JUST AGRICULTURE
multidisciplinary e-Newsletter

CRISPR: A Tool for Crop Improvement

Deepak Kumar
Department of Genetics and Plant Breeding, College of Agriculture, CCS Haryana Agricultural University, Hisar, Haryana

ARTICLE ID: 012

Summary

Unlike first-generation genome editing tools, CRISPR/Cas9 genome editing involves simple designing and cloning methods, with the same Cas9 being potentially available for use with different guide RNAs targeting multiple sites in the genome. After proof-of-concept demonstrations in crop plants involving the primary CRISPR-Cas9 module, several modified Cas9 cassettes have been utilized in crop plants for improving target specificity and reducing off-target cleavage (e.g., Nmcas9, Sacas9, and Stcas9). Further, the availability of Cas9 enzymes from additional bacterial species has made available options to enhance specificity and efficiency of gene editing methodologies. CRISPR/Cas9 genome editing used in many agricultural plant species, by targeting varied genes of interest for improved nutritionary price, increased sickness resistance and improved tolerance against drought, organic phenomenon and abiotic stress.

Introduction

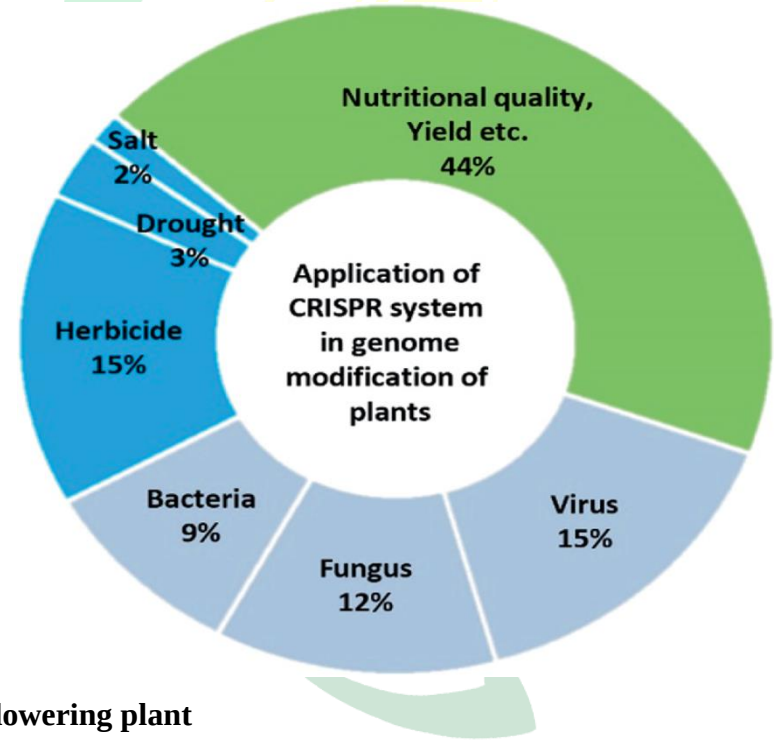
Crop quality has been a larger concern of customers since it's directly related to human health by providing multiple nutrients like proteins, fiber, vitamins, minerals, and bioactive compounds. Scientists and breeders have additionally step by step shifted their focus from increasing production to rising quality.

This technology employed in totally different traits in agriculture as well as infective agent resistance, plant development and morphology, fiber development, abiotic and organic

JUST AGRICULTURE
multidisciplinary e-Newsletter

phenomenon stress, even secondary metabolites. Additionally to flowering plant crops, CRISPR-Cas9 has been extensively used for attribute improvement in cereals like maize, rice, wheat. CRISPR was initial known in E. coli in 1987, as an immune mechanism to fight against invasive microorganism and inclusion body polymer.

In recent years, CRISPR/Cas systems have developed to become the foremost in style GE technology. Compared with different SDNs, the CRISPR/Cas systems square measure a lot of economical and simple for ordination redaction as a result of the specificity of redaction is determined by ester complementarily of the guide polymer to a selected sequence while not advanced macromolecule engineering. Therefore, several researchers have applied CRISPR/Cas tools to cistron useful analysis. Once introduced into crop improvement field, GE will considerably accelerate the progress of desired traits' insertion and greatly save labor and different prices.



CRISPR in flowering plant

Cotton

Recently, cotton genome has also been edited for targeted mutagenesis through CRISPR/Cas for improved lateral root formation. In addition, an efficient and fast method has been developed to evaluate guide RNAs transiently in cotton. The targeted disruption of undesirable genes or metabolic pathway can be achieved to increase quality of cotton. Undesirable metabolites like gossypol in cottonseed can be targeted efficiently using ENs for

JUST AGRICULTURE
multidisciplinary e-Newsletter

seed-specific low-gossypol cotton (Rathore *et al.*, 2020). Moreover, CRISPR/Cas is also helpful in gene stacking for herbicide resistance, insect resistance, and abiotic stress tolerance. Recently developed CRISPR/Cpf1 system is a highly specific and efficient system in plant genome editing, which will be a very promising alternative of the CRISPR/Cas9 system in cotton (Li *et al.*, 2019).

Rice

CRISPR technology will so be probably accustomed target any attribute of interest within the rice ordination within the close to future. incontestable sequence-specific CRISPR/Cas9 mediate genomic modification of 3 rice genes, phytoene desaturase (OsPDS), alkaloid organic compound dehydrogenase (OsBADH2) and mitogen-activated macromolecule enzyme (OsMPK2) genes that square measure concerned in rice.

Wheat

Targets were found within the mutant bread of wheat population. AN extended protocol of RNP delivery has been created out there by Liang et al. (2018). This DNA-free piece of writing methodology avoids time overwhelming procedures like copulate breeding for the removal of the transgene and permits to get transgenefree plants at T0. If these limitations are often overcome, the RNP methodologies are AN economical approach to attain CRISPR/Cas9 primarily based ordering piece of writing in crop species, particularly perennial crops.

Maize

Maize is one in every of the foremost vital cereal crops within the world. High economical and correct sequence modification would profit in maize biological science study and breeding. Multiplex ordering piece of writing in maize was incontestable by ki *et al.* (2016) employing a tRNA-RNA process system. A multiplex piece of writing vector will incorporate a cluster of gRNAs separated by spacers in an exceedingly polycistron, manufacturing multiple gRNAs from one primary transcript. The study targeted 3 transcription issue genes (MADS, MYBR, and AP2) for simplex piece of writing and 3 alternative genes (RPL, PPR, and IncRNA) for multiplex piece of writing. Enlarged piece of writing potency (upto 100%) was determined for t-RNA process primarily based multiplex piece of writing. Current high yielding maize varieties area unit the results of hybrid maize seed production and also the production of hybrid maize needs sterilization to avoid

JUST AGRICULTURE
multidisciplinary e-Newsletter

impregnation. In Maize thermosensitive sequence male-sterile five (ZmTMS5), celebrated to cause male sterility was targeted for ordering piece of writing by CRISPR/Cas9 approach (Li *et al*., 2017b).

Compare to alternative endogen crop in maize (Zea mays) phytic acid constitutes over seventieth of the maize seed. It's believed to be anti-nutritional because it isn't digestible by monogastric animals and is additionally an environmental waste matter. Liang *et al.* (2014) have reportable targeted knock out of genes concerned in phytic acid synthesis (ZmIPK1A, ZmIPK, and ZmMRP4) in Z. mays. Similarly, Zhu *et al.* (2016) incontestable sequence piece of writing of phytoene synthase sequence (PSY1) exploitation maize U6 snRNA promoter. PSY1 is concerned in antioxidant biogenesis and its mutant (psy1) ends up in white kernels and unusual person seedlings. Among fifty 2 T0 lines obtained by Agrobacterium-mediated transformation, seven lines were reportable to hold the psy1 knockout attribute and every one seven lines were deep sequenced to grasp the sort of variation and to guage the mutation potency. The results showed that no off-target sites were altered and stable psy1 mutants were obtained.

Conclusion

The new breeding procedures make it easier and faster to produce new features in crops than traditional breeding methods. The most significant accomplishments were the development of disease-resistant crops, improved grain quality, nutritional price, and stress-free agricultural development. Since the previous five years, sequence writing by CRISPR has proven to be one of the most advanced techniques, and it is unquestionable in a variety of agricultural industries. Exploiting this technology in a suitable manner to write the sequence of plants in order to overcome a variety of features and, potentially, to overcome the feed of an ever-increasing human population.

References

Chilcoat, D., Liu, Z. B., & Sander, J. (2017). Use of CRISPR/Cas9 for crop improvement in maize and soybean. *Progress in molecular biology and translational science*, *149*, 27-46.

Jaganathan, D., Ramasamy, K., Sellamuthu, G., Jayabalan, S., & Venkataraman, G. (2018). CRISPR for crop improvement: an update review. *Frontiers in plant science*, *9*, 985.

Li B, Rui H, Li Y, Wang Q, Alariqi M, Qin L, Sun L, Ding X, Wang F, Zou J, Wang Y, Yuan D, Zhang X, Jin S. (2019). Robust CRISPR/Cpf1 (Cas12a)-mediated genome editing in allotetraploid cotton (Gossypium hirsutum). *Plant Biotechnol J.*, 17(**10**):1862-1864. doi: 10.1111/pbi.13147.

Liu, Q., Yang, F., Zhang, J., Liu, H., Rahman, S., Islam, S, & She, M. (2021). Application of CRISPR/Cas9 in Crop quality improvement. *International Journal of Molecular Sciences*, *22*(8), 4206.

Rathor, K. S., Pandeya, D., Campbell, L. M., Wedegaertner, T. C., Puckhaber, L., Stipanovic, R. D., and Hake, K. (2020). Ultra-Low Gossypol Cottonseed: Selective Gene Silencing Opens Up a Vast Resource of Plant-Based Protein to Improve Human Nutrition. *Critical Reviews in Plant Sciences*, *39*(**1**), 1-29.

Zhang, D., Zhang, Z., Unver, T., & Zhang, B. (2021). CRISPR/Cas: A powerful tool for gene function study and crop improvement. *Journal of Advanced Research*, *29*, 207-221.