Review Article

Status and scenario of genome editing device CRISPR-Cas9 in crop advancement

Abstract

Plant breeding systems have a extensive antiquity and they had been employed meanwhile the commencement of subjugation of the initial agricultural crop plants. Subsequently, innumerable novel procedures have and are being advanced to added upsurge the profitable worth and harvest of plants. The newest crop enhancement method recognized as genome editing is a method which empowers accurate alteration of the crop genome *via* bashing out unwanted genes or permitting genes to advance novel occupation. Genome sequencing of many plants and advancement in genome editing methodologies has unlocked prospects to breed advantageous traits. Innovations in genome editing machineries for instance have created it feasible for biotechnologists to mark a specific gene of consideration more proficiently. The first-generation CRISPR/Cas9 genome editing entails modest conniving and cloning approaches, which can be accessible and applicable for various guide RNAs to edit various positions in the genome of targeted organism. It is more willingly recognized in the marketplace economically. The tradition of genome editing has verified to be reimbursements and theatres an encouraging part in upcoming crop development endeavors. So, in presented review article, it is intended to emphasize the advancement and usage of genome editing procedures, in regard, the CRISPR/Cas9 for crop improvement.

Key words: Crop Enhancement, CRISPR-Cas 9, Genome editing, Plant genome

Introduction

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) is bacteria immune arrangement through which it can resist against virus attack [1]. Once a virus annexes a bacterium the CRISPR DNA of bacteria produces one or two small RNAs named cRNA and tracer RNA. The produced RNAs predestined to Cas proteins and deliberate campuses that has ability to cut the DNA of the virus with entering intension and these mechanisms defend the bacteria [2]. In 2012, a novel system was discovered to exclusively alter the DNA order of any creature with excessive affluence. The two RNAs, crRNA and tracer RNA pair up with Cas9 protein and directed to the aimed DNA, with the possibility of the corresponding hybridization of bases of the crRNA and the target DNA. Further, Cas9 cleaves both the DNA stands [3]. This cleavage happens at extremely specific position that's imposed by the sequence in crRNA molecule. Application of CRISPR-Cas-9 for the purpose of genome editing in plants is one among the leading promptly promising techniques in bioscience since it's becoming manipulator responsive device for advancement of non-transgenic genome editing [4]. CRISPR-Cas-9 is simpler, cost effective, faster, and extremely competent in editing genome even at multiplex level [5]. CRISPR genome editing technology facilitates gene-splicing in a targeted living organism at the position of DNA where replacement, deletion, or insertion took place within the genome. Currently, three types of endonucleases are being applied in the experiments based on plant genome editing. Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR-Cas9 [6-9]. Examples are available on application of ZFN approach for editing lengthy leap of double stranded DNA [10,11].

Genome editing, free from DNA can be done with the help of protoplast-mediated transformation and particle bombardment Woo *et al.* [12]. Furthermore, Malnoy *et al*. [13] successfully inserted mutation with the use of CRISPR/Cas9 RNPs into protoplasts of grape as well as apple. An effectual, regenerable arrangement from protoplast and somatic embryos [14-16] is pre-requisite. Regrettably, this is not accessible for numerous agriculturally imperative higher plant species [17,18]. The invention of genome editing system especially CRISPR/Cas9 has opened the windows for multidimensional research in animal as well as plants [19]. However, the acronym CRISPR was first devised in 2002 by Jansen *et al*. [20] and was first examined in the downstream of *Escherichia coliiap* [21]. Further improvements in this field were taken in the year 2005, when the homology of these non-repetitive sequences was identified with foreign DNA sequences obtained 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].

Clustered Regularly Interspaced Palindromic Repeats (CRISPR/Cas9) based genome editing method

Different components are involved in the CRISPR cleavage approach. The first component is a 20-nucleotide synthetic gRNA sequence with the ability to bind with target DNA. The second component is Cas9 nuclease enzyme, with the ability to cut 3-4 bases after the protospacer adjacent motif. This Cas9 nuclease consists of two important parts as RuvC-like domains and an HNH domain. Both of these domains have abilities to cut one strand of DNA. The use of CRISPR in plants and animals has been widely documented in more than 5000 articles. The steps involve: (i) identification of specific sequence, (ii) synthesis of a single gRNA (sgRNA), (iii) selection of suitable vector for cloning the sgRNA, (iv) transformation (v) screening of transformants and (vi) final validation. A laboratory with basic facilities for plant transformation can be utilized for genome editing related experiments. This may be due to the straight forward steps in CRISPR/Cas9 mediated (Fig.1 & Fig.2) genome editing (CMGE). CRISPR/Cas9 approach has proven its efficiency in the last half decade, owing to their ease of use.

Benefits of CRISPR-CAS system

- Modest cost.
- It doesn't involve any protein engineering step.
- The simple mechanism of the CRISPR nuclease makes it superior toll for genome editing.
- The main practical advantage is that it may be applied to edit several genes simultaneously.
- Free software exists to guide RNA to focus on any preferred gene.

Limitations

- It is ambiguous, for instance, how specific the guide RNAs are for just the genes they're alleged to target.
- There are often considerable off-target consequences.
- Non-target DNA resembling the guide RNA can be, activated, or deactivated.
- Release is a huge contest

Application of CRISPR Technique in Crop Improvement

CRISPR/Cas9 gene editing technology is being used in different crops. However, till date most successful experiments have been done in about 20 crop species [24]. These crops were targeted for the improvement of important traits including yield. Development of high yielding, biotic and abiotic stress tolerant/resistant crops is another target. Most of the research papers based on mechanism of knock out specific genes involved in tolerance against abiotic and biotic stresses are available publically. These publications also describe applications of CRISPR/Cas9 in enhancement of tolerance processes. Genome editing approaches demonstrated their significance in the development of biotic stress resistant crops [25]. In this sequence, crops with tolerance against various abiotic stresses like drought and salinity have also been improved with the applications of this technology.

Among genome editing technologies, application of CRISPR-Cas9 have been published by various research groups. In this sequence reports are available on enhancement of metabolic pathways, development of crops against fungal, bacterial, and viral diseases, or cold, drought, and salt stresses (Table 1). Literatures are also available on improvement of nutritionally superior crops with higher yield, and grain quality in various crops. Apart from these, few works are reported on production of haploid seeds. Among these improvements, few may be detailed here as thermo-sensitive genic male sterility in maize [26] and wheat [27], nutritional parameters in sorghum and wheat [28,29], tolerance or resistance against diseases [30,31], and herbicide resistance [32,33].

Due to the simplicity of CRISPR-Cas9 method, it may be applied for the modifications of any targeted gene in plants. This approach provides faster results in comparison to the other approaches. Some of the features of this approach make it superior over others as its simplicity, effectiveness and low cost. This technology is being adopted for the improvement of neglected crops also [34,35]. CRISPR-Cas9 applied to knock down the gene responsible for granule-bound starch synthase (GBSS) in potato plants. It has given tremendous results in just one cycle of transfection with the production of amylopectin starch. The improvement of this trait in potato has given it high commercial values [36]. In cucumber, the activity of eukaryotic translation initiation factor gene elF4E turned off with this technology which resulted in the non-transgenic homozygotic mutant plants [37]. Engineering for viral and other pathogen resistant in crops have been done successfully [38,39,40]. These experiments demonstrate the efficiency and ability of genome editing technology in the field of crop improvement. This technology is not only suitable for cereals, but also for horticultural and other groups of crops.

Improvements in yield and quality of crops using CRISPR technology

Vegetables

Tomato

Use of genome editing technology has been demonstrated in vegetables including tomato. Application of CRISPR/Cas9 in improvement of tomato was published by Brooks et al. [41]. Other researchers have detailed about applications of genome editing in tomato [42]. Works on use of CRISPR/Cpf1(Cas12a)- a new addition to the CRISPR/Cas genome editing systems [43] are also in progress for the improvement of tomato crop. Apart from this CRISPR/Cpf1 system is now being employed in a wide range of plant species. The utilization of CRISPR/Cpf1 improved genome editing effectiveness in tomato [44]. Use of multiplex sgRNAs given better results instead of single sgRNAs in tomato [45]. CRISPR/Cas9 increased anthocyanin synthesis gene so, that developed tomato plants can be easily identified by their colour [46,47]. For the first time in tomato, two hormone signalling genes *DELLA* and *ETR1* were edited successfully [48]. Edited acetolactate synthase (ALS) gene resulted in chlorsulfuron resistance [49,50]. Since the introduction of genome editing techniques, particularly CRISPR/Cas9, in tomato, multiple traits have been improved [51]. Researchers used CRISPR/Cas9 technology for edition of promoter sequence in tomato [52].

For the first time, food that has been genome-edited using CRISPR–Cas9 technology has been sold on the open market. Since September 2021, Sanatech Seed, based in Tokyo, has been selling Sicilian Rouge tomatoes directly to Japanese consumers, which have been genetically modified to have high quantities of -aminobutyric acid (GABA). When taken orally, GABA, according to the company, can help lower blood pressure and enhance relaxation (https://www.nature.com/articles/d41587-021-00026-2).

Cereals

Rice

New breeding technology such as genome editing has played significant role in the improvement of rice. Rice was the first crop to use the CRISPR/Cas system [53] for its improvement. Most recent improvements in rice [54,55,56] have been published by research groups. CRISPR/Cas9 technology has been applied on genes governing rice grain appearance and quality. Genes including Gn1a, which affects grain amount, and GS3, which controls grain length in rice, have recently been effectively edited [57]. Edited plants produced longer grain and higher thousand grain weights. In other study, genome editing of *GW2*, *GW5*, and *TGW6* genes enhanced grain size [58]. Various reports are available on relations of genes with important characteristics of rice grain. Wang et al. [59] used numerous CRISPR sgRNAs to efficiently remove the segments of the dense and erect panicles in rice. Further, gene that controls grain length were edited to raise thousand kernel weights considerably [60].

Wheat

Similar to rice crop, wheat grain length and width were increased by knocking down *GW2*, that is responsible for grain weight [61]. In an experiment, Shan et al. [62] applied a CRISPR/Cas9 approach to edit the *TaMLO* gene in wheat (Mildew resistance locus O). The first reported application of CRISPR-Cas9 in wheat was development of resistance to the powdery mildew disease [63]. An experiment was conducted by Wang et al. [61] on utilization of CRISPR/Cas9 for enhancement of seed size in wheat crop. During their experiment they worked on *TaGW2*, a gene thought to be a negative seed size regulator. In some other experiments, the system has been used to generate low-gluten wheat [64], to increase grain weight [65], for meiotic homologous crossover [66], *TaQsd1* gene for postharvest sprouting reduction [67], *TaMTL* and *CENH3* [68] for haploid plant. Furthermore, a specific mutation of *TaSBEIIa* resulted in high amylose wheat with significantly greater starch content [69]. These applications of CRISPR/Cas9 in wheat indicate the scope of utilization of genome editing technology to boost wheat yields and grain quality.

Maize

Genome editing for dwarf gene in maize was done to obtain semidwarf seedling of maize. CRISPR/Cas9 technology was applied to edit *GA20ox3* gene,and targeted results were obtained [70]. Second experiment to edit *PSY1* gene in maize was done to alter carotenoid production [71]. Development of Fast-Flowering Mini-Maize (FFMM) Lines A and B may help in reduction of generation time. McCaw et al. [72] used an *Agrobacterium*-mediated conventional transformation technique to successfully introduce CRISPR-Cas9 reagents into immature embryos, resulting in expected transgenic and mutant lines of maize.

Oilseed crop

Soybean

In the first attempt of genome editing in soybean [73], two *DICER-LIKE* genes, *DCL4a* and *DCL4b* were targeted. Furthermore, two fatty acid desaturase genes, *FAD2-1A* and *FAD2-1B*, were altered to generate a high oleic acid soybean variety [74]. Various research groups utilized CRISPR/Cas platform to edit different beneficial genes in soybean. Mutation efficiency was also evaluated in these experiments [75-79]. In an experiment, Du et al. [80] examined the effectiveness of TALENs and CRISPR/Cas9 in modifications of two phytoene desaturase genes (*GmPDS11* and *GmPDS18*) and proved the better performance of CRISPR/Cas9 between both approaches. Furthermore, researchers focused on alteration of a range of GmU6 promoters for driving sgRNA production in soybean and *Arabidopsis thaliana*. The results revealed that the GmU6-8 and GmU6-10 promoters were the most efficient, resulting in improved editing efficiency [81].

CRISPR-Cas9 method was applied to mutate *GmFT2a*, a soybean integrator by Cai et al. [82]. However, late flowering was observed in the matured soybean plants with increased vegetative growth. This technology has also improved the profile of soybean seed oil [83] as well as the unpleasant flavour of soybean seed products [84]. Alterations in the content of isoflavones and resistance to soybean mosaic virus [85] have also been achieved after utilization of genome editing technology in soybean.

Horticultural crops

Banana

There are several examples of utilization of genome editing approaches in improvement of banana crop. Among various beneficial bioactive compounds, Carotenoid affects physiological processes in plants [86]. In this sequence, phytoene desaturase genes i.e. *RAS-PDS1* and *RAS-PDS2* were modified using CRISPR/Cas9 in banana. The production of ethylene should be considered during development of postharvest preservation technology in banana. Reduction in ethylene production could be very efficient for slowing the ripening process [87]. To achieve these targets, researchers utilized CRISPR/Cas9 for generation of modified *MaACO1* plants. Resultant mutant fruits were noticed with decreased ethylene synthesis which has positive effects on slow ripening. These findings suggest that *MaACO1* is a good candidate for using the CRISPR/Cas9-mediated editing approach to produce fruit with a longer shelf life. Newly produced germplasm would significantly reduce postharvest losses and improve the commercial value of the banana sector by prolonging the shelf life of banana fruit [88]. Tripathi et al. [89] recently compiled the known reports on CRISPR/Cas9-based genome editing in banana crop.

Cassava

Odipio et al. [90] used CRISPR/Cas9 technology to create *MePDS* mutants in cassava plants with 22 to 47% success rate. Cassava brown streak disease (CBSD), is a serious problem in East and Central Africa, as well as in West Africa. To overcome this virus CRISPR/Cas9-mediated genome editing was employed to construct *ncbp-1*, *ncbp-2*, and *ncbp-1/ncbp-2* mutants in a recent experiment done by Gomez et al. [91]. When challenged with CBSV, *ncbp-1/ncbp-2* mutants showed delayed and attenuated CBSD aerial symptoms, as well as decreased the intensity and incidence of storage root necrosis. The successful application of genome editing approaches in cassava confirms the possibility of modifications in multiple genes in cassava with disease tolerance/resistance.

Prospects of CRISPR-Cas9

- It will be necessary to identify a suitable delivery method for future experiments on crops. The CRISPR/Cas9 system is too vast to be packed into viral vectors due altering its size. Crop improvement will be aided by a small CRISPR system.
- It is important to develop such mechanism that should focus on targeted genes with high efficiency. This will be helpful to achieve the target.
- It is important to gain a better understanding of the hazards associated with this technology. Antimicrobials based on CRISPR-Cas could have unintended consequences: If a strain is eliminated from a population, or if the removal of a specific plasmid affects its growth or metabolism, other, potentially more clinically hazardous species may overtake it.

- During deployment of gene-editing systems in the environment, it is important to exercise on all aspects and dangers. It has been argued that revised nongovernmental rules on the release of CRISPR-Cas and other gene-editing structures that can be used to alter the genetic material of environmental populations, as well as guidance and law from national and international agencies, are needed.
- Adaptation of technically approved guidelines may be followed by community engagement.
- To tackle the off-targeting problem with CRISPR, researchers are now combining the technology with Cas-clover. Cas-CLOVERTM is a revolutionary gene-editing tool that is a true dimeric system, unlike standard single-guided Cas9 and dual-guided Cas9-nickase systems. Cas-CLOVER exhibits great fidelity with no off-targets while keeping high editing efficiency.

Conclusion

Research works on use of genome editing technology to refine the CRISPR/Cas9 protocols in order to make them more user-friendly are being carried out. Researchers are also focused to make this technology widely accessible for research and practical applications in tropical areas, in order to have a stronger impact on agriculture in such places. With the growing use of CRISPR, we will be able to find an alternative to transgenic technology and, hopefully, put an end to the moral difficulties surrounding transgenic crops. New CRISPR Cas-clover approaches are frequently used to address the problem of off-targeting. CRISPR remains an upmarket approach to embrace in India, but its application can still lead to significant agricultural improvement.

References

1. Rath D, Amlinger L, Rath A, Lundgren M (2015) The CRISPR-Cas immune system: Biology, mechanisms and applications. Biochimie, 117: 119-128.
2. Loureiro, A., da Silva, G. J. (2019). CRISPR-Cas: Converting A Bacterial Defence Mechanism into A State-of-the-Art Genetic Manipulation Tool. Antibiotics (Basel, Switzerland), 8(1), 18. https://doi.org/10.3390/antibiotics8010018.

3. Hsu, P. D., Lander, E. S., Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. Cell, 157(6), 1262–1278. https://doi.org/10.1016/j.cell.2014.05.010.
4. Zhu, H., Li, C. & Gao, C. Applications of CRISPR–Cas in agriculture and plant biotechnology. *Nat Rev Mol Cell Biol* **21,** 661–677 (2020). https://doi.org/10.1038/s41580-020-00288-9.
5. Lino, C. A., Harper, J. C., Carney, J. P., Timlin, J. A. (2018). Delivering CRISPR: a review of the challenges and approaches. Drug delivery, 25(1), 1234–1257. https://doi.org/10.1080/10717544.2018.1474964
6. Malzahn, A., Lowder, L., & Qi, Y. (2017). Plant genome editing with TALEN and CRISPR. *Cell & bioscience*, *7*(1), 1-18.
7. Shah, T., Andleeb, T., Lateef, S., Noor, M. A. (2018). Genome editing in plants: advancing crop transformation and overview of tools. *Plant Physiol. Biochem.* 131, 12–21.
8. Zhang, Y., Li, D., Zhang, D., Zhao, X., Cao, X., Dong, L., *et al*. (2018a). Analysis of the functions of TaGW2 homoeologs in wheat grain weight and protein content traits. *Plant J.* 94, 857–866.
9. Bao A, Burritt DJ, Chen H, Zhou X, Cao D, Tran LP. (2019) The CRISPR/Cas9 system and its applications in crop genome editing. Crit Rev Biotechnol. 2019: 39(3):321-336.
10. Shukla, V. K., Doyon, Y., Miller, J. C., Dekelver, R. C., Moehle, E. A., Worden, S. E., *et al*. (2009). Precise genome modification in the crop species Zea mays using zinc-finger nucleases. *Nature* 459, 437–441.
11. Gallego-Bartolome, J., Liu, W., Kuo, P. H., Feng, S., Ghoshal, B., Gardiner, J., *et al*. (2019). Co-targeting RNA polymerases IV and V promotes efficient de novo DNA methylation in *Arabidopsis*. *Cell*. 176, 1068–1082.
12. Woo, J. W., Kim, J., Kwon, S. I., Corvalán, C., Cho, S. W., Kim, H. & Kim, J. S. (2015). DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. *Nature biotechnology*, *33*(11), 1162-1164.

13. Malnoy M, Viola R, Jung MH, Koo OJ, Kim S, Kim JS, *et al*. DNA-free genetically edited grapevine and apple protoplast using CRISPR/Cas9 ribonucleoproteins. Front Plant Sci. 2016; 7:1904.
14. Patidar SL, Tripathi MK, Tiwari G, Patel RP, Ahuja A. Standardization of an efficient and reproducible embryogenic cell suspension culture protocol for production of secondary metabolites in *Plumbago zeylanica* Linn. Ecol. Environ. Conserv. 2017; 23:373–380.
15. Bele D, Mishra N, Tiwari S, Tripathi MK, Tiwari G.Massive*in vitro* cloning of sandalwood (*Santalum album* linn.)*via* cultured nodal segments. Current Journal of Applied Science and Biotechnology; 2019; 33(1):1-14.
16. Tripathi MK, Tripathi N, Tiwari S, Tiwari G, Mishra N, Bele D, Patel R.P, Sapre, S, Tiwari S. Optimization of different factors for initiation of somatic embryogenesis in suspension cultures in sandalwood (*Santalum album* L.). *Horticulturae.* 2021;*7*(5): 118.
17. Adlak T, Tiwari S, Tripathi MK, Gupta N, Sahu VK, Bhawar PC, Kandalkar VS. Biotechnology: An advanced tool for crop improvement. Current Journal of Applied Science and Technology. 2019;33(1):1-11.
18. Tripathi MK, Mishra N, Tiwari S, Singh S, Shyam C, Ahuja A. Plant tissue culture technology: sustainable option for mining high value pharmaceutical compounds.International Journal of Current Microbiology and Applied Sciences.2019; 8(2):102-110.
19. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., and Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science* 337, 816–821.
20. Jansen, R., Embden, J. D. V., Gaastra, W., and Schouls, L. M. (2002). Identification of genes that are associated with DNA repeats in prokaryotes. *Mol. Microbiol.* 43, 1565–1575.
21. Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., and Nakata, A. (1987). Nucleotide sequence of the IAP gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. *J. Bacteriol.* 169, 5429–5433.

22. Mojica, F. J., Diez-Villasenor, C., Garcia-Martinez, J., and Soria, E. (2005). Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J. Mol. Evol.* 60, 174–182.

23. Liu, X., Wu, S., Xu, J., Sui, C., and Wei, J. (2017). Application of CRISPR/Cas9 in plant biology. *Acta Pharm. Sin. B* 7, 292–302.

24. Ricroch, A., Clairand, P., and Harwood, W. (2017). Use of CRISPR systems in plant genome editing: toward new opportunities in agriculture. *Emerg. Top. Life Sci.* 1, 169–182.

25. Oerke, E. C. (2005). Crop losses to pests. *J. Agric. Sci.* 144, 31–43.

26. Li, J., Zhang, H., Si, X., Tian, Y., Chen, K., Liu, J., *et al*. (2017). Generation of thermosensitive male-sterile maize by targeted knockout of the ZmTMS5 gene. *J. Genet. Genomics* 44, 465–468.

27. Okada, A., Arndell, T., Borisjuk, N., Sharma, N., Watson-Haigh, N. S., Tucker, E. J. &Whitford, R. (2019). CRISPR/Cas9-mediated knockout of Ms1 enables the rapid generation of male-sterile hexaploid wheat lines for use in hybrid seed production. *Plant biotechnology journal*, *17*(10), 1905-1913.

28. Li, A., Jia, S., Yobi, A., Ge, Z., Sato, S. J., Zhang, C., *et al*. (2018a). Editing of an Alpha-Kafirin gene family increases, digestibility and protein quality in Sorghum. *Plant Physiol.* 177, 1425–1438.

29. Zhang, Y., Massel, K., Godwin, I. D., Gao, C. (2018b). Applications and potential of genome editing in crop improvement. *Genome Biol.* 19, 210.

30. Zhang, Y., Bai, Y., Wu, G., Zou, S., Chen, Y., Gao, C., *et al*. (2017b). Simultaneous modification of three homoeologs of TaEDR1 by genome editing enhances powdery mildew resistance in wheat. *Plant J.* 91, 714–724.

31. Pyott, D. E., Sheehan, E., Molnar, A. (2016). Engineering of CRISPR/Cas9-mediated potyvirus resistance in transgene-free *Arabidopsis* plants. *Mol. Plant Pathol.* 17, 1276–1288.

32. Endo, M., Mikami, M., Toki, S. (2016). Biallelic gene targeting in rice. *Plant Physiol.* 170, 667–677.

33. Sun, Y., Zhang, X., Wu, C., He, Y., Ma, Y., Hou, H., *et al*. (2016). Engineering Herbicide-resistant rice plants through CRISPR/Cas9-mediated homologous recombination of Acetolactate synthase. *Mol. Plant* 9, 628–631.
34. Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., *et al*. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science* 339, 819–823.
35. Mali, P., Aach, J., Stranges, P. B., Esvelt, K. M., Moosburner, M., Kosuri, S., *et al*. (2013). CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nat. Biotechnol.* 31, 833–838.
36. Andersson, M., Turesson, H., Nicolia, A., Falt, A. S., Samuelsson, M., Hofvander, P. (2017). Efficient targeted multiallelic mutagenesis in tetraploid potato (Solanum tuberosum) by transient CRISPR-Cas9 expression in protoplasts. *Plant Cell Rep.* 36, 117–128.
37. Chandrasekaran, J., Brumin, M., Wolf, D., Leibman, D., Klap, C., Pearlsman, M., *et al*. (2016). Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. *Mol. Plant Pathol.* 17, 1140–1153.
38. Luria, N., Smith, E., Reingold, V., Bekelman, I., Lapidot, M., Levin, I., *et al*. (2017). A new Israeli tobamovirus isolate infects tomato plants harboring Tm-22 resistance genes. *PloS One* 12, e0170429.
39. Garcia-Ruiz, H. (2018). Susceptibility genes to plant viruses. *Viruses* 10, 484.
40. Wamaitha, M. J., Nigam, D., Maina, S., Stomeo, F., Wangai, A., Njuguna, J. N., *et al*. (2018). Metagenomic analysis of viruses associated with maize lethal necrosis in Kenya. *Virol. J.* 15, 90.
41. Brooks, C., Nekrasov, V., Lippman, Z. B., and Van Eck, J. (2014). Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. Plant Physiol. 166, 1292–1297. doi: 10.1104/pp.114.247577.
42. Wang, T., Zhang, H. & Zhu, H. CRISPR technology is revolutionizing the improvement of tomato and other fruit crops. Hortic Res 6, 77 (2019). https://doi.org/10.1038/s41438-019-0159-x.

43. Makarova, K. S., &Koonin, E. V. (2015). Annotation and Classification of CRISPR-Cas Systems. Methods in molecular biology (Clifton, N.J.), 1311, 47–75. https://doi.org/10.1007/978-1-4939-2687-9_4.

44. Vu, T.V., Sivankalyani, V., Kim, E.J., Doan, D.T.H., Tran, M.T., Kim, J., et al. (2020) Highly efficient homology-directed repair using CRISPR/Cpf1-geminiviral replicon in tomato. Plant Biotechnol. J. 18: 2133–2143.

45. Martínez, M.I.S.; Bracuto, V.; Koseoglou, E.; Appiano, M.; Jacobsen, E.; Visser, R.G.; Wolters, A.-M.A.; Bai, Y. CRISPR/Cas9-targeted mutagenesis of the tomato susceptibility gene PMR4 for resistance against powdery mildew. BMC Plant Biol. 2020, 20, 284.

46. Hu, T.; Wang, Y.; Wang, Q.; Dang, N.; Wang, L.; Liu, C.; Zhu, J.; Zhan, X. The tomato 2-oxoglutarate-dependent dioxygenase gene SlF3HL is critical for chilling stress tolerance. *Hortic. Res.* 2019, *6*, 1–12.

47. Feder, A., Jensen, S., Wang, A., Courtney, L., Middleton, L., Van Eck, J., et al. (2020). Tomato fruit as a model for tissue-specific gene silencing in crop plants. Hortic. Res. 7:142. doi: 10.1038/s41438-020-00363-4.

48. Shimatani, Z.; Kashojiya, S.; Takayama, M.; Terada, R.; Arazoe, T.; Ishii, H.; Teramura, H.; Yamamoto, T.; Komatsu, H.; Miura, K. Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. Nat. Biotechnol. 2017, 35, 441–443.

49. Yu, S., Ligang, C., Liping, Z., and Diqiu, Y. (2010). Overexpression of OsWRKY72 gene interferes in the abscisic acid signal and auxin transport pathway of Arabidopsis. J. Biosci. 35, 459–471. doi: 10.1007/s12038-010-0051-1.

50. Veillet, F.; Perrot, L.; Chauvin, L.; Kermarrec, M.-P.; Guyon-Debast, A.; Chauvin, J.-E.; Nogué, F.; Mazier, M. Transgene-free genome editing in tomato and potato plants using agrobacterium-mediated delivery of a CRISPR/Cas9 cytidine base editor. Int. J. Mol. Sci. 2019, 20, 402.

51. Xu, X.-X.; Hu, Q.; Yang, W.-N.; Jin, Y. The roles of cell wall invertase inhibitor in regulating chilling tolerance in tomato. BMC Plant Biol. 2017, 17, 195.

52. Rodríguez-Leal, D., Lemmon, Z. H., Man, J., Bartlett, M. E., & Lippman, Z. B. (2017). Engineering quantitative trait variation for crop improvement by genome editing. *Cell*, *171*(2), 470-480.

53. Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, Zhang K, Liu J, Xi JJ, Qiu JL, Gao C (2013) Targeted genome modification of crop plants using a CRISPR-Cas system. Nat Biotechnol 31(8):686–688.
54. Zong Y, Wang Y, Li C, Zhang R, Chen K, Ran Y, Qiu JL, Wang D, Gao C (2017) Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. Nat Biotechnol 35(5):438–440.
55. Li C, Zong Y, Wang Y, Jin S, Zhang D, Song Q, Zhang R, Gao C (2018) Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion. Genome Biol 19(1):59
56. Endo M, Mikami M, Endo A, Kaya H, Itoh T, Nishimasu H, Nureki O, Toki S (2019) Genome editing in plants by engineered CRISPR-Cas9 recognizing NG PAM. Nat Plants 5(1):14–17
57. Shen, C.; Que, Z.; Xia, Y.; Tang, N.; Li, D.; He, R.; Cao, M. Knock out of the annexin gene OsAnn3 via CRISPR/Cas9-mediated genome editing decreased cold tolerance in rice. J. Plant Biol. 2017, 60, 539–547.
58. Xu, R., Yang, Y., Qin, R., Li, H., Qiu, C., Li, L. & Yang, J. (2016). Rapid improvement of grain weight via highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. *Journal of Genetics and Genomics= Yi chuanxue bao*, *43*(8), 529-532.
59. Wang, Y., Geng, L., Yuan, M., Wei, J., Jin, C., Li, M., Yu, K., Zhang, Y., Jin, H., Wang, E., Chai, Z., Fu, X., & Li, X. (2017). Deletion of a target gene in Indica rice via CRISPR/Cas9. *Plant cell reports*, *36*(8), 1333–1343.
60. Zhang, Y., Liang, Z., Zong, Y., Wang, Y., Liu, J., Chen, K., Qiu, J.L., and Gao, C. (2016). Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. Nat.Commun. 7:12617.
61. Wang, W., Simmonds, J., Pan, Q. et al. Gene editing and mutagenesis reveal inter-cultivar differences and additivity in the contribution of TaGW2 homoeologues to grain size and weight in wheat. Theor Appl Genet 131, 2463–2475 (2018). https://doi.org/10.1007/s00122-018-3166-7
62. Shan, Q., Wang, Y., Li, J., and Gao, C. (2014). Genome editing in rice and wheat using the CRISPR/Cas system. *Nat. Protoc.* 9, 2395–2410.

63. Wang Y., Cheng X., Shan Q., Zhang Y., Liu J., Gao C., *et al.* . (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat. Biotechnol. 32, 947–951.
64. León SS, Gil-Humanes J, Ozuna CV, Giménez MJ, Sousa C, Voytas DF, et al. Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. Plant Biotechnol J. (2018) 16:902–10. doi: 10.1111/pbi.12837
65. Li C, Zong Y, Wang Y, Jin S, Zhang D, Song Q, Zhang R, Gao C (2018) Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion. Genome Biol 19:59. https://doi.org/10.1186/s13059-018-1443-z
66. Rey, M.-D., Martín, A. C., Smedley, M., Hayta, S., Harwood, W., Shaw, P., & Moore, G. (2018). Magnesium increases homoeologous crossover frequency during meiosis in ZIP4 (Ph1 gene) mutant wheat-wild relative hybrids. Frontiers in Plant Science, 9, 509– 509. doi: 10.3389/fpls.2018.00509.
67. Liu, H., Wang, K., Jia, Z., Gong, Q., Lin, Z., Du, L.,Ye, X. (2019). Efficient induction of haploid plants in wheat by editing of TaMTL using an optimized Agrobacterium-mediated CRISPR system. Journal of Experimental Botany, 71(4), 1337– 1349. doi: 10.1093/jxb/erz529.
68. Lv, J., Yu, K., Wei, J., Gui, H., Liu, C., Liang, D., Kelliher, T. (2020). Generation of paternal haploids in wheat by genome editing of the centromeric histone CENH3. Nature Biotechnology, 38, 1397– 1401. doi: 10.1038/s41587-020-0728-4.
69. Li, J., Jiao, G., Sun, Y., Chen, J., Zhong, Y., Yan, L., Jiang, D., Ma, Y.,and Xia, L.(2020b). Modification of starch composition, structureand properties through editing of TaSBEIIain both winter and springwheat varieties by CRISPR/Cas9. Plant Biotechnol. J.19:937–951.https://doi.org/10.1111/pbi.13519.
70. Zhang J, Zhang X, Chen R, Yang L, Fan K, Liu Y, Wang G, Ren Z, Liu Y (2020) Generation of Transgene-Free Semidwarf Maize Plants by Gene Editing of Gibberellin-Oxidase20-3 Using CRISPR/Cas9. Frontiers in Plant Science.11:1048. DOI0.3389/fpls.2020.01048.
71. Zhu, J., Song, N., Sun, S., Yang, W., Zhao, H., Song, W., & Lai, J. (2016). Efficiency and inheritance of targeted mutagenesis in maize using CRISPR-Cas9. Journal of Genetics and Genomics, *43*(1), 25-36.

72. McCaw Morgan E., Lee Keunsub, Kang Minjeong, Zobrist Jacob D., Azanu Mercy K., Birchler James A., Wang Kan (2021) Development of a Transformable Fast-Flowering Mini-Maize as a Tool for Maize Gene Editing. Frontiers in Genome Editing, 2:27. DOI 0.3389/fgeed.2020.622227.
73. Curtin, S.J., Michno, J.M., Campbell, B.W., Gil-Humanes, J., Mathioni, S.M.,Hammond, R., Gutierrez-Gonzalez, J.J., Donohue, R.C., Kantar, M.B., Eamens, A.L.,et al., 2016. MicroRNA maturation and MicroRNA target gene expression regulationare severely disrupted in soybean dicer-like1 double mutants. G3; Genes|genomes|genetics 6, 423–433.
74. Haun, W., Coffman, A., Clasen, B. M., Demorest, Z. L., Lowy, A., Ray, E., et al. (2014). Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. *Plant Biotechnol. J.* 12, 934–940. doi: 10.1111/pbi.12201
75. Cai, Y., Chen, L., Liu, X., Sun, S., Wu, C., Jiang, B., *et al*. (2015). CRISPR/Cas9-mediated genome editing in soybean hairy roots. PLoS One 10:e0136064. doi: 10.1371/journal.pone.0136064
76. Jacobs, T.B., LaFayette, P.R., Schmitz, R.J., Parrott, W.A., 2015. Targeted genomemodifications in soybean with CRISPR/Cas9. BMC Biotechnol. 15, 16.
77. Li, Z.S., Liu, Z.B., Xing, A.Q., Moon, B.P., Koellhoffer, J.P., Huang, L.X., Ward, R.T.,Clifton, E., Falco, S.C., Cigan, A.M., 2015. Cas9-guide RNA directed genome editingin soybean. Plant Physiol. 169, 960–970.https://doi.org/10.1104/pp.15.00783.
78. Michno, J.M., Wang, X.B., Liu, J.Q., Curtin, S.J., Kono, T.J., Stupar, R.M., 2015. CRISPR/Cas mutagenesis of soybean andMedicagotruncatulausing a new web-tool and amodified Cas9 enzyme. GM Crops Food 6, 243–252.
79. Sun, X.J., Hu, Z., Chen, R., Jiang, Q.Y., Song, G.H., Zhang, H., Xi, Y.J., 2015. Targetedmutagenesis in soybean using the CRISPR-Cas9 system. Sci. Rep. 5, 10342.https://doi.org/10.1038/srep10342.
80. Du, H.Y., Zeng, X.R., Zhao, M., Cui, X.P., Wang, Q., Yang, H., Cheng, H., Yu, D.Y., 2016.Efficient targeted mutagenesis in soybean by TALENs and CRISPR/Cas9.J.Biotechnol. 217, 90–97.
81. Di, Y.H., Sun, X.J., Hu, Z., Jiang, Q.Y., Song, G.H., Zhang, B., Zhao, S.S., Zhang, H., 2019.Enhancing the CRISPR/Cas9 system based on multiple GmU6 promoters in

soybean.Biochem. Biophys. Res. Commun. 519, 819–823.https://doi.org/10.1016/j.bbrc.2019.09.074.

82. Cai, Y., Chen, L., Liu, X., Guo, C., Sun, S., Wu, C., *et al*. (2018). CRISPR/Cas9-mediated targeted mutagenesis of GmFT2a delays flowering time in soybean. *Plant Biotechnol. J.* 16, 176–185. doi: 10.1111/pbi.12758
83. al Amin, N., Ahmad, N., Wu, N., Pu, X.M., Ma, T., Du, Y.Y., Bo, X.X., Wang, N., Sharif, R.,Wang, P.W., 2019. CRISPR-Cas9 mediated targeted disruption ofFAD2-2microsomalOmega-6 desaturase in soybean (*Glycine max*. L). BMC Biotechnol. 19, e9.https://doi.org/10.1186/s12896-019-0501-2.
84. Wang, S., Yokosho, K., Guo, R., Whelan, J., Ruan, Y. L., Ma, J. F., et al. (2019). The soybean sugar transporter GmSWEET15 mediates sucrose export from endosperm to early embryo. Plant Physiol. 180, 2133–2141. doi: 10.1104/pp.19.00641
85. Zhang, P.P., Du, H.Y., Wang, J., Pu, Y.X., Yang, C.Y., Yan, R.J., Yang, H., Cheng, H.,Yu, D.Y., 2019. Multiplex CRISPR/Cas9-mediated metabolic engineering increasessoya bean isoflavone content and resistance to soya bean mosaic virus. PlantBiotechnol. J., e13302https://doi.org/10.1111/pbi.13302.
86. Kaur, N., Alok, A., Kaur, N., Pandey, P., Awasthi, P., & Tiwari, S. (2018). CRISPR/Cas9-mediated efficient editing in phytoene desaturase (PDS) demonstrates precise manipulation in banana cv. Rasthali genome. *Functional & integrative genomics*, *18*(1), 89-99.
87. Elitzur, T., Yakir, E., Quansah, L., Zhangjun, F., Vrebalov, J., Khayat, E., Giovannoni, J.J. et al. (2016) Banana MaMADS transcription factors are necessary for fruit ripening and molecular tools to promote shelf-life and food security. Plant Physiol. 171, 380– 391.
88. Hu C, Sheng O, Deng G, He W, Dong T, Yang Q, Dou T, Li C, Gao H, Liu S, Yi G, Bi F (2020) CRISPR/Cas9-mediated genome editing of MaACO1 (aminocyclopropane-1-carboxylate oxidase 1) promotes the shelf life of banana fruit. Plant Biotechnol J. https://doi.org/10.1111/pbi.13534.
89. Tripathi L, Ntui VO, Tripathi JN (2020) CRISPR/Cas9-based genome editing of banana for disease resistance. Current Opinion in Plant Biology, 56: 118-126, https://doi.org/10.1016/j.pbi.2020.05.003.

90. Odipio J., Alieai T., Ingelbrecht I., Nusinow D. A., Bart R., Taylor N. J. (2017). Efficient CRISPR/Cas9 genome editing of phyteone desaturase in cassava. Front. Plant Sci. 8:1780.
91. Gomez MA, Lin ZD, Moll T, Chauhan RD, Hayden L, Renninger K, Beyene G, Taylor NJ, Carrington JC, Staskawicz BJ, Bart RS. Simultaneous CRISPR/Cas9-mediated editing of cassava eIF4E isoforms nCBP-1 and nCBP-2 reduces cassava brown streak disease symptom severity and incidence. Plant Biotechnol J. 2019 Feb;17(2):421-434. doi: 10.1111/pbi.12987.
92. Iqbal Z., Sattar M. N., Shafiq M. (2016). CRISPR/Cas9: a tool to circumscribe cotton leaf curl disease. Front. Plant Sci. 7:475.
93. Janga, M. R., Campbell, L. M., & Rathore, K. S. (2017). CRISPR/Cas9-mediated targeted mutagenesis in upland cotton (*Gossypium hirsutum* L.). *Plant Molecular Biology*, *94*(4), 349-360.
94. Li, C., Unver, T., and Zhang, B. (2017c). A high-efficiency CRISPR/Cas9 system for targeted mutagenesis in Cotton (*Gossypium hirsutum* L.). Sci. Rep. 7:43902.
95. Jiang W., Zhou H., Bi H., Fromm M., Yang B., Weeks D. P. (2013). Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice.
96. Feng, C., Yuan, J., Wang, R., Liu, Y., Birchler, J. A., and Han, F. (2016). Efficient targeted genome modification in maize using CRISPR/Cas9 system. *J. Genet. Genomics* 43, 37–43.
97. Li, X., Xie, Y., Zhu, Q., & Liu, Y. G. (2017). Targeted genome editing in genes and cis-regulatory regions improves qualitative and quantitative traits in crops. *Molecular Plant*, *10*(11), 1368-1370.
98. Shi, J., Gao, H., Wang, H., Lafitte, H. R., Archibald, R. L., Yang, M., *et al*. (2017). ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotechnol. J.* 15, 207–216.
99. Wang F., Wang C., Liu P., Lei C., Hao W., GAO Y., *et al*., (2016). Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. PLoS ONE 11:0154027.

100. Lacchini E, Kiegle E, Castellani M, Adam H, Jouannic S, *et al.* (2020) CRISPR-mediated accelerated domestication of African rice landraces. PLOS ONE 15(3): e0229782.

101. Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., *et al*. (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat. Biotechnol. 32, 947–951.

102. Kim D., Alptekin B., Budak H. (2018). CRISPR/Cas9 genome editing in wheat. Funct. Integr. Genomics 18, 31–41.

103. Liang, Z., Chen, K., Li, T., Zhang, Y., Wang, Y., Zhao, Q. & Gao, C. (2017). Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. *Nature communications*, *8*(1), 1-5.

104. Cai, Y., Chen, L., Zhang, Y., Yuan, S., Su, Q., Sun, S., Wu, C., Yao, W., Han, T.and Hou, W. (2020) Target base editing in soybean using a modified CRISPR/Cas9 system. Plant Biotechnol.

105. Yuan, M., Zhu, J., Gong, L., He, L., Lee, C., Han, S., ... & He, G. (2019). Mutagenesis of FAD2 genes in peanut with CRISPR/Cas9 based gene editing. *BMC biotechnology*, *19*(1), 1-7.

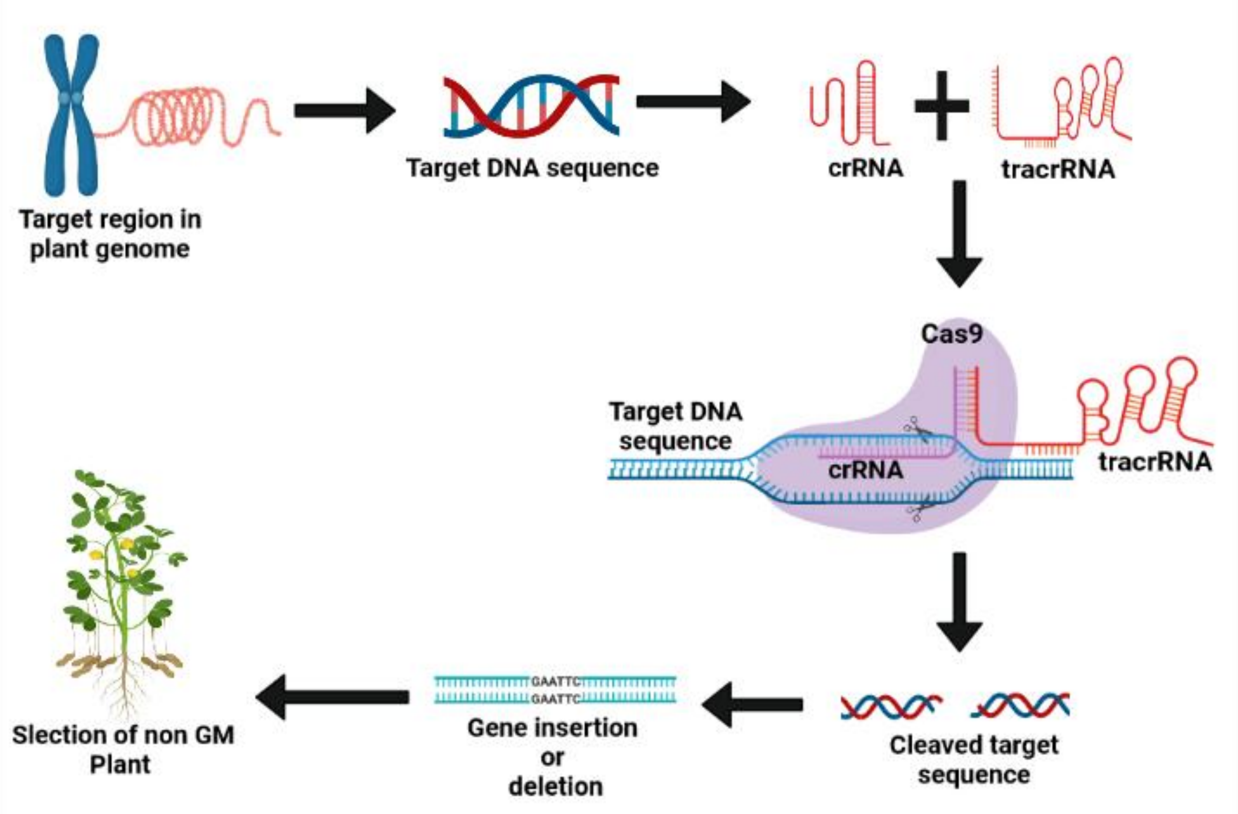

Fig 1. Overview of CRISPR-Cas 9 genome editing

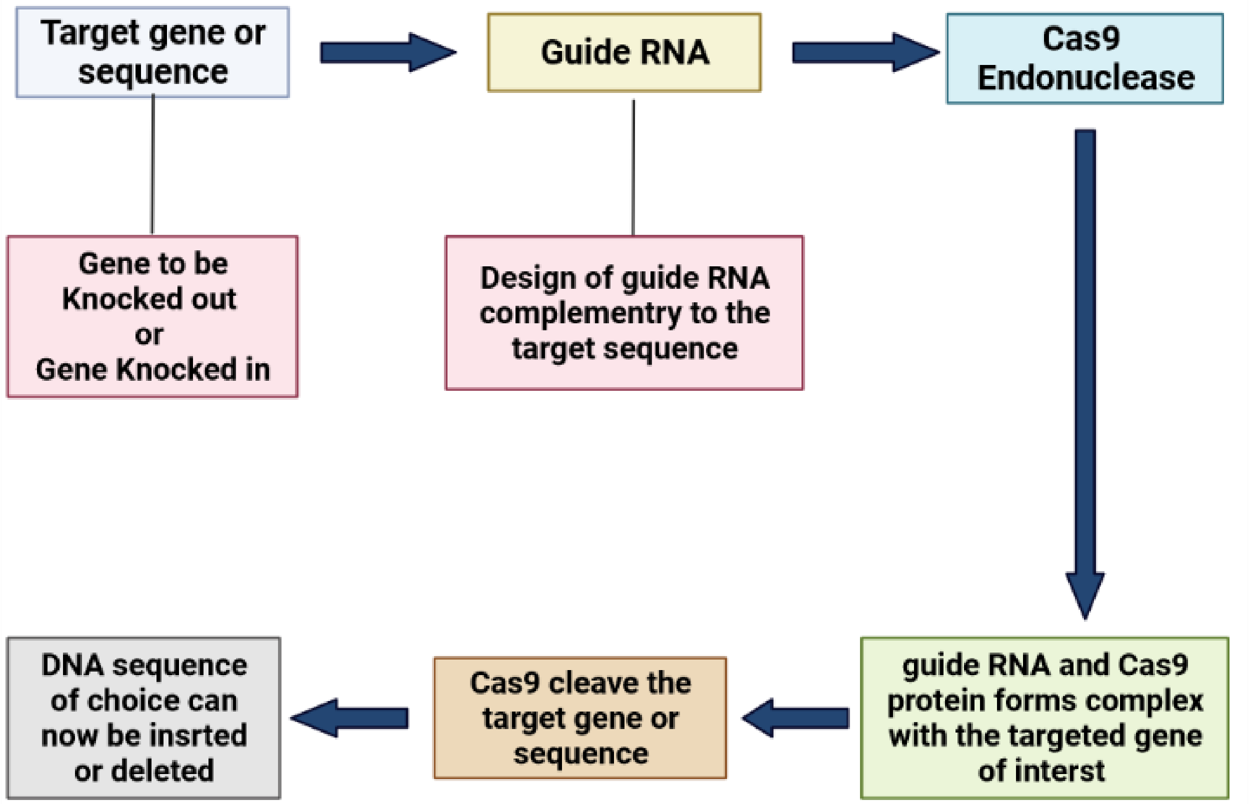

Fig 2. Illustration of involvement of steps in CRISPR/Cas9 based genome editing

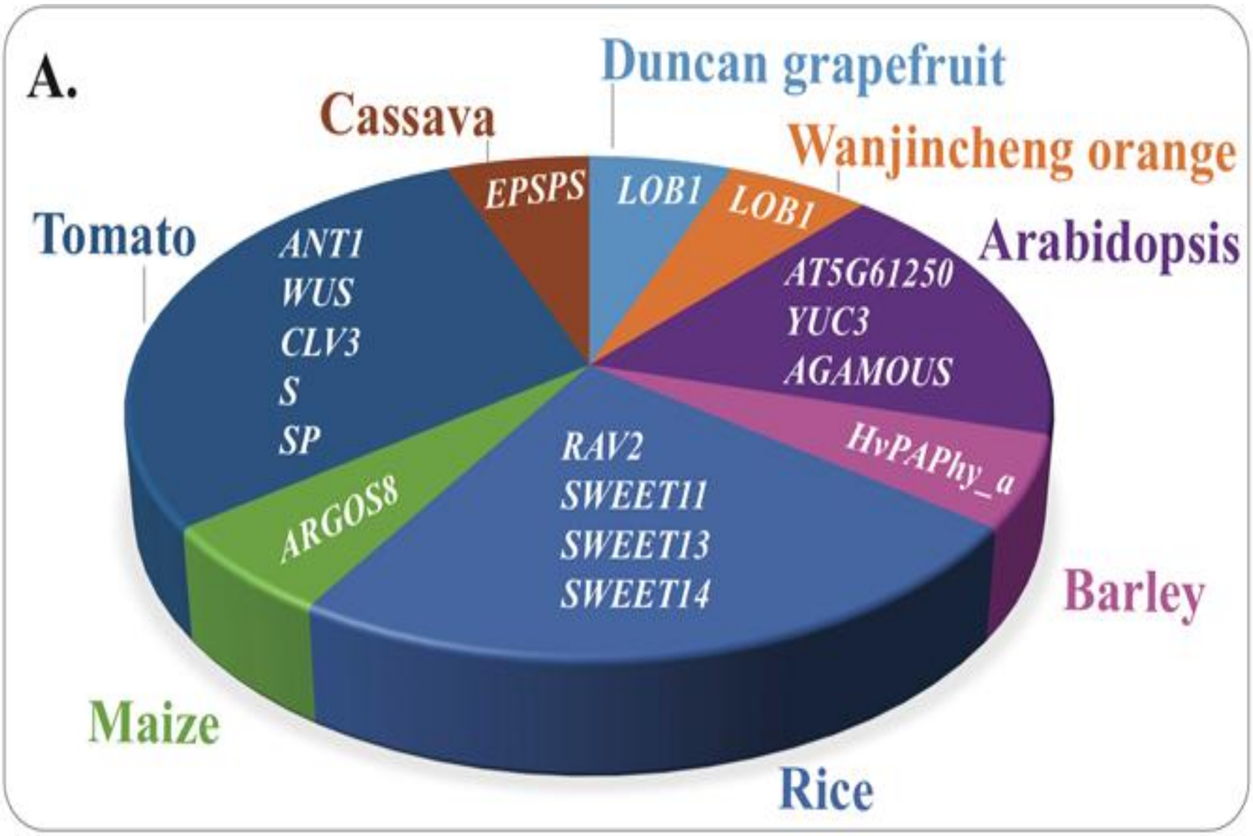

Fig. 3. Presentation of applications of CRISPR/Cas-mediated *cis*-engineering in plants

Table 1 List of CRISPR-Cas9 studies for plant improvement

Crop	**Gene (s) targeted**	**Traits targeted**	**Obtained results**	**References**
Cassava	Phytoene desaturase	Trial for CRISPR	Examination of phenotype	[90]
Cassava	*elF4E, nCBP-1&nCBP-2*	CBSD resistance	Resistance to CBSD	[91]
Cotton	CLCuD IR and Rep	Cotton leaf curl disease	Plants with reduced infection	[92]
Cotton	Green fluorescent protein (GFP)	Phenotypic classification	Insertion of targeted DNA sequence	[93]
Cotton	GhMYB25-like A and GhMYB25-like D	Fiber development	Fiber development improved	[94]
Rice	*OsSWEET11, OsSWEET14*	Bacterial blight resistance	Bacterial blight resistant plants	[95]
Maize	Zmzb7	Albino related gene	Albino plant	[96]
Maize	*ZmTMS5*	Male sterility	Knockout the gene	[97]
Maize	ARGOS8	Hybrid development	Hybrids produced	[98]
Rice	*OsERF922*	Resistance to rice blast	Resistance enhanced	[99]
Rice	*HTD1, GS3, GW2* and *GN1A*	Agronomic prospective	Mutants with improved seed yield	[100]
Wheat	*TaMLO-A1, TaMLO-B1* and *TaMLO-D1*	Resistance to powdery mildew	No infection in edited plants	[101]
Wheat	*TaDREB2* and *TaERF3*	Initial trial	Abiotic stress tolerance	[102]
Wheat	*TaMLO*	Resistance against powdery mildew	Resistant plants	[62]
Wheat	(*TaGW2* and *TaGASR7*)	Ribonucleoproteins	Transgene-free plants	[103]
Soybean	GmFEI2 and GmSHR)	Hairy root system	Successful examination of use of hairy root system	[72]
Soybean	*GmFT2a* and *GmFT4*	Flowering time	Successfully induced single base substitution	[104]
Groundnut	*FAD 2*	Oleic acid content	Three mutations identified	[105]