GENOME EDITING IN PLANTS CRISPR/CAS-9

Abstract

The prokaryotes' genome is shielded against infectious viruses and plasmids by the RNA represent as CRISPR and Cas proteins. This is a tool for site-specific alteration, genome engineering, crop improvement, and molecular therapy because of its low cost, versatility, less time consuming, and efficiently modify endogenous genes in a wide variety of cell types and in organisms that are being manipulated genetically. Therefore, CRISPR-Cas 9 offers a quick and effective way to introduce specific mutations into a target DNA strand with high accuracy and efficacy. RNA-guided nuclease Cas 9 induce double-strand break (DSB) and the breaks are corrected by cellular DNA repair mechanisms and mediate genome modifications. It results in gene modifications by both Homology directed repair (HDR) and Non-Homologous end-joining repair (NHEJ) mechanisms. CRISPR-Cas 9 was recently established in plants by stable transformation. This chapter discusses the use of Cas9 as a plant genome editing tool to advance crop research, plant breeding to satisfy future agricultural demand growth.

Keywords: CRISPR/CAS-9, DSB, NHEJ, Crop Improvement

Authors

Anjali Gaur
Research Scholar
Department of Centre for Rural Development and Technology (CRDT)
IIT Delhi.
Indian Institute of Delhi
Hauz Khas, New Delhi, India.
anjaligaur827@gmail.com

Saurabh Pandey
Project Scientist
Department of Centre for Rural Development and Technology (CRDT)
IIT Delhi.
Indian Institute of Delhi
Hauz Khas, New Delhi, India.
saurabhpandey28@gmail.com

Copyright © 2024 Authors

I. INTRODUCTION

Clustered regularly interspaced short palindromic repeats, or "CRISPR" for short, are DNA sequences found in prokaryotes that offer acquired resistance against attacks from viruses or plasmids as well as genetic alteration that may be present in the environment. Its derived from the Streptococcus pyrogens (Jinek et al., 2012, Weeks et al., 2015) and it contains of 3 genes – 1 encoding Cas 9 nuclease and 2 non-coding RNA genes (tracr-RNA and pre-crRNA) (Statello et al., 2021). RNA- guided endonuclease multiple gene mutations and then germ-line communication has been accomplished (Statello et al., 2021, Li D et al., 2013, Li W et al., 2013, Wang H et al., 2014) and modified endogenous genes of interest in several organisms (Jinek et al., 2012, Cong W.C et al., 2022, Li et al., 2013, Montecillo et al., 2020, Wang et al., 2014, Statello et al., 2021).

Its covers of three phases – Adaptation (Garneau et al., 2010, LA Maraffini 2010), Immunization (Howarth P et al., 2010, Bhaya et al., 2011) and Spacer acquisition (Karginov FV et al., 2010, Vander Oost et al 2009). In this defense process, foreign nucleotide fragments are recognized as non-self and are combined into the host genome flanked by short nucleotide repeats. These repeats in conjunction through Cas proteins identify the foreign DNA and finish them. The pre-crRNA comprises spacers interspaced via identical shortest which are managed to form crRNA in assortment by tracr-RNA. The two RNA nucleotide can be substituted by one RNA nucleotide using a single g-RNA which has a designed hairpin that impersonators cr-RNA-tracr-RNA complex. Both the PAM nucleotides and gRNA-DNA base pairing immediately downstream of the target area determine the binding selectivity of Cas 9 with the target DNA (Statello et al., 2021, Statello et al., 2021).

Earlier introducing a heterologous DNA into plants was usually done by Agrobacterium-mediated transformation and biolistic methods invented in the 1980s. Nowadays, many approaches are used for gene editing and targeting that are more precise. ZFNs (Zinc Engineered Nucleases), TALENs (Transcription activator-like effector nucleases), HR (Homologous Recombination) and EMN (Engineered mega nucleases) are mostly used for editing. ZFNs and TALENs, both are created on DNA protein interactions and CRISPR which is an RNA-guided DNA endonuclease (Brooks, Nov 2014, Wada et al 2020). ZFNs and TALENs have successfully revolutionized the concept of Plant Breeding and allow rapid progress for the improvement of crops.

CRISPR-Cas 9 has wide applications for bacterial, animal systems, and most importantly plant systems due to its easy construction design and assembly. CRISPR/CAS 9 efficacy has been reported in the Arabidopsis (*Gao* et al., 2016) and *Nicotiana benthamiana* (Belhaj K et al., 2013), Sorghum, wheat, and rice.

The tomato is very efficient for CRISPR/Cas 9 gene editing due to its efficient transformation availability (Montecillo et al.,2020), and diploid genome.

The problem faced with the techniques ZFNs and TALENs is that their role as dimers and protein factor are compulsory. Designing ZFNs is difficult due to the intricated nature of the interaction between Zinc fingers and DNA (González et al., 2021).

Copyright © 2024 Authors

90% of Archaeal and 48% of bacterial genomes have the CRISPR-Cas (Rousseau et al., 2009). Cas 9 is a versatile nature that binds and cleaves DNA targets (Jinek et al., 2014, Nishimasu et al., 2014, Sternberg SH et al., 2014, Anders C et al., 2014). First Cas proteins and a second CRISPR locus that holds genetic memory. The second component is primarily the sg-RNA of CRISPR/Cas 9 which is an artificial RNA chimera twisted via fusing cr-RNA with tracr-RNA (Voytas DF 2013).

II. APPLICATION IN CROP IMPROVEMENT

1. **Rice:** Rice is cropping species to go through gene editing employing TALENs (Shen et al., 2013a) and Cas 9/sg RNA (Feng et al., 2013, Jiang et al 2013, Shan et al 2013b, Weeks et al., 2015). The capability to find bi-allelic alterations in a simple generation (Feng et al 2013, Xu et al., 2014, Zhou et al.m 2014) and chance to remove huge number of nucleotide in chromosomes (Zhou et al., 2014, Weeks et al., 2015) and gene replacement accessibility over Homologous recombination (Feng et al., 2013, Weeks et al., 2015).

2. **Maize:** Multiple genes in maize have been precisely edited using maize TALENs and CRISPR/Cas9 systems (Li et al., 2014; Weeks et al., 2014). It has been established that Cas9/sg-RNA can effective gene knockouts and and replacements.

3. **Soya Beans:** This system has also been used for Soyabean (Jacobs et al., 2015). Either using Agrobacterium rhizogenes-derived hairy root and somatic embryo cultures, GFP and nine endogenous loci were edited (Weeks et al., 2015).

4. **Wheat:** One of the first plants to be altered with CRISPR/Cas9 was wheat (Shan et al., 2013). Subsequential demonstrated knockouts of Inositol oxygenase and phytoene desaturase genes (Upadhyay et al., 2015). It exposed the simultaneous expression of two multiplexed sgRNAs genes targeting two closely spaced target in wheat genome caused nucleotide segment between the both sites to be proficiently deleted (Weeks et al., 2015).

5. **Tomato:** Due to the availability of an effective transformation methodology, tomato is frequently used to investigate gene editing in a dicot (Van Eck et al., 2006). In their findings, (Brooks et al., 2014) showed how the CRISPR/Cas9 produced several target mutations in tomatoes that were stable transgenics by using two sgRNAs that permitted homozygous deletions of a suitable size in the first progeny. Cermak et al., 2015 investigated that the geminivirus vectors is competent apparatus for gene targeting in tomato.

III. RECENT RESEARCH STUDIES ABOUT CRISPR/CAS 9 SYSTEM

A study shows that transgenic upregulated Cas 9 expression are virus and symptom-free. so this investigation suggested that it has been great potential to enhance plant immunity, as it might be used for several targets at viral genomes (Jix et al., 2015). Delivering sg-RNA and Cas 9 protein into the target cells is necessary for CRISPR/Cas 9-based genome editing (Kumar & Jain, 2014). plant-codon optimized version of the cas9 approach is using by many scientists and experts in genetic engineering (Jiang et al., 2013b, Li et al., 2013, Miao et al., 2013, Shan et al., 2013b, Kumar & Jain 2014). Many experiments and investigations using human codon-optimized version in CRISPR/Cas9 systems in plants

Copyright © 2024 Authors

(Feng et al., 2013, Mao et al., 2013, Nekrasov 2013, Upadhyay et al., 2013, Xie & Yang 2013). A few promoters are reported such as EFIA, CMV, VBO, and LTR to derive Cas 9 expression in plants (Kumar & Jain 2014). 35s promoter of cauliflower mosaic viruses (CMV) has been mostly reported (Belhaj 2013, Kumar & Jain 2014).

IV. CHALLENGES

The NHEJ repair mechanism is not accurate and produces indels at the cut site, resulting in endogenous gene disruption/ mutagenesis (Lloyd et al., 2005, Zhang et al., 2010, Battles et al., 2014, Belhaj et al., 2015). Battles et al., 2014 suggested an efficient and facile vector using Gemini-virus (a large family of plant viruses circular, ss-DNA which infects both monocots and dicots) that replicates to overcome the lack of NHEJ mechanism. An off-target mutation is one of the biggest drawbacks of CRISPR/Cas technology which is yet to overcome. CRISPR-Cas technology recognizes targets using Watson-Crick DNA. With the help of nucleotide data analysis, off-target sites can be predicted more precisely (Cho et al., 2014, Bortesi & Fischer 2015, Basak & Nithin 2015). Strategies considered for minimizing off-target mutations are- Source-(Basak & Nithin 2015) such as,

- Accurate and precise target selection.
- Elongation of gRNAs and Cas9 mutations.
- Additionally, on-target mutations often precede off-target mutations which result in the loss of novel mutations after regeneration.

V. CONCLUSION

Genome editing of model organisms is vitally important to study gene function, which is detrimental to human health and agricultural production of more sustainable food yields. It is important for the development of a fundamental understanding of crop sciences as well as for producing agricultural and innovative qualities for farmers and consumers. This technique is an intriguing approach to genome editing in plant biology because of its robustness and understandability. To date, the chief application of the CRISPR/Cas 9 is to knockout. Additional modifications of the CRISPR/Cas 9 will help the application in agriculture. However, its application in gene editing is still surging in plants. New enhanced strategies will further improve CRISPR/Cas-9 to develop ncRNA which is a vital regulator of biological mechanisms which include developmental processes and diseases. Successful applications of the CRISPR/Cas-9 in editing nc-RNA in plants will support to the elucidation and decode action thus opening a boulevard in plant biology.

REFERENCES

[1] Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A: Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. J Bacteriol 1987, 169: 5429-5433.

[2] Jinek M, Jiang F, Taylor DW, Sternberg SH, Kaya E, Ma E, Anders C, Hauer M, Zhou K, Lin S et al.: Structures of Cas9 endonucleases reveal RNA-mediated conformational activation. Science 2014, 343:1247997

[3] Nishimasu H, Ran FA, Hsu PD, Konermann S, Shehata SI, Dohmae N, Ishitani R, Zhang F, Nureki O: Crystal structure of Cas9 in complex with guide RNA and target DNA. Cell 2014, 156:935-949.

[4] Sternberg SH, Redding S, Jinek M, Greene EC, Doudna JA: DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. Nature 2014, 507:62-67.

[5] Anders C, Niewoehner O, Duerst A, Jinek M: Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. Nature 2014, 513:569-573.

Copyright © 2024 Authors

[6] Voytas DF: Plant genome engineering with sequence-specific nucleases. Annu Rev Plant Biol 2013, 64:327-350.
[7] Zetsche, Bernd; Gootenberg, Jonathan S.; Abudayyeh, Omar O.; Slaymaker, Ian M.; Makarova, Kira S.; Essletzbichler, Patrick; Volz, Sara E.; Joung, Julia; van der Oost, John; Regev, Aviv; Koonin, Eugene V.; Zhang, Feng (October 2015). "Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System". Cell 163 (3): 759–771. doi:10.1016/j.cell.2015.09.038
[8] Cas9 gets a classmate Erik J Sontheimer & Scot A Wolfe Nature Biotechnology 33,1240–1241 (2015) doi:10.1038/nbt.3426 Published online 09 December 2015.
[9] Xu Y, Li Z. CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. Comput Struct Biotechnol J. 2020 Sep 8;18:2401-2415. doi: 10.1016/j.csbj.2020.08.031. PMID: 33005303; PMCID: PMC7508700.
[10] P. Raffeiner et al., An MXD1-derived repressor peptide identifies noncoding mediators of MYC-driven cell proliferation. Proc. Natl. Acad. Sci. U.S.A. 117, 6571–6579 (2020).
[11] M. Jinek et al., A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337, 816–821 (2012)
[12] T. Wang, J. J. Wei, D. M. Sabatini, E. S. Lander, Genetic screens in human cells using the CRISPR-Cas9 system. Science 343, 80–84 (2014).
[13] Statello, L., Guo, CJ., Chen, LL. et al. Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol 22, 96–118 (2021).
[14] Montecillo, Jake Adolf V., Luan Luong Chu, and Hanhong Bae. 2020. "CRISPR-Cas9 System for Plant Genome Editing: Current Approaches and Emerging Developments" Agronomy 10, no. 7: 1033. https://doi.org/10.3390/agronomy10071033
[15] Gan WC, Ling APK. CRISPR/Cas9 in plant biotechnology: applications and challenges. BioTechnologia (Pozn). 2022 Mar 24;103(1):81-93. doi: 10.5114/bta.2022.113919. PMID: 36605382; PMCID: PMC9642946.
[16] Wada, N., Ueta, R., Osakabe, Y. et al. Precision genome editing in plants: state-of-the-art in CRISPR/Cas9-based genome engineering. BMC Plant Biol 20, 234 (2020). https://doi.org/10.1186/s12870-020-02385-5
[17] Liu L, Fan XD. CRISPR-Cas system: a powerful tool for genome engineering. Plant Mol Biol. 2014 Jun;85(3):209-18. doi: 10.1007/s11103-014-0188-7. Epub 2014 Mar 18. PMID: 24639266.
[18] Liu X, Wu S, Xu J, Sui C, Wei J. Application of CRISPR/Cas9 in plant biology. Acta Pharm Sin B. 2017 May;7(3):292-302. doi: 10.1016/j.apsb.2017.01.002. Epub 2017 Mar 11. PMID: 28589077; PMCID: PMC5443236.
[19] Gao X, Chen J, Dai X, Zhang D, Zhao Y. An Effective Strategy for Reliably Isolating Heritable and Cas9-Free Arabidopsis Mutants Generated by CRISPR/Cas9-Mediated Genome Editing. Plant Physiol. 2016 Jul;171(3):1794-800. doi: 10.1104/pp.16.00663. Epub 2016 May 15. PMID: 27208253; PMCID: PMC4936589.
[20] Jiang W, Yang B, Weeks DP. Efficient CRISPR/Cas9-mediated gene editing in Arabidopsis thaliana and inheritance of modified genes in the T2 and T3 generations. PLoS One. 2014 Jun 11;9(6):e99225. doi: 10.1371/journal.pone.0099225. PMID: 24918588; PMCID: PMC4053344.
[21] Belhaj, K., Chaparro-Garcia, A., Kamoun, S. *et al.* Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. *Plant Methods* **9**, 39 (2013). https://doi.org/10.1186/1746-4811-9-39
[22] Montecillo, Jake Adolf V., Luan Luong Chu, and Hanhong Bae. 2020. "CRISPR-Cas9 System for Plant Genome Editing: Current Approaches and Emerging Developments" Agronomy 10, no. 7: 1033. https://doi.org/10.3390/agronomy10071033
[23] González Castro, Nicolás, Jan Bjelic, Gunya Malhotra, Cong Huang, and Salman Hasan Alsaffar. 2021. "Comparison of the Feasibility, Efficiency, and Safety of Genome Editing Technologies" International Journal of Molecular Sciences 22, no. 19: 10355. https://doi.org/10.3390/ijms221910355
[24] Nemudryi AA, Valetdinova KR, Medvedev SP, Zakian SM. TALEN and CRISPR/Cas Genome Editing Systems: Tools of Discovery. Acta Naturae. 2014 Jul;6(3):19-40. PMID: 25349712; PMCID: PMC4207558.
[25] Gupta RM, Musunuru K. Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. J Clin Invest. 2014 Oct;124(10):4154-61. doi: 10.1172/JCI72992. Epub 2014 Oct 1. PMID: 25271723; PMCID: PMC4191047.

Copyright © 2024 Authors