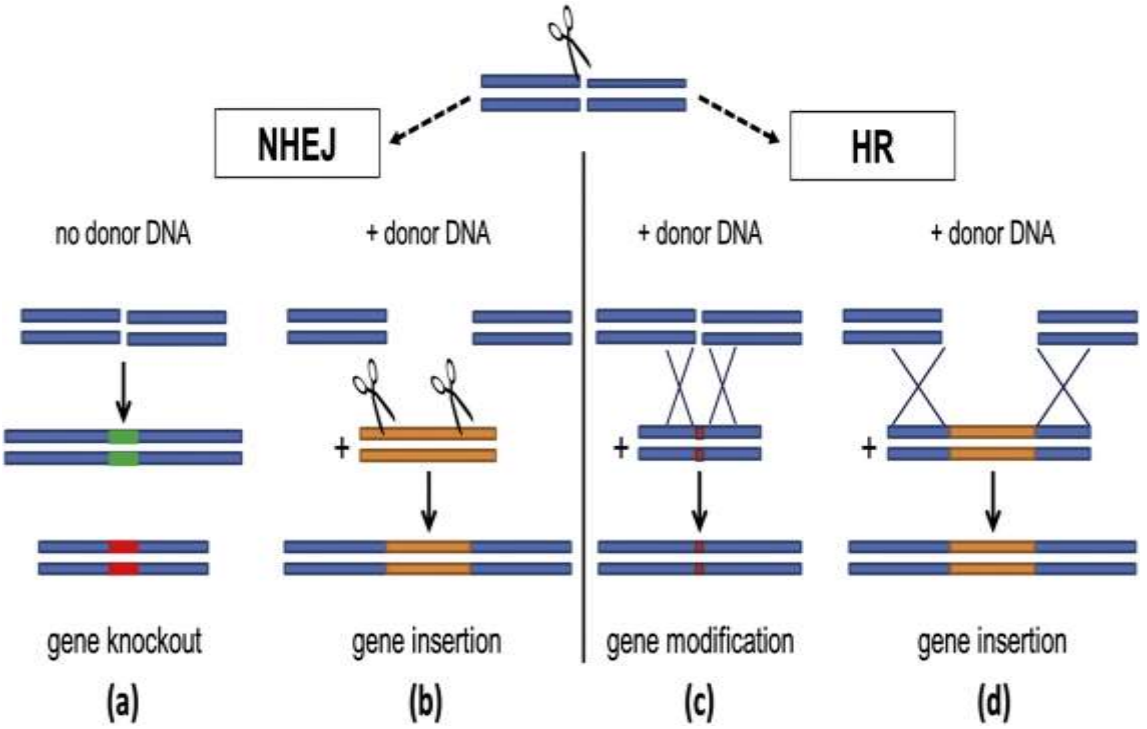

Fig. 1 Typical genome editing with site-specific nucleases. (Source: Maresca *et al.*, 2013)

Figure 2 explains the relevance of the CRISPR/Cas system beyond gene editing. CRISPR/Cas9 technology is suitable for other exciting applications, such as gene regulation, cargo delivery and RNA cleavage. From Fig. 2(a), the catalytically inactive dead Cas9 can be fused to either activator (right) or a transcriptional repressor (left) during gene regulation. When the dCas9-repressor fusion is conscripted by a coding sequence of an endogenous gene or gRNA that matches the promoter, 5′ untranslated region, it can block transcription initiation, elongation, or the binding of transcription factors. The specific expression of the endogenous gene is stimulated when the dCas9-activator fusion is targeted to a promoter. However, in Cargo delivery (Fig.2b), it explained that the catalytically inactive dCas9 could deliver diverse cargos to specific genomic locations as a programmable DNA-binding protein.

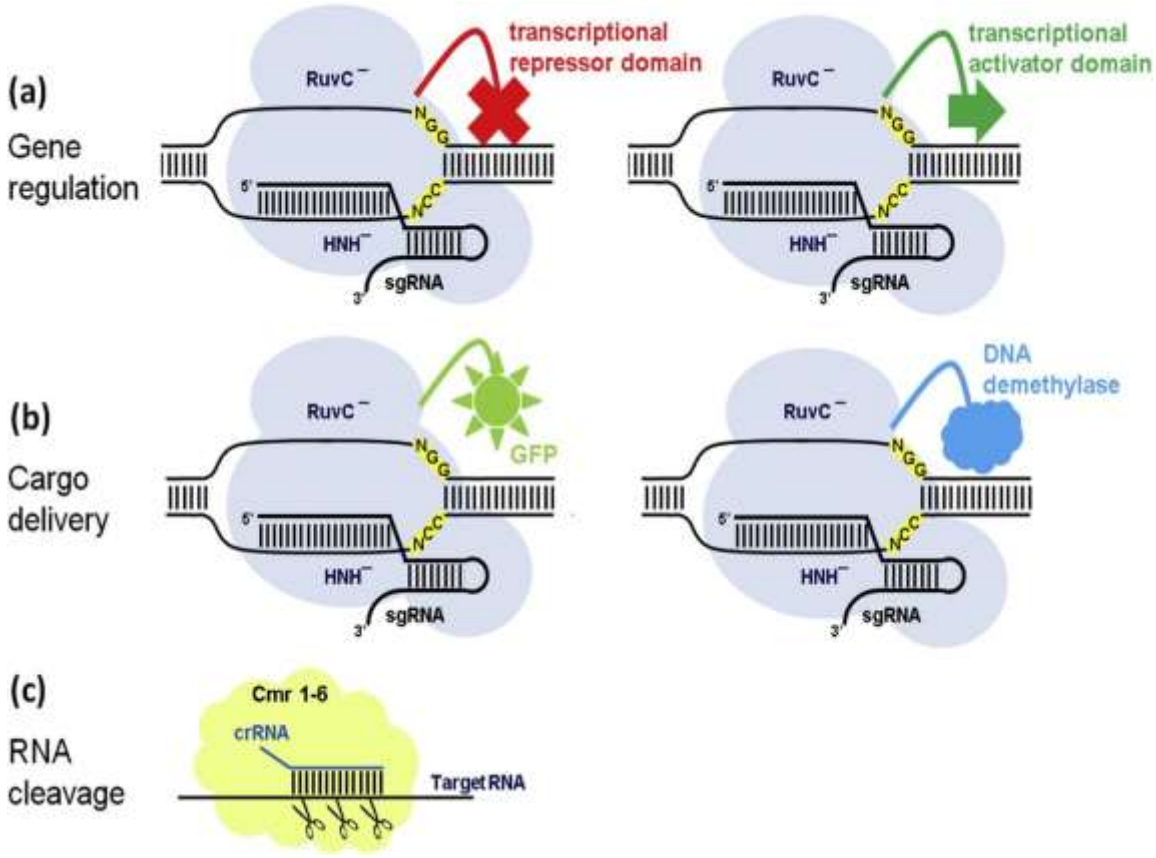

Fig. 2: Designed target sites of the specific gene for the Cpf1 and Cas9 systems. (Source: *Yin et al.*, 2017)

For instance, fusion with a demethylase (right) can be used for targeted epigenome editing while fusion with a green fluorescent protein (left) provides a tool for visualising chromosome structure/dynamics. Fig. 2C represents the RNA cleavage with a unique RNA silencing system and Type III-B CRISPR-Cas system that is composed of nucleases (from Pyrococcusfuriosus) and form the so-called Cmr complex (yellow). The Cmr-crRNA complex can degrade complementary RNA sequences cleaving them at multiple sites and targets invading RNA in a PAM-independent process.

Using both nucleases, Cas9 and Cpf1 for targeted exon-1 of the specific gene allows robust genome editing in cell-lines and can target A/T-rich areas of the genome which resulted in the increase the number of locations that can be edited. The target sequence can be formed by annealing a pair of oligos that had compatible sticky ends to the digested backbone of the nucleases vector. The Cpf1 target sequence may be chosen very close to the Cas9 target sequence for a more accurate comparison (Fig. 3). In the case of mutually Cpf1 and Cas9 targets chosen, the cleavage site should be after the start codon (indicated by underlined in fig. 3). The result of this would be the disruption of the targeted gene.

Fig. 3: Three Functions of the CRISPR/Cas technique beyond genome editing.

The alignment of the target region of both Cpf1 and Cas9against other members of the other gene family will indicate the outcome result and similarity with the off-target effect. Perhaps, several studies (Hunt *et al.,* 2010: Yin *et al.,* 2017: Konermann *et al.,* 2018) did not find significant result by using both Cas9 and Cpf1, most likely the induced DNA double-strand break is not repaired by the NHEJ repair pathway and the effect by local micro-homologous DNA sequences in both nucleases are not collaborated. This opened a new phase of research for nucleases combination effect.

Gene Editing Application for Crops Improvement

Due to the potential contributions of genetic crop improvement, there is considerable excitement around the rapid emergence of gene-editing technology (Govindaraj *et al.,* 2015; Arora *et al.,* 2017). The conventional breeding approaches limit what can be attained with the availability of beneficial alleles in nature. Rice plant was the first staple crop used in the application of TALEN-mediated genome editing for crop improvement, the gene OsSWEET14 (for bacterial blight susceptibility) was disrupted and resulted in mutant rice resistant to bacterial blight (see Table 2 for more examples). The nutritional profiles of crops can be modified using TALENs: soybeans with low linoleic acid and high oleic acid contents were generated by disrupting fatty acid desaturase (FAD) genes, hence, improving heat stability and shelf life of soybean oil (Haun *et al.,* 2014; Demorest *et al.,* 2016). The concentration of reducing sugars in potato tubers during cold storage influences the quality of the product, also knocking out the vacuolar invertase (VInv) gene resulted in undetectable levels of problematic reducing sugars in the tubers (Clasen *et al.,* 2016). Crops flavour is essential with the aid of TALEN technology; fragrant rice has been yielded by disrupting the betaine aldehyde dehydrogenase (BADH2) gene (Shan *et al.,* 2015). Furthermore, crop improvement by TALEN-mediated gene insertion is well exemplified in tomato, an active promoter was inserted in the upstream gene controlling anthocyanin biosynthesis, and resulting in purple tomatoes with high anthocyanin levels while integrating TALENs and donor DNA into geminivirus replicons notably increased their copy number and thus the efficiency of homologous recombination enhanced (Čermák *et al.,* 2015). These examples revealed the immense potential of TALEN tool for crop improvement. However, the construction of TALE repeats endures a challenge and the efficiency of gene targeting with TALENs is inconstant. NHEJ-mediated gene knockouts are the most straightforward form of targeted modification that could be used to remove genes that negatively affect crop quality to confer susceptibility to pathogens or to divert metabolic flux away from valuable end-products. For example, Wang *et al.,* (2014) successfully knocked out all three MLOhomoeoalleles generating wheat plants resistant to powdery mildew disease by the used of both TALEN and CRISPR/Cas9 technologies to target the genes of the mildew-resistance locus (MLO). Also, several studies have successfully transformed plant species phenotype through knockout gene processes, these plants include cucumber, potato, poplar, tobacco, grapes and petunia (Fan *et al.,* 2015; Wang *et al.,* 2015; Gao *et al.,* 2015; Zhang *et al.,* 2016; Wang *et al.,*

2018). Precise nucleotide exchanges such as oligonucleotide donor sequences had been used to modify the regulatory sequences upstream of genes for improving crop yields and determine agricultural performance. For example, CRISPR/Cas9 was used to dislocate the coding region of *CsLOB1*, resulting in Duncan grapefruits that had no canker signs, and when the eukaryotic translation initiation factor 4E (eIF4E) gene was disrupted in cucumber, the plants were shown to be immune to an *Ipomovirus* (Cucumber Vein Yellowing Virus); the broad virus resistance was generated and were resistant to Papaya ringspot mosaic virus-W and the potyviruses Zucchini yellow mosaic virus and (Chandrasekaran *et al.*, 2016: Waltz, 2016: Jia *et al.*, 2017).

The insertion of the larger sequence by HR or NHEJ would not interfere with the activity of endogenous genes and could allow the introduction of transgenes at defined loci that promote high-level transcription (Farboud & Meyer, 2015). Site-specific nucleases also allow the addition of several genes in close vicinity to an accessible transgenic locus. This makes it feasible to present multiple traits into crops with a low risk of segregation or even conventional genetic engineering which is difficult to achieve by classical breeding (Ainley *et al.*, 2017), the entire array of transgenes can be assembled into other germplasm by crossing once stacking has been achieved since it acts as a single locus. In maize, the endogenous gene ZmIPK1 was disrupted by the insertion of PAT gene cassettes using ZFNs and this resulted in the advancement of herbicide tolerance and changes of the inositol phosphate (Ipa) profile of developing maize seeds (Shukla *et al.*, 2009). Additionally, ZFN-mediated targeted transgene integration was used to assemble several useful traits for greater potential crop improvement (Ainley *et al.*, 2017) and ZFNs used for gene integration in rice to identify safe regions that serve as reliable loci for further gene insertion and trait stacking (Cantos *et al.*, 2014). As a proven technology, the design of ZFNs remains a complicated, technically challenging process and often has low efficacy. It is possible to target integration via programmable nucleases joint with precise NHEJ or HR does not leave behind any footprints linked with the integration method; these aims using site-specific recombination such as loxP or attB sequences. Eight agronomic genes have been successfully edited using one binary vector for individual genetic transformation in rice through intermediate vector method ligated to those genes (Shen *et al.*, 2017). This study showed that a cascade of sgRNAs might not affect the mutation rate of CRISPR/Cas9 and reduced off-targets.

There are numerous ways to create transgene-free mutated plants using programmable nucleases involving the transient expression of the nuclease components using viral vectors or agro-infiltration, the delivery of the components directly as functional gRNA Cas9 protein or Cas9 transgenes on a separate chromosome to the targeted locus so that they can be removed by segregation and the incorporation of the gRNA. Although the specificity of the CRISPR/Cas9 technology is still indescribable in detail, the frequency of off-target mutations caused by physical and chemical mutagenesis techniques that are well below and it is already clear (Podevin *et al.*, 2013). Indeed, the random integration of transgenes can be address by the use of site-specific nucleases deletion of many regulatory burdens associated with transgenic plants and is one of the leading causes of concern and the resulting potential for unintended effects such as disrupting host metabolism and/or producing toxic or allergenic compounds (Podevin *et al.*, 2013). The potential to introduce transgenes at a specific and predetermined chromosomal position using site-specific nucleases should all but eliminate the risk of unpredictable events. Quite a lot of traits have been manipulated using the CRISPR/Cas9 tool. In many vegetables and fruits, the enzyme (Polyphenol oxidase: PPO) that causes browning, by knocking out the gene related to this enzyme, Waltz, (2016) industrialised a non-browning mushroom. CRISPR/Cas9-engineered mutations in tomato SELF-PRUNING 5G (SP5G) result in rapid flowering (Soyk *et al.*, 2017). Recently, a dual amino-acid promoter and swap substitutions were achieved at specific and generating glyphosate tolerance locus in cassava (Hummel *et al., 2018*). In addition to generating herbicide-resistant crops, CRISPR/ Cas9-mediated gene replacement and insertion methods have produced drought-resistant characteristics in maize (Shi *et al.*, 2017).

A new system for genome editing: CRISPR–Cpf1: Cpf1 enzymes have been shown to have minor rates of off-target activity related to Cas9 nucleases (Malnoy *et al.*, 2016). Several studies have reported this new system to be a useful DNA-free genome-editing tool for plant genome editing (Malnoy *et*

al., 2016; Jia *et al.,* 2017). These higher features make it a more suitable editing system in crop plants as match up to SpCas9. Most recently, Cpf1 system was used in rice to generated stable and heritable mutations by selecting two genome targets in the OsPDS and OsBEL genes, also for multiplexed gene editing by editing four OsBEL genes was successfully achieved (Wang *et al.,* 2016; Jia *et al.,* 2017). Scientists have successfully revealed that both LbCpf1 and FnCpf1 can generate indel mutations as well as precise gene insertions in the rice genome combined with the repairing template DNA and crRNA. This study clearly indicates the broad adoption of Cpf1 genome editing technology to make a significant impact on crop improvement. CRISPR–Cpf1 system has full applications in plant genome editing like functional screening based on transcriptional repression, transcriptional activation using dCpf1 fused with a transcription activator domain or gene knockouts using catalytically inactivated Cpf1 (dCpf1), epigenome editing with dCpf1 fused to epigenetic modifiers, and the tracking of cell lineages with DNA-barcoding techniques. These advanced functions will enhance the improvement of quality and yield of crops and help in attaining food security and sustainability.

Alteration in Polyploid Crops using Gene Editing

Polyploid crops are the primary food and fibre crops of the world, include potato, wheat, cotton, peanut, apple, citrus and brassica oilseeds: rape, canola and Camelina (Renny-Byfield & Wendel, 2014). The existence of triploids, tetraploids and hexaploids having three, four and six sets of chromosomes, respectively, present significant challenges to conventional plant breeding (Weeks, 2017). However, recent studies with gene editing techniques in several polyploid crops have shown easy editing of some genes targeted for change on homologous chromosomes (Wei *et al.,* 2017). These changes have allowed improvements in plant breeding procedures, disease resistance, seed oil composition, food nutrition, weed protection and food safety (Otto & Whitton, 2002). Such technology that creates precise mutations and leaves no transgene footprint holds potential promise for supporting the significant diminution of regulatory processes that currently inconvenience approvals of conventional transgenic crops. Recent studies provided ample evidence for successfully rapid and efficient modification of several polyploid plant species. Examples are presented in (Table 1) for both academic studies and potential commercial applications. Briefly, in the first report of successful use of the CRISPR/Cas9 gene-editing system in bread wheat (*Triticum aestivum*) cells, two genes, phytoene desaturase (PDS) and inositol oxygenase (inox) were targeted. In each case, efficient production of deletions and insertions were observed in wheat cell suspension cultures with sgRNA constructs, TALEN-induced mutation of all three TaMLO gene homoeologs could be achieved and confer heritable resistance to fungal disease and powdery mildew. Another advanced technique for use in wheat callus tissues was used on exogenous DNAs, and transiently RNAs present do not persist in mutagenized callus cells neither regenerated in wheat plants. Recently, extended the technology to hexaploid wheat used portions of dwarf virus of wheat as the base viral replicon for transmitting the Cas9/sgRNA gene-editing system; as a result, a 110-fold increase in expression of a reporter gene relative to a conventional was achieved. Furthermore, to target the fatty acid desaturase 2 (FAD2) genes for knockout system in so doing, synthesis of both linolenic acid and linoleic acid should be blocked, and oleic acid should increase as their precursor. The advantage of the FAD2 genes of Camelina is highly homologous to the FAD2 gene in the diploid for Arabidopsis thaliana. These were a critical first demonstration that a crop with a complex allohexaploid genome could be efficiently engineered using recently developed gene-editing techniques.

Gene Editing as a Novel Defence in the Array to Fight Plant Diseases

The utilisation of gene editing tools for the viral forbearance has been accomplished by targeting either the host susceptibility factors or the viral genome (Table 2). For example, nearly all of the CRISPR/Cas9 mediated viral resistance has been succeeded by marking the ssDNA of the *Geminiviruses* with bi-partite or mono genome encompassing the genes encoding proteins required for viral replication, movement and suppressor of host defence machinery. Moreover, the identification of specific ERFs as undesirable regulators of plant protection made them hypothetical targets for genome editing. In rice, targeting the OsERF922 gene using CRISPR/Cas9 technology showed resistance to blast disease. The wild-type was similar to the T2 mutant lines rice plants about several agronomic traits (Langner *et al.,* 2018).

Table 1: Application of gene editing tools for some successfully mutated polyploid crops

Crop	Edit Tool	Target Gene(s)	Trait	Citation
Wheat	Cas9/sgRNA	PDS and inox	Chlorophyll syn	Upadhyay *et al.,* (2013)
	Cas9/sgRNA	*TaGASR7*	Grain length and weight	Zhang *et al.,* (2016)
	Cas9/sgRNA RNPs	*TaGASR7*	Grain length and weight	Liang *et al.,* (2017)
Camelina	Cas9/sgRNA	FAD2	Seed oil composition (high oleic and low polyunsaturated FAs)	Jiang *et al.,* (2017); Morineau *et al.,* (2017)
Watermelon	Cas9/sgRNA	*ClPDS*	mosaic albino phenotype	Tian *et al.,* (2017)
Oilseed rape	ssODNs	ALS	Herbicide resistance	Zhang *et al.,* (2015)
	TALEN	Endogenous constit. promoter	Herbicide resistance	Sovová *et al.,* (2017)
	TALEN and geminivirus	ALS	Herbicide resistance	Butler *et al.,* (2016)
Potato	TALEN	Vacuolar invertase	No reducing sugars and improved food safety	Andersson *et al.,* (2017)
	TALEN and Cas9/sgRNA	Granule-bound starch synthase	Altered starch composition	Nicolia *et al.,* (2016)
	Cas9/sgRNA	S-genes	*Phytophthora infestans* resistance	Schaart *et al.,* (2016)
Cotton	Cas9/sgRNA	Viral and satellite DNAs	Cotton leaf curl disease	Iqbal, Sattar, & Shafiq, (2016)
Peanut	Various	Alergen genes	Peanut alergens	Chandran *et al.,* (2015)
	Various	Aflatoxin genes	Peanut mycotoxins	Arias, Dang, & Sobolev, (2015)
	Various	*Ahwrky13*	pod size segregation	Zhuang *et al.,* (2019)
Sugar cane	TALEN	Caffeic acid O-methyltransferase	Reduced lignin improved biofuel prod	Jung & Altpeter, 2016; Kannan *et al.,* (2018)
Citrus	Cas9/sgRNA	CsLOB1	Canker resistance	Jia *et al.,* (2017)
Apple	Cas9/sgRNA RNPs	DIPM-1, DIPM-2, and DIPM-4 genes	Resistance to fire blight disease	Malnoy *et al.,* (2016)

Table 2: Application of Gene Editing Tools for pest and disease control

Resistance against organism	Plant species	Target gene	Gene Function	Citation
Viral resistance				
Beet severe curly top virus (BSCTV)	*Arabidopsis thaliana, Nicotiana benthamiana*	Coat protein, replication initiator protein, and intergenic region	Rolling circle replication	Ji *et al.,* (2015)
Bean yellow dwarf virus (BeYDV)	*Nicotiana benthamiana*	Rep binding site. Hairpn, Invariant nonanucleotide sequence within the replication stem-loop arKl Rep motifs I, II, and Ill	Rolling circle replication	Baltes *et al.,* (2015)
Cucumber mosaic virus (CMV), Tobacco mosaic virus (TMV),	*Nicotiana benthamiana Arabidopsis thaliana*	ORF1, 2, 3, CP and 3' UTR	Viral replication	Zhang *et al.,* (2018)
Rice tungro spherical virus (RTSV)	Oryza sativa (Indica)	elF4G	Host susceptibility for viral translation	Macovei *et al.,* (2018)
Fungal resistance				
Rice blast disease *(Mangnaporthe oryzae)*	Oryza sativa (Japonica)	OsERF922	Transcription factor involved in multiple stress responses	Wang *et al.,* (2016)
Bacterial resistance				
Bacterial blight (*Xanthomonas oryzae*)	Oryza sativa	SWEET13	Sucrose transporter	Zhou *et al.,* (2015)
Pseudomonas syringae, Xanthomonas gardneri, Phytophthora capsici	*Solanum lycopersicum*	Exon-3, S/DMR6-1	Susceptibility factor in Pseudomonas syringae, Phytophthora capsica	deToledo *et al.,* (2016)

Rep, replication initiator protein; IR, intergenic region; ORF, open reading frame; UTR, untranslated region; elF4G, eukaryotic translation initiation factor 4G; ERF922; ethylene-responsive factor; SWEET, sugar will eventually be exported transporter; DMR6, Downy mildew resistance 6.

FUTURE PERSPECTIVE AND CONCLUSION

With the greater development and expanded applications of gene-editing technology, it's an indispensable tool for precise and efficient gene control. To date, template free NHEJ is more efficient than HR repair of DSBs using template donor DNA, making it challenging to induce single nucleotide replacements in plants. Conversely, genome-wide association studies have shown that single-base changes are typically responsible for variations in elite traits in crop plants. Hence, efficient techniques for producing precise point mutations in crops are needed urgently. Genome editing technologies cannot modify all genes in all genomes, GMOs developed by transgenesis are still needed with newly added genes into the genome to fight pests and diseases.

To have a greater impact on agriculture in tropical areas, further efforts are needed to optimize the CRISPR protocols for making it more user-friendly and freely accessible for research and practical applications. The development of an efficient transformation system for major tropical crops and crops in tropical climates would facilitate crops improvement. CRISPR mediated genome-edited: deleted or disruption of undesirable genes/sequences, were mostly conducted in the laboratory, crop plants should be considered as non-GMO for rapid application and acceptance of this technology at the field level. We foresee the application of CRISPR technology in various crops to revolutionise agriculture in a second green revolution to ensure food and nutritional security of the ever-increasing population of tropical countries.

In conclusion, gene-editing tools have thrived as technology and transformed the field of agriculture and crop improvement in plants in the past five years. CRISPR/Cas9 has appeared as the most ensuring approach owing to its ease of use, simplicity, versatility, and tolerable off-target properties. The genome-editing technology sustains excellent promise in producing crop varieties with enhanced, improved yield and quality, disease resistance and novel agronomic traits, which will be helpful for consumers and farmers. Addressing sustainability for society, genome editing is promising for the development of new plant varieties to meet the present challenges of food production which is in sharp increase and demand globally while preserving the environment. The technology has been effectively used for targeted mutagenesis in many model crops. Most recently, CRISPR–Cpf1 has been employed as a new and advanced method for plant genome editing, which can overcome some limitations of CRISPR/Cas9, such as the PAM site requirement, and therefore, widen the scope of genome editing in crops. Yet, cumulative case-studies suggest that the CRISPR/Cas9 is a proficient and frequently-used technology that can speed up applied and basic research towards crop improvement.

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