Review Article

Status and scenarios of genome editing tool CRISPR-Cas9 in crop improvement

Abstract

Crop improvement systems have a long history and they had been applied since the commencement of domestication of the first agricultural plants. Since then, innumerable new techniques have and are being advanced to further upsurge the commercial value and yield of crops. The latest crop improvement technique known as genome editing is a method that empowers accurate alteration of the plant genome *via* bashing out unwanted genes or permitting genes to advance novel function. Genome sequencing of many crops and advancement in genome editing methodologies has opened prospects to breed advantageous traits. Innovations in genome editing technologies for instance zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) have created it feasible for molecular biologists to target any gene of interest more efficiently. However, these methodologies are time-consuming and pricy as they involve convoluted steps that require protein engineering. Unlike first-generation genome editing tools, CRISPR/Cas9 genome editing entails simple designing and cloning methods, with the same Cas9 being potentially available for application with various guide RNAs targeting multiple sites in the genome. It is more readily accepted in the market commercially. The usage of genome editing has proven to be benefits and plays an encouraging role in future crop improvement endeavors. Therefore, in this review article, we intend to emphasize the advancement and usage of genome editing techniques, in regard, the CRISPR/Cas9 system as a powerful genome editing tool for crop improvement. In addition, the challenges, and prospects of this technology for crop improvement will also be reviewed.

Key words: Crop Improvement, CRISPER-Cas 9, Genome editing, Plant genome

Introduction

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) is bacteria immune system by which it can resist against virus attack [1]. When a virus annexes a bacterium the CRISPR DNA of bacteria produces one or two small RNAs called cRNA and tracer RNA. These RNAs bound to Cas proteins and formed complexes that cut the DNA of the invading virus, thus protecting the bacteria from infection [2]. In 2012, a group of scientists discovered a new technique to exclusively change the DNA sequence of any organism with great ease. This was published in Science, in an article titled "A programmable dual-RNA guided DNA endonuclease". The two RNAs, crRNA and tracer RNA pair up and form Cas9 protein and directed to compel the target DNA, *via* the complementary bae pairing between the crRNA and the target DNA, Cas9 cleaves both the DNA stands [3]. This cleavage happens at extremely specific position that's imposed by sequence in crRNA molecule. Now if can mimic the structure of the CRISPR RNA and tracer RNA bound together that might guide Cas9 to chop DNA at a selected location. Application of CRISPR-Cas-9 in genome editing in plants is one among the leading promptly promising technologies in bioscience since it's becoming user friendly tool for development of non-transgenic genome editing [4]. CRISPR-Cas-9 is simpler, cost effective, faster, and highly efficient in editing genome even at multiplex level. CRISPR are often employed differently reliable with need like. Gene Knock-Out -Gene silencing using CRISPR starts with the utilization of one guide RNA (sgRNA) to focus on genes and instruct a double stranded break utilizing the Cas9 endonuclease. DNA-Free Gene Editing -CRISPR are often applied for DNA-free gene editing without the deployment of DNA vectors, necessitating only RNA or protein components. A DNA-free gene editing system are often a straightforward choice to evade the likelihood of detrimental genetic alterations [5]. The CRISPR-induced double-strand break also can be won't to create a gene "knocking" by exploiting the cells' homology-directed repair. In CRISPR genome editing technology facilitates gene-splicing where DNA is replaced, deleted, or inserted within the genome of a living organism, and therefore the advent of CRISPR-Cas9 system has further speeded up the acceptance of exact genetic alterations.

Three types of programmable endonucleases are presently being employed for plant genome editing. Zinc finger nucleases, transcription activator-like effector nucleases (TALENs), and CRISPR-Cas9 [6-9]. Zinc finger nucleases are chimeric proteins comprised of a synthetic zinc finger DNA binding domain and a DNA cleavage domain. The zinc finger DNA binding domain can be modified to explicitly target any long stretch of double stranded DNA of interest.

Zinc finger nucleases have been employed to edit the genomes of several species, including maize, rice and *Arabidopsis* [10,11]. DNA-free genome editing has been accomplished by means of both protoplast-mediated transformation and particle bombardment. The first profitable report of DNA-free genome editing in plants was reported by Woo *et al.* [12] who transfected CRISPR/Cas9 ribonucleoproteins (RNPs) into protoplasts of *Arabidopsis*, tobacco, lettuce, and rice. Similarly, Malnoy *et al*. [13] created targeted mutations by delivering purified CRISPR/Cas9 RNPs into protoplasts of both grape and apple. An efficient, regenerable systems from protoplast and somatic embryos [14-16] is pre-requisite. Regrettably, this is not available for several agriculturally important higher crop species [17,18], and consequently there has been a quest for other DNA-free genome editing methods.

Clustered Regularly Interspaced Palindromic Repeats (CRISPR/Cas9)

The invention of CRISPR/Cas9 gene editing system has transformed research in animal and plant biology with its effectiveness in genome editing being first exhibited in 2012 in mammalian cells [19]. Unlike ZFNs and TALENs, CRISPR genome editing is more uncomplicated and involves designing a guide RNA (gRNA) of about 20 nucleotides complementary to the DNA stretch within the target gene. The acronym CRISPR, first devised in 2002 by Jansen *et al*. [20] implies to tandem repeats flanked by non-repetitive DNA stretches that were first examined in the downstream of *Escherichia coli* iap genes [21]. In 2005, these non-repetitive sequences were discovered to be homologous with foreign DNA sequences derived from plasmids and phages. Consequently, the mechanism of homology-dependent cleavage was investigated for genome editing and the technology of CRISPR/Cas9 cleavage 'arrived' as a capable genome editing tool [22,23].

The CRISPR cleavage methodology needs (i) a short synthetic gRNA sequence of 20 nucleotides that bind to the target DNA and (ii) Cas9 nuclease enzyme that cleaves 3-4 bases after the protospacer adjacent motif (PAM; generally, 5′ NGG). The Cas9 nuclease is composed of two domains, (a) RuvC-like domains and (b) a HNH domain, with each domain cutting one DNA strand. Following the development of the CRISPR cleavage methodology, it has been extensively applied in plant and animal genome editing. Between 2010 and 2018, nearly 5000 articles have been published detailing the use of CRISPR. Implementing a CRISPR project involves simple steps *viz*., (i) identifying the PAM sequence in the target gene, (ii) synthesizing a

single gRNA (sgRNA), (iii) cloning the sgRNA into a suitable binary vector, (iv) introduction into host species/cell lines transformation tracked by (v) screening and (vi) validation of edited lines. The simple steps engaged in CRISPR/Cas9 mediated genome editing (CMGE) permits even a small laboratory with a fundamental plant transformation set up to carry out genome editing projects (Fig.1 & Fig. 2). CRISPR/Cas9 techniques have been employed more comprehensively to edit plant genomes in the last half decade compared to ZFNs/TALENs and are reflective of its ease of application.

Application of CRISPR Technique in Crop Improvement

CRISPR/Cas9 method of gene editing has been adopted in nearly 20 crop species so far [24] for various traits including yield improvement, biotic and abiotic stress management. Many of the published articles are deemed as proof-of-concept studies as they describe the application of CRISPR/Cas9 system by knocking out specific reported genes playing an important role in abiotic or biotic stress tolerant mechanisms. Biotic stress inflicted by pathogenic micro-organisms pose severe challenges in the development of disease-resistant crops and account for more than 42% of potential yield loss and contribute to 15% of global failures in food production [25]. CRISPR/Cas9-based genome editing has been employed to increase crop disease resistance and also to improve tolerance to major abiotic stresses like drought and salinity.

Inspiring genetic modifications have been accomplished with CRISPR-Cas9 to augment metabolic pathways, tolerant to biotic (fungal, bacterial, or viral pathogens), or abiotic stresses (cold, drought, salt), improve nutritional content, increase yield, and grain quality, obtain haploid seeds, herbicide resistance, and others (Table 1). Prominent cases include thermo-sensitive genic male sterility in maize [26] and wheat [27], improved nutritional properties in sorghum and wheat [28,29], tolerance or resistance to pathogens [30,31], and resistance to herbicides [32,33].

CRISPR/Cas9-based genome editing has been utilized to extend crop disease resistance and to develop tolerance to major abiotic stresses like drought and salinity. Genome editing with CRISPR-Cas9 is amendable to edit any gene in any plant species (Fig. 1 & Fig. 2). Owing to its ease, effectiveness, low-cost, and therefore the chance to attention on multiple genes, it permits faster genetic modification than other techniques. It can also be employed to develop genetically modify plants that were earlier overlooked. The capability that this signifies for crop breeding and hence the development of sustainable agriculture is incommensurable [34,35].

In potato CRISPR-Cas9 was accustomed to knockout the gene encoding granule-bound starch synthase (GBSS) in one round of transfection leading to the event of potato plants that produce amylopectin starch, a highly desirable commercial trait [36]. In cucumber CRISPR-Cas9 system was used to inactivate the eukaryotic translation initiation factor gene elF4E. The resulting non-transgenic homozygotic mutant plants were immune to Cucumber vein yellowing virus (Genus Ipomo virus) and resistant to the potyviruses Zucchini yellow mosaic virus and Papaya ring spot mosaic virus [37]. Engineering genetic resistance to viruses and other pathogens has immense potential to manage diseases that no natural resistance has been detected, like maize lethal necrosis disease and tomato brown rugose fruit virus [38,39,40].

Improving yields and quality of crops using CRISPR technology

Tomato

According to Brooks et al. [41], CRISPR/Cas9 has been employed in tomato as the primary genome editing technique, with applications ranging from gene function analysis to precision plant breeding [42]. CRISPR/Cpf1(Cas12a), a type V member of the CRISPR/Cas genome editing systems, is a new addition to the CRISPR/Cas genome editing systems [43]. The CRISPR/Cpf1 system has gradually been used in a variety of plant species, including tomato. In particular, when compared to a Cas9-based single replicon system, the combination of CRISPR/Cpf1 and geminiviral multireplicons significantly increased (approximately threefold) the homology-directed repair (HDR)-based genome editing efficiency in tomato [44], demonstrating good prospects for CRISPR/Cpf1 in tomato genome editing. When multiplex sgRNAs are used instead of single sgRNAs, the likelihood of significant deletions in tomato mutants created by CRISPR/Cas9 increases [45]. Including an expression cassette for overexpressing the anthocyanin intensification gene PAP1/MYB75 in a CRISPR/Cas9 construct speeds up the isolation of transgene-free tomato plants that can be easily identified by their colour [46]. Cas9 expression is directed by a fruit-specific promoter (phosphoenolpyruvate carboxylase 2 gene promoter) in a spatiotemporally regulated CRISPR/Cas9 toolkit that confers fruit-specific gene editing in tomato [47]. In 2017, cytidine base editors (CBE) were used for the first time in tomato, efficiently editing the two hormone signaling genes DELLA and ETR1 with a base edition efficiency of 26.2 percent to 53.8 percent [48]. The acetolactate synthase (ALS) gene is implicated in the branched-chain amino acid biosynthesis pathway, and a mutation of the

Proline-186 residue in tomato ALS1 can give chlorsulfuron resistance [49]. Chlorsulfuron-resistant tomato plants were successfully generated via a CBE-mediated mutation of the tomato ALS1 gene [50].

Several tomato genes important in development and ripening, fruit yield and quality, and stress responses have been functionally validated utilising genome editing-mediated targeted mutagenesis since the establishment of several genome editing tools, particularly CRISPR/Cas9, in tomato [51]. The use of CRISPR/Cas9 to re-evaluate tomato transcription factors and cell wall modifying enzymes in fruit ripening underlines the need to re-examine numerous aspects of tomato fruit ripening.

Using CRISPR/Cas9 technology, a gaggle of scientists at Cold Spring Harbor Laboratory (CSHL) precisely engineered the promoter sequence of quantitative genes in tomato [52]. By making small changes within the promoter regions in genes that control quantitative traits like LOCULE NUMBER (control fruit shape and size), FASCINATED (responsible for giant fruit size), COMPOUND INFLORESCENCE (control flower proliferation) and SELF PRUNING (control flowering time and hence growth habit) in tomato, researchers generated a good range of latest alleles that improved fruit shape, size also as plant architecture.

For the first time, genome-edited food generated with CRISPR–Cas9 technology is being marketed on the open market. Sanatech Seed, based in Tokyo, has been selling the Sicilian Rouge tomatoes, which have been genetically modified to contain high levels of γ-aminobutyric acid (GABA), directly to consumers in Japan since September. GABA, according to the business, can help decrease blood pressure and increase relaxation when taken orally (https://www.nature.com/articles/d41587-021-00026-2).

Rice

The key quality feature that impacts rice market acceptability is grain appearance. Another key quality aspect is grain chalkiness, which is an unfavourable quality factor that leads to low market acceptability. Grain shape is considered a yield component and plays an important role in determining rice grain quality. In rice various genes responsible for rice grain appearance and quality have been found, and CRISPR/Cas9 technology has the potential to tap into them. Rice was the first crop to use the CRISPR/Cas system [53], and also the first to use current

advancements to the system[54,55,56] . According to the recent research reports, various genes as Gn1a, which controls the quantity of grains, and GS3, which controls grain length, have recently been effectively modified in four rice types [57]. In comparison to the wild type, the transgene-free T1 plants had longer grain lengths and higher thousand grain weights. Similarly, CRISPR/Cas9-mediated multiplex genome editing was used to target three more key genes, GW2, GW5, and TGW6, which are negative regulators of grain weight. The results showed that genome editing of these genes enhanced grain size and thousand grain weight significantly [58]. For these features, numerous genes have been found, and their interactions and functions have been thoroughly investigated. Panicle1 (DEP1) gene within the Indica rice line IR58025B was edited by Wang et al. [59] where several CRISPR sgRNAs were used to effectively delete the fragments of the dense and erect panicles in rice. Enhancements in yield-related traits, like dense and erect panicles and reduced plant height, were observed within the produced mutant plants.

Wheat

Many negative regulatory genes have been knocked out using CRISPR/Cas9 to increase wheat yields and quality. GASR7, for example, is a gibberellin-regulated gene in rice that regulates grain length. Regardless of the varietal background, simultaneous targeting of all three TaGASR7 homoeologs dramatically increased the thousand kernel weight [60]. Similarly, GW2, which encodes a RING-type E3 ligase that regulates rice grain weight, was knocked out to increase wheat grain length and width, and thus grain yields [61]. Shan *et al*. [62] utilized CRISPR/Cas9 strategy in wheat protoplasts for TaMLO gene (Mildew resistance locus O). The CRISPR TaMLO knockout was also shown to confer resistance to mildew disease caused by *Blumeria graminis* f. sp. Tritici (Btg). Wang *et al*. [61] used CRISPR/Cas9 to extend the seed size in wheat. They knocked out the function of all homologs of TaGW2, a gene which is understood as negative regulator of seed size. Correspondingly, transgene-free low-gluten wheat has recently been engineered with CRISPR/Cas9. The targeted knockout of the Mildew Locus O (Mlo), conferring resistance to the powdery mildew pathogen, was the first reported use of CRISPR-Cas9 to produce a stably genome edited wheat plant. This was achieved in combination with an earlier gene editing technology, transcription activator-like effector nucleases [63]. Since the first report, CRISPR-Cas9 technology has been used to target wheat genes of agronomical and fundamental scientific interest, such as -gliadin genes to reduce gluten grain content [64],

TaGW2 to increase grain weight [65], TaZIP4-B2 for meiotic homologous crossover [66], TaQsd1for the reduction in postharvest sprouting, TaMTL and CENH3 for haploid plant [67,68]. Furthermore, CRISPR/Cas9-mediated targeted mutation of TaSBEIIa resulted in high amylose wheat with considerably higher resistant starch content [69]. As a result, CRISPR/Cas9 can be used to improve wheat yields and quality attributes.

Soybean

In 2011, genome editing technique induced heritable mutations in two homologous DICER-LIKE genes, DCL4a and DCL4b, in the first case of genome editing in soybean [70]. Further this technology was first applied in soybean to develop a high oleic acid soybean variety by mutating two fatty acid desaturase genes i.e. FAD2-1A and FAD2-1B [71]. CRISPR/Cas technology was applied in soybean by different research groups to evaluate mutation efficiency in different [72-76]. Further, the effectiveness of TALENs and CRISPR/Cas9 in altering two phytoene desaturase genes (GmPDS11 and GmPDS18) in hairy roots was evaluated by Du et al., [77]. CRISPR/Cas9 was substantially found more effective than TALENs at targeting two alleles at the same time. Subsequently, investigation on a variety of GmU6 promoters in soybean hairy roots and *Arabidopsis thaliana* to find the best ones for driving sgRNA expression, and discovered that the GmU6-8 and GmU6-10 promoters had the most activity, resulting in improved editing efficiency [78].

Cai *et al*. [79] applied the CRISPR-Cas9 system to induce mutations on GmFT2a, an integrator within the soybean. The developed soybean plants demonstrated late flowering, leading to increased vegetative size. The mutation was also found to be stably inherited within the subsequent generation. CRISPR/Cas9 technology has also enhanced soybean seed oil profile [80], disagreeable beany flavour of soybean seed product [81], and isoflavone content and resistance to soybean mosaic virus [82]. The experiments mentioned above showed that genome editing technology has a lot of potential for improving soybeans.

Maize

For dwarf maize breeding, understanding the function of GA biosynthesis genes might be beneficial. In a study, CRISPR/Cas9 technology was used to alter the maize GA20ox3 gene and

created semidwarf maize seedlings. The dwarf phenotypes were recovered by exogenous gibberellin, demonstrating that the mutants were gibberellin deficient [83].

Zhu *et al*. [84] exhibited gene editing during PSY1 is involved in carotenoid biosynthesis and its mutant (psy1) leads to white kernels and albino seedlings. Among fifty-two T_0 lines achieved by *Agrobacterium*-mediated transformation, seven lines were reported to hold the psy1 knockout trait and everyone seven lines were deep sequenced to know the sort of variation and to gauge the mutation efficiency.

Lines A and B of Fast-Flowering Mini-Maize (FFMM) were recently developed as an open-source tool for maize research, reducing space requirements and generation time. Neither FFMM line was capable of genetic transformation using traditional methods, which was a need for its status as a comprehensive toolset for public maize genetic research. Recently, McCaw et al. [85] effectively introduced the CRISPR-Cas9 reagents into immature embryos using an *Agrobacterium*-mediated conventional transformation approach and created transgenic and mutant lines with the expected mutant phenotypes and genotypes of maize.

Banana

Genome editing approaches are reported in banana crop by several researchers. Kaur *et al*. [86] studied that carotenoid play a crucial role in many physiological processes in plants and therefore the phytoene desaturase genes encode important enzymes within the carotenoid biosynthesis pathway. Phytoene desaturase genes, RAS-PDS1 and RAS-PDS2 were just mutated by the appliance of CRISPR/Cas9 with a 59% success rate in bananas. The generation of ethylene, which is the first component examined when creating postharvest preservation technology, is intimately tied to the shelf life of bananas. Reduced endogenous ethylene production or ethylene signal transduction impairment caused by genetic alteration could be highly effective strategies for delaying the ripening process [87]. In a recent study conducted on this aspect, researchers developed multiple MaACO1-disrupted plants with distinct editing patterns using the CRISPR/Cas9 technology. Under natural ripening circumstances, the mutant fruits had lower ethylene synthesis and a longer shelf life. Furthermore, MaACO1-deficient fruit was responsive to ethephon and ripened normally after treatment with ethephon. Furthermore, the MaACO1-disrupted line's vegetative growth, lifespan, and fruit quality were equivalent to wild-type plants, with the exception of somewhat decreased height and yield. These findings show that MaACO1

is an excellent target for employing the CRISPR/Cas9-mediated editing technique to create fruit with an extended shelf life. By extending the shelf life of banana fruit, newly developed germplasm would considerably reduce postharvest losses and boost the economic value of the banana sector [88]. CRISPR/Cas9 technology can also be used to generate disease-resistant variants. Recently, Tripathi et al. [89] summarized the reports available on CRISPR/Cas9 based genome editing in banana.

Cassava

Odipio *et al*. [90] applied CRISPR/Cas9 technology, to generate MePDS mutants in cassava, which exhibited albino or partial albino at cotyledonary-stage somatic embryos. This phenotype was observed in over 95% of the mutant cassava. More importantly, the somatic embryo lines successfully produced plantlets with the mutations (22-47% success rate).

Cassava brown streak disease (CBSD) is a serious constraint on cassava yields in East and Central Africa, and it affects production in West Africa. Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) are two positive-sense RNA viruses in the family Potyviridae, genus Ipomovirus, that cause CBSD. The interaction of viral genome-linked protein (VPg) with host eukaryotic translation initiation factor 4E (eIF4E) isoforms is required for diseases caused by the Potyviridae family. In a recent experiment conducted by Gomez et al. [91] on cassava cultivar 60444, CRISPR/Cas9-mediated genome editing was used to create ncbp-1, ncbp-2, and ncbp-1/ncbp-2 mutants. When challenged with CBSV, ncbp-1/ncbp-2 mutants demonstrated delayed and attenuated CBSD aerial symptoms, as well as reduced storage root necrosis severity and occurrence. In comparison to wild-type controls, lower viral titre in storage roots was associated with suppressed disease symptoms. Genome editing results in this study show that it is possible to change numerous genes in cassava at the same time to acquire CBSD tolerance.

Benefits of CRISPR-CAS system

- Modest cost.
- It doesn't involve any protein engineering step.
- The simplicity of the CRISPR nuclease, with only three required components (Cas9 alongside the crRNA and trRNA) makes this technique amenable to adaptation for genome editing.

- The main practical advantage is that the simple multiplexing, which may be used to edit several genes at same time.
- Large genomic deletions or inversions are often accomplished by targeting two extensively spaced cleavage sites on an equivalent chromosome.
- Free software exists to style guide RNA to focus on any preferred gene.

Limitations

- The only limitation today is people's ability to consider creative ways to harness CRISPR.
- It's unclear, for instance, how specific the guide RNAs are for just the genes they're alleged to target.
- There are often significant off-target effects.
- Non-target DNA resembling the guide RNA can become cut, activated, or deactivated.
- Delivery is a huge contest.

Prospects of CRISPER-Cas9

- Future identification of an appropriate delivery method.
- Simple reprogramming of CRISPR-Cas constructs to focus on particular genes of interest will greatly enhance the efficiency with which this will be accomplished.
- Optimise the spread of CRISPR-Cas in additional realistic microbial communities and to know the risks related to this technology.
- Active position with communities and development of clear guidelines to manage its sensible and safe use.
- Avoid a number of the problems related to the discharge of genetically engineered organisms.
- Risk-free implementation of this technology.
- To overcome the off targeting of CRISPR technique, now use of CRISPR technique with Cas-clover is getting employed.

Conclusion

To have a greater impact on agriculture in tropical areas, further efforts are required to optimize the CRISPR/Cas9 protocols for designing it more user-friendly and freely accessible for research

and practical applications. With the emerging application of CRISPR we will find an alternate to transgenic technique and optimistically stop the moral issues regarding transgenic crops. the obstacle regarding off-targeting is often overcome by new emerging CRISPR cas-clover techniques. In India CRISPR remains an upscale technique to adopt but still its use can give us great achievements in crop improvement.

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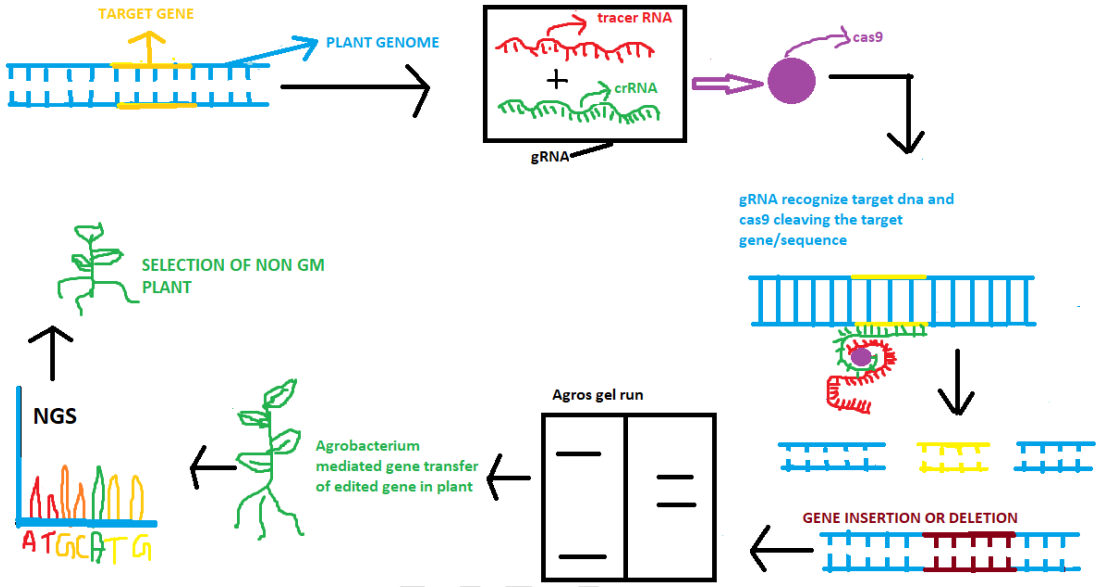

Fig 1. Overview of CRISPER-Cas 9 genome editing

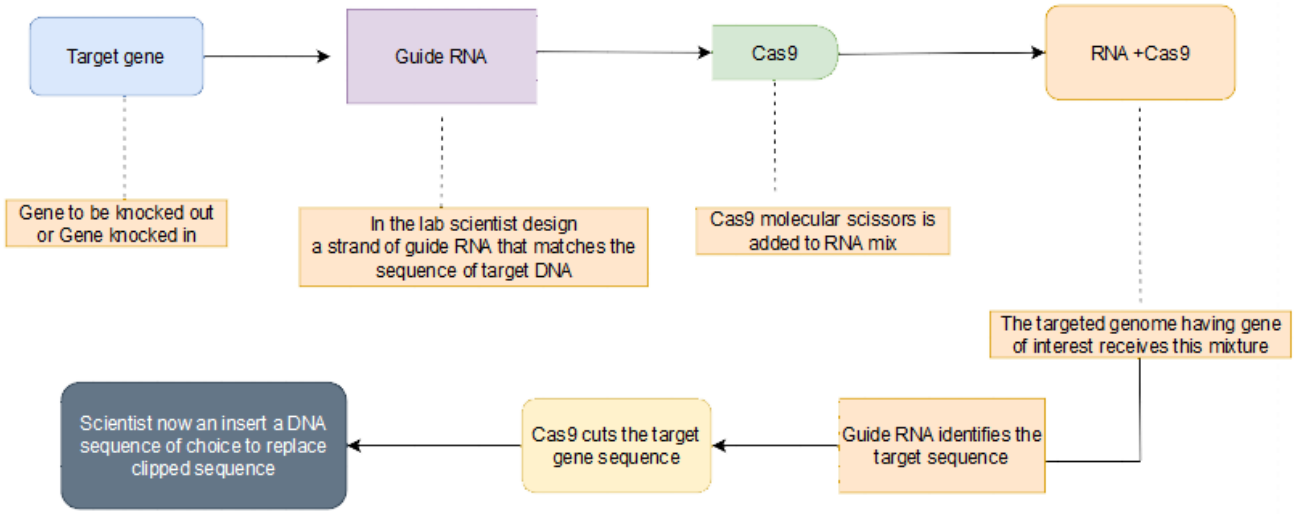

Fig 2. Flow chart describing the steps involved in CRISPR/Cas9 based genome editing

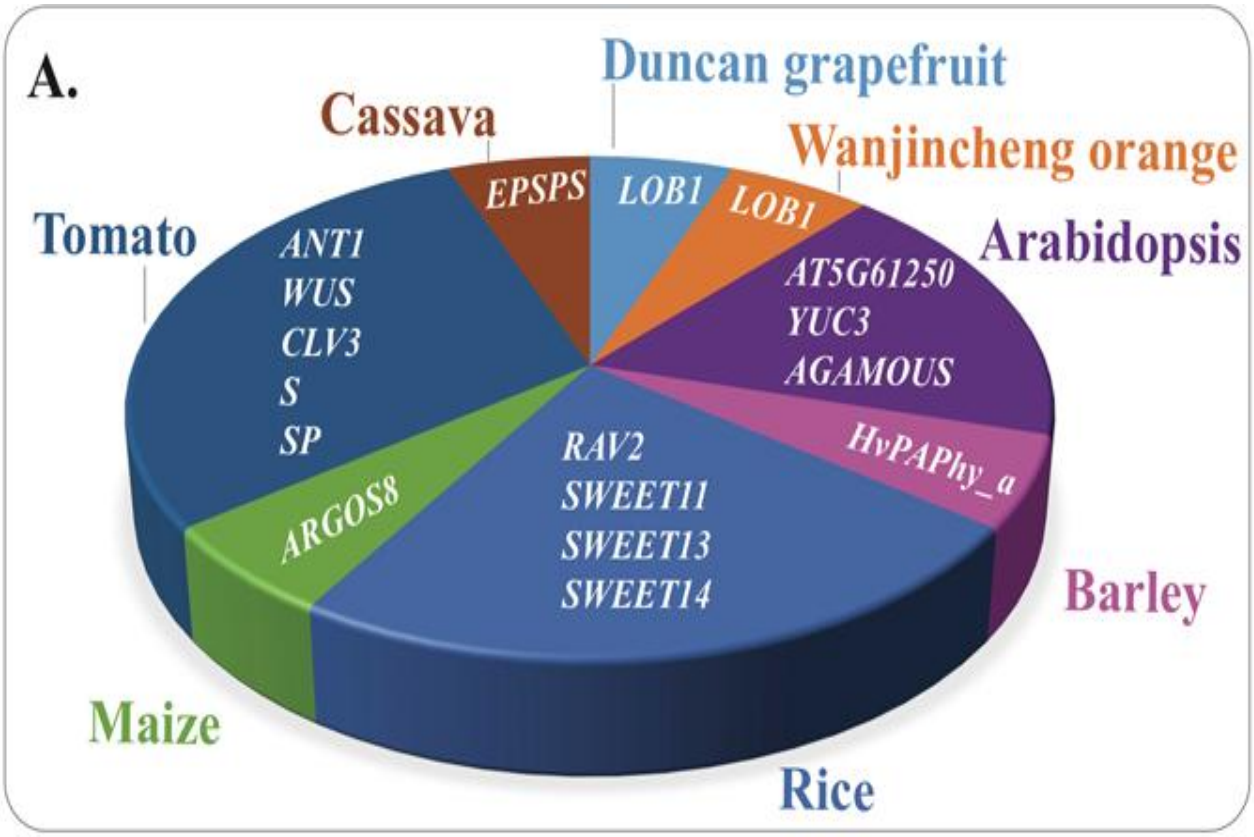

Fig. 3. Summarization of current applications of CRISPR/Cas-mediated *cis*-engineering in plants.

Table 1 List of CRISPR-Cas9 studies for plant improvement

Crop	Target gene(s)	Target traits	Type of edit	Results	References
Cassava	Phytoene desaturase	Trial for CRIPSR	Gene disruption	Observation of albino phenotype	[90]
Cassava	*elF4E* isoforms *nCBP-1* & *nCBP-2*	Resistance to cassava brown streak disease	Gene disruption	Elevated resistance to cassava brown streak disease	[91]
Cotton	CLCuD IR and Rep regions	Resistance to cotton leaf curl disease	Viral gene disruption	Targeted cleavage of mixed infections by multiple viruses and associated DNA satellites, such as CLCuD-complex	[92]
Cotton	Green fluorescent protein (GFP)	phenotypic characterization	Indels	The ability to introduce DSB at a precise target site has been further extended to create a precise nucleotide substitution or insertion of the desired DNA sequence through homology-dependent repair	[93]
Cotton	GhMYB25-like A and GhMYB25-like D)	fiber development controlling GhMYB25 homoeologous genes	Truncation event	demonstrated CRISPR/Cas9-induced specific truncation events in the cotton	[94]
Rice	*OsSWEET11*, *OsSWEET14* (rice bacterial blight susceptibility genes)	Resistance to bacterial blight	Promoter disruption	The promoter of the blight susceptibility gene was disrupted	[95]
Maize	Zmzb7	Albino marker gene	Promotor disruption	Targeted the albino marker gene, Zmzb7 in a protoplast system. Knockout of Zmzb7 results in albino plant, with the sgRNA designed to target a region in the eighth exon of Zmzb7 and maize U3 promoter was used for expression.	[96]

Maize	*ZmTMS5*	male sterility	Gene disruption	Three gRNAs were used to knockout the gene, with one sgRNA targeting the first exon and the other two sgRNAs targeting the second exon.	[97]
Maize	ARGOS8	Hybrid production	Allelic variant	Two genome edited variants (ARGOS8-v1 and ARGOS8-v2) were used to produce hybrids and evaluated in the field in multi-location trials.	[98]
Rice	*OsERF922* (ethylene responsive factor transcription factor)	Resistance to rice blast	Gene disruption	Resistance to *M. oryzae* was enhanced	[99]
Rice	*HTD1 gene*, three loci shown to control seed size and/or yield (*GS3*, *GW2* and *GN1A*) were targeted.	agronomic potential	multiple gene knockouts	This resulted in mutants with significantly improved seed yield.	[100]
Wheat	*TaMLO-A1*, *TaMLO-B1* and *TaMLO-D1*	Resistance to powdery mildew	Gene disruption	The number of mildews microcolonies formed on the leaves was significantly reduced against the control and no apparent fungal growth was observed on the leaves of edited plants	[101]
Wheat	*TaDREB2* and *TaERF3*	Trial for CRISPR	Gene disruption	Provide a deep insight about their functioning in abiotic stress response	[102]
Wheat	*TaMLO*	Powdery mildew disease	Protoplast targeted	The CRISPR TaMLO knockout was also shown to confer resistance to powdery mildew disease caused by *Blumeria graminis* f. sp. Tritici (Btg).	[62]

Wheat	(*TaGW2* and *TaGASR7*)	Ribonucleoproteins	biolistic delivery	This DNA-free editing method avoids time consuming procedures such as backcross breeding for the removal of the transgene and allows to obtain transgene-free plants at T0	[103]
Soybean	GmFEI2 and GmSHR)	Hairy root system	Knockout	Using a single sgRNA for a transgene (bar) and six sgRNAs that targeted different sites of two endogenous soybean genes (GmFEI2 and GmSHR) and examined efficacy of the sgRNAs in a hairy root system.	[72]
Soybean	*GmFT2a* and *GmFT4*	Flowering time	There were C to T mutations and C to G mutations, both types occurred at position 7 of the target sequence	Induce single base substitution in soybean	[104]
Groundnut	*FAD 2*	Oleic acid contents		As a result of CRISPR/Cas9 activity, three mutations were identified - G448A in ahFAD2A, and 441_442insA and G451T in ahFAD2B.	[105]