

Potential Application of Genome Editing in Crop Science: Challenges and Opportunities

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INTRODUCTION

Recent advancement in crop science has supplanted the impediments of conventional and molecular breeding of crop improvement. The accelerated genome-editing tools like ZFNs, TALENs, Cas-9 based CRISPR have been utilized in a wide variety of plant species to characterize gene functions and improve agricultural traits (Anzalone, et al., 2019). These genome-editing tools have ability to alter the plant genome by addition or deletion of base sequences on the various genome regions. Using genome-editing tools, several crop species have been improved through precise targeted mutagenesis and gene targeting. Site-specific nucleases (SSNs) are one of basic platform to accelerate the genome-editing tools by changing the location of genes present in the genome.

Genome-editing tools have been used by researches since last two decades for the alteration of plant genome for agronomical traits, nutritional profile, and stress resistance (Ainley, et al., 2013). Genome editing approaches are more cost efficient, quicker, and precise in achieving desired crop improvement as compared to traditional methods and other biotechnological approaches. In crop species, several metabolic pathways have been altered using these genome-editing tools. Considering the importance of crop species for global food security, genome-editing techniques have been widely employed for their improvement to advance novel varieties with enhanced produce and superiority. Genome-editing tools are well suited for those crops, whose genome has been completely sequenced. Due to availability of complete genomic data of the plant we can successfully apply the genome editing tools for alteration of genomes. Cereal (rice, wheat, maize, barley, and sorghum), pulses, oil yielding crops, vegetables, fruits and cash crops have been improved for various purposes using genome-editing tools (Armario, et al., 2019).

In addition, hybrid seed production has also been improved through targeting cytoplasmic male sterility in many crops. The crop plants improved using genome-editing techniques might be established as non-genetically modified crops and have no any risk in the environment. Zinc Finger Nucleases (ZFNs) is the first technique for highly targeted genome engineering, the discovery of zinc finger nucleases (ZFN) improved the effectiveness of gene targeting in several ways.

Genome editing tools

Dr. Emmanuelle Carpenter, one of scientific founders, co-invented CRISPR/Cas9 gene editing. He has explored the targeted nucleases to explore the ability to manipulate genomic sequence. Jennifer Doudna is the biggest name in the world of genome editing. CRISPR-Cas9, a powerful technology discovered in 2012 by American scientist Jennifer Doudna, French scientist Emmanuelle Charpentier (Makarova, et al., 2015).

The genome editing tools including ZFNs, TALENs and CRISPR would be helpful to the researchers for altering the genome for crop improvement (Li et al., 2011). Introduction of genome editing into modern breeding programs should facilitate rapid and precise crop improvement. ZFNs are the first genome editing tools generated by fusing zinc finger DNA-binding domains to the cleaved DNA domain (Shukla et al., 2009; Fiaz et al., 2019). Zinc finger domain is able to target desired DNA sequences with in the complex genomic region. The DNA binding domains associated with zinc finger nuclease associated with three and six zinc finger nucleases. This is one of the important tools used in the genome editing of several crops for alteration of various genomic region.

TALENs are another genome editing tools applicable for genome editing of crop plants. This genome editing technology is known to function in a variety of host systems, including bacteria, yeast, plants, insects, zebrafish, and mammals. They are almost

similar to the ZFNs. TALENs are fusions of transcription activator-like (TAL) proteins and a FokI nuclease (Li, et al., 2012). TAL proteins are composed of 33-34 amino acid repeating motifs with two variable positions that have a strong recognition for specific nucleotides (Boch, et al., 2009; Doyle, et al., 2012).

Cas-9 based CRISPR technology of genome editing is one of the advanced system frequently applied in many crop plant for alteration of genomic region for the development of smart crop cultivars (Shmakov et al., 2017). CRISPR-Cas9 uses a specially designed RNA molecule to guide an enzyme called Cas9 to a specific sequence of DNA (Jansen et al., 2002). Cas9 then cuts the strands of DNA at that point and removes a small piece, causing a gap in the DNA where a new piece of DNA can be added for the alteration of nucleotide sequences. The system consists of two parts i.e. the Cas9 enzyme and a guide RNA. Rapidly translating a revolutionary technology into transformative therapies (Ishino et al., 1987).

Application of genome editing

Genome editing is one of the wonderful techniques used to make precise and targeted changes in the DNA of organism. Most of the genome editing tools can be applied to develop more resilient crops and for the improvement of agronomically important traits as well as quality traits. Potential application of genome editing includes more accurate and faster diagnosis, more targeted alteration of genome by addition or deletion of nucleotide sequences, and prevention of diseases and disorders.

Crop being improved more rapidly when genome-editing tools come in to existence. Genome-editing tools are useful and able to provide information on genome size, gene number, gene mapping, gene sequencing, crop plant evolution, gene cloning, DNA marker recognition, marker assisted selection, transgenic breeding, linkage map creation and QTL mapping. They are also used in gene

therapy, cell therapy, and the screening and development of new drugs.

Some genome editing tools like Cas-9 based CRISP technology able to utilize site-specific genome editing has great therapeutic potential for achieving long-term, stable gene expression. Viral diseases in several crops have also been targeted using Cas-9 based CRISPR technology for the development of resistance against viral diseases through alteration of nucleotide sequences on the various genomic regions. Some important crop cultivars improved for various traits using genome-editing tools are given in the table.

CONCLUSION

Genome editing is the wonderful tools of the crop improvement. Crops including cereals, fruits, vegetables, pulses and oil yielding crops needs to be improve for insect, pests, fungal pathogen, bacterial pathogens and other quality parameters. These area of crop improvement cannot be achieved by molecular breeding and transgenic approaches due to several limitations. However, Genome editing tools play a crucial role in the coming future within the committed period for the strategic improvement of crop plants to fulfill the requirements.

REFERENCES

- Ainley, W.M., Sastry-Dent, L., Welter, M.E. (2013). Trait stacking via targeted genome editing. *Plant Biotechnol J* 11:1126–1134.
- Anzalone, A.V., Randolph, P.B., Davis, J.R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* 576(7785):149–157.
- Armario Najera, V., Twyman, R.M., Christou, P. (2019). Applications of multiplex genome editing in higher plants. *Curr Opin Biotechnol* 59:93–102.
- Boch, J., Scholze, H., Schornack, S., Landgraf, A., Hahn, S., Kay, S., Lahaye, T., Nickstadt, A., Bonas, U. (2009). Breaking the code of DNA binding specificity of TAL-type III effectors. *Science*. 326, 1509–1512.
- Doyle, E.L., Booher, N.J., Standage, D.S., Voytas, D.F., Brendel, V.P., VanDyk, J.K., Bogdanove, A.J. (2012). TAL Effector-Nucleotide Targeter (TALEN) 2.0: Tools for TAL effector design and target prediction. *Nucleic Acids Res.* 40, W117–W122.
- Fiaz, S., Ahmad, S., Noor, M.A., Wang, X., Younas, A., Riaz, A., Riaz, A., Ali, F. (2019). Applications of the CRISPR/Cas9 system for rice grain quality improvement: Perspectives and opportunities. *Int. J. Mol. Sci.* 20, 888.
- Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., 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.
- Jansen, R., Embden, J.D.V., Gaastra, W., Schouls, L.M. (2002). Identification of genes that are associated with DNA repeats in prokaryotes. *Mol. Microbiol.* 43, 1565–1575.
- Li, T., Huang, S., Zhao, X., Wright, D.A., Carpenter, S., Spalding, M.H., Weeks, D.P., Yang, B. (2011). Modularly assembled designer TAL effector nucleases for targeted gene knockout and gene replacement in eukaryotes. *Nucleic Acids Res.* 39, 6315–6325.
- Li, T., Liu, B., Spalding, M.H., Weeks, D.P., Yang, B. (2012). High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nat. Biotechnol.* 30, 390.
- Makarova, K.S., Wolf, Y.I., Alkhnbashi, O.S., Costa, F., Shah, S.A., Saunders, S.J., Barrangou, R., Brouns, S.J., Charpentier, E., Haft, D.H. (2015). An updated evolutionary classification of CRISPR–Cas systems. *Nat. Rev. Microbiol.* 13, 722.

Shmakov, S., Smargon, A., Scott, D., Cox, D., Pyzocha, N., Yan, W., Abudayyeh, O.O., Gootenberg, J.S., Makarova, K.S., Wolf, Y.I. (2017). . Diversity and evolution of class 2 CRISPR–Cas systems. *Nat. Rev. Microbiol.* 15, 169.

Shukla, V.K., Doyon, Y., Miller, J.C., DeKolver, R.C., Moehle, E.A., Worden, S.E., Mitchell, J.C., Arnold, N.L., Gopalan, S., Meng, X. (2009). Precise genome modification in the crop species *Zea mays* using zinc-finger nucleases. *Nature.* 459, 437.