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CRISPR or CAS: Potential tool for Crop Improvement

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SUMMARY

Crop improvement has introduced various techniques for enhancing desirable traits in our cultivated crops. CRISPR/Cas mediated genome editing system is such a recently emerging plant breeding tool for crop improvement. To prevent gene expressions and to insert the desirable genes in targeted locations, a single guide RNA along with the suitable Cas protein molecule can be used for targeted gene editing. This technique can also be employed for studying plant functional genomics and enhancing traits such as quantity, quality, stress resistance (both biotic and abiotic) and to create genetic variability in cultivated crops. CRISPR/Cas has attained widespread popularity in the field of genome editing technology. However, in addition to its benefits, CRISPR too has some limitations viz., designing extremely specific guide RNA, a suitable vector and a threat of disastrous misuse.

INTRODUCTION

Clustered regularly interspaced short palindromic repeats (CRISPR) are usually segments of prokaryotic DNA containing short repetitions of base sequences (Deveau *et. al.,* 2010). Each repetition is followed by short segments of "spacer DNA" from previous exposures to a bacteriophage virus or plasmid. CRISPR/Cas system is naturally found in most of the prokaryotes such as bacteria and archaea (Horvath *et. al.,* 2010), which aids in developing adaptive immune mechanisms against foreign genes of many bacteriophages and plasmids invading them. There are around 40 different Cas protein families found in nature, and depending on their actions they are categorised into 3 types i.e., Type I, Type II and Type III (Makarova *et. al.,* 2011). Cas9 protein belongs to Type II is widely popularised in genome editing technology (Garneau *et. al.,* 2010). Cas9 was the first nuclease discovered, followed by Cpf1, which was discovered in the CRISPR/Cpf1system of *Francisella novicida*. By transferring the Cas9 nuclease complexed with a synthetic guide RNA (gRNA) into a cell, the cell's genome can be cut at the desired location, allowing the addition and removal of genes. The most advantageous thing about CRISPR/Cas9 system is its applicability amongst diverse organisms. Editing carried out for research purposes may not necessitate the same level of inflexibility as those for therapeutic applications. However, one should need to carefully examine any plants or animals undergoing genome editing. The first description about CRISPR was made by Yoshizumi Ishino from Osaka University in 1987, who accidentally cloned part of a CRISPR together with the *iap* gene, the target of interest (Ishino *et. al.,* 1987). The organization of the repeats was unusual because repeated sequences are typically arranged consecutively along with DNA. The function of the interrupted clustered repeats was unknown at the time and it was revealed later. CRISPR/Cas technology has got a wide range of applications in different fields such as gene therapy, gene tagging, gene mapping, gene sequencing to study functional genomics and gene transformation (Yashwanth *et. al.,* 2021).

Components of CRISPR

- Protospacer adjacent motif (PAM)
- CRISPR-RNA (crRNA)
- trans-activating crRNA (tracrRNA)

Table 1. Traits improved in few crops by CRISPR/ CAS gene-editing technique

S. N	Crop	Target gene	Improved trait
1.	Rice	*OsMPK5*	Resistance to various biotic and abiotic stresses
2.	Wheat	*TaMLO*	Powdery mildew resistance
3.	Rice	*OsERF922*	Resistance to blast
4.	Maize	*ARGOS8*	Higher yield under stress conditions
5.	Tomato	*5P5G*	Enhanced yield attributes
6.	Tomato	*SIMAPK3*	Drought tolerance

7.	Soybean	*Rj4*	Increased root nodulation
8.	Lettuce	*NcED4*	Higher seed germination
9.	Orange	*CsLOB1* promoter	Resistance to citrus canker
10.	Cotton	*Gh14-3-3d*	Broad disease resistance
11.	Banana	*RAS-PDS*	Carotenoid synthesis
12.	Potato	*GBSS*	High amylopectin and Low amylose
13.	Cotton	*Rep, βC1*	Resistance to cotton leaf curl virus (CLCuV)
14.	Wheat	*TaERF3*	Ethylene responsive factor
15.	Cucumber	*eIF4E*	Resistance to cucumber vein yellowing virus (CVYV)

CRISPR/Cas system can be regarded as one of the ideal genome engineering technology because of:

- Highly specificity
- Target multiple sites in the genome at a time
- High cleavage efficiency
- Broad applicability to both *in vivo* and *in vitro* application
- Precise transgene integration at specific loci.
- Developing biotic and abiotic resistant traits in crop plants.
- A potential tool for developing virus-resistant crop varieties
- Multifunctional programmability
- Simple editing tools allow unparalleled ability to scale and optimize at speed
- Can create a high degree of genetic variability at a precise locus in the genome of the crop plants
- A potential tool for multiplexed reverse and forward genetic study

CONCLUSION

CRISPR/Cas is a magnificent tool that must be used along with conventional breeding methods for alluring results. It necessitates being a part of conventional methods rather than merely an alternative. It very well may be utilised to improve the desirable characters and to create desirable variation. This precise tool helps to enhance the quantity, quality, resistance to various abiotic and biotic stresses, storage ability, early maturity and many other traits in crop improvement programmes. It also contributes to food security and thus aids in decreasing hunger and malnutrition. Thus, it can be very well stated that it is an exact mutagenesis procedure that has a significant role in crop improvement and genome editing programmes.

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