Biological Forum – An International Journal **13(1): 26-29 (2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Role of Clustered Regularly Interspaced Short Palindromic Repeats in Crop Improvement - A review

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(Received 02 November 2020, Accepted 28 January, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Genome editing for desirable traits is one of the essential techniques for crop improvement. CRISPR/Cas mediated genome editing system is such a recently emerging plant breeding tool for crop improvement, which is a natural adaptive immune mechanism in most bacteria and archaea. A single guide RNA along with the suitable Cas protein molecule can be used for targeted gene editing to prevent gene expressions and to insert the desirable genes in targeted locations. This precise method can be used for studying plant functional genomics and enhancing morphological traits, quantity, quality, resistance to biotic and abiotic stress and to create genetic variability in both field and horticultural crops. CRISPR/Cas has been a practically successful mechanism in the field of genome editing technology. Here, we describe its origin and applications in crop improvement. However, CRISPR too has some limitations viz., designing highly specific guide RNA, a capable vector and fear of catastrophic misuse. This tool must be used along with conventional breeding techniques to create desirable genotypes of interest rapidly, saving time and resources.

Key words: CRISPR/Cas, Crop improvement, Genome editing, sgRNA.

INTRODUCTION

Development of cultivars with resistance to biotic and abiotic stresses along with sound quality and quantity is the principal role of plant breeding. There are different techniques to reach the desirable results of crop improvement, but low precision and high time consumption are the major constraints after the unavailability of gene resources. Genome editing technology can be used to overcome such limitations. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) - associated Cas protein system is the new genome editing technology that is widely used these days. CRISPR/Cas system is naturally found in most of the prokaryotes such as bacteria and archaea (Horvath *et al.,* 2010), which helps in building up adaptive immune mechanisms against foreign genes of many bacteriophages and plasmids invading them.

CRISPR/Cas genome editing system depends on small RNA sequences that are specific and complementary to some part of invading foreign genes and Cas proteins can cleave the genes thereby silencing their effect. Thus, this mechanism of sequence-specific DNA binding domain along with non-specific DNA cleave domain system is adapted in the process of genome editing. The crispr rna sequences which are specific to the target gene with tracr rna together known as single guide rna (sgRNA) helps in the identification of target sequence and Cas protein helps to cleave the sequence at specific sites. This tool can be used to suppress undesirable genes, to insert and over express a desirable gene, thereby it can be used for deletion, insertion, and substitution of genes precisely. There are around 40 different Cas protein families found in nature, based on their actions they are divided into 3 types Type I, Type II and Type III (Makarova *et al.,* 2011). Cas9 protein belongs to Type II is widely used in genome editing technology.

CRISPR/Cas technology has a wide range of applications in different fields such as crop improvement, gene therapy, gene sequencing, gene tagging, gene mapping to study functional genomics and gene transformation. This technology has a precise action that helps to produce accurate results in a very short span of time compared to many other genome editing technologies and breeding techniques. It has a high efficiency to improve phenotype through genotype and produce a good result in any crop improvement programme.

Role in Crop Improvement

CRISPR/Cas associated genome editing system can be extensively used in crop improvement to create genotypes with ability to produce a good quantity and quality yields. It can also be used to produce genotypes with resistance and tolerance to many biotic and abiotic stresses and thus increase the productivity with many other desirable agronomic characters. This tool helps to identify the function of specific gene sequences by knockout mechanism and thus the function of gene is known. This tool has been used to find out the function of many genes in different crops.

Yield is the most important trait which is generally governed by large number of genes. Using CRISPR/Cas, few genes governing yield are found in rice but a lot of genes to be known yet. This tool can be used for complex genome editing, which may help to increase the yield by creating precise mutations. New allelic variations of *ARGOS8* genes are used in hybrid seed production of high yielding under stress conditions is done in maize (Shi *et al.,* 2017). The complex genome editing using CRISPR/Cas9 in *5P5G* gene increased the yields in tomato (Soyk *et al.,* 2017). Programmed editing of *OsSPL16* gene improved grain yield in rice (Usman *et al.,* 2020).

Quality is the other important trait to be improved. Increasing the nutritional value of genotype is the efficient way to reduce the malnutrition. The improved quality helps to manage the health issues. In maize, the phytic acid is the major component of the seed, which reduces the digestive ability. Knockout of genes involved in phytic acid synthesis helped to increase the digestive ability. *lncRNA1459* mutants of tomato resulted in late ripening due to the inhibition of ethylene and carotenoid synthesis in tomato (Li *et al.,* 2018). Knockout of all genes of *GBSS* in potato reduced the amylose and increased the amylopectin content (Anderson *et al.,* 2017). Knockout of *RAS-PDS* genes in banana effected the carotenoid synthesis (Kaur *et al.,* 2018).

Many biotic (plant pathogens, insects, and pest) and abiotic stresses (unfavourable conditions) are main reason for loss of yields in almost all crops. Certain genes mutated using CRISPR/Cas helped to maintain yield even under such stress conditions. Knockout mutants of *OsSWEET13* gene in IR24, rice genotype provided resistance against bacterial blight disease caused by *Xanthomonas sps* (Zhou *et al.,* 2015). The CRISPR/Cas targeted mutation in *OsERF922* (ethylene responsive factor) gene provided resistance to blast resistance to blast in rice (Liu *et al.,* 2012). Knockout study of *OsMPK5* gene in rice showed its ability to provide resistance against various biotic and abiotic stresses (Xie and Yang 2013). In wheat knockout of *TaMLO* gene using CRISPR/Cas9 technology was susceptible to mildew hence providing proof that *TaMLO* gene is mildew resistance locus (Wang *et al.,* 2014). The CRISPR mediated genome editing of *Gh14-3-3d* provided a broad disease resistant cultivar in cotton (Zhang *et al.,* 2018). In cucumber, *eIF4E* gene (Chandrasekaran *et al.,* 2016) provided resistance to cucumber vein yellowing virus (CVYV), zucchini yellow mosaic virus (ZYMV), and papaya ring spot mosaic virus (PRSV-W). CRISPR/Cas13a is used for interference in turnip mosaic virus (TuMV). *CsLOB1 promoter* gene provides resistance to citrus canker in orange (Peng *et al.,* 2017). Resistance to cotton leaf curl virus (CLCuV) in cotton can be extend by using CRISPR technology (Khan *et al.,* 2020). The knockout mutants of wheat for *TaDREB2* gene explained it as dehydration response protein gene (Kim *et al.,* 2018). Knockout of *GmFT2* delayed flowering even in favourable conditions in soybean (Cai *et al.,* 2018). Knockout of *SIMAPK3* genein tomato provided it as gene responsible for drought tolerance (Wang *et al.,* 2017). Knockout of *NcED4* gene increased seed germination even under high temperatures in lettuce (Bertier *et al.,* 2018).

Crop	**Target Gene**	**Target Trait**	**References**
Maize	*ARGOS8*	Increased yields under stress conditions	Shi *et al.,* 2017
Tomato	*5P5G*	Increased yields	Soyk *et al.,* 2017
Rice	*OsSPL16*	Improved yields	Usman *et al.,* 2020
Tomato	*lncRNA1459*	Late ripening	Li *et al.,* 2018
Potato	*GBSS*	Low amylose and High amylopectin	Andersson *et al.,* 2017
Banana	*RAS-PDS*	Carotenoid synthesis	Kaur *et al.,* 2018
Rice	*OsSWEET13*	Resistance to bacterial blight	Zhou *et al.,* 2015
Rice	*OsERF922*	Resistance to blast	Liu *et al.,* 2012
Rice	*OsMPK5*	Resistance to various biotic and abiotic stresses.	Xie and Yang 2013
Wheat	*TaMLO*	Resistance to powdery mildew	Wang *et al.,* 2014
Cotton	*Gh14-3-3d*	Broad disease resistance	Zhang *et al.,* 2018
Cucumber	*eIF4E*	Resistance to cucumber vein yellowing virus (CVYV), zucchini yellow mosaic virus (ZYMV), and papaya ring spot mosaic virus (PRSV-W)	Chandrasekaran *et al.,* 2016
Orange	*CsLOB1* promoter	Resistance to citrus canker	Peng *et al.,* 2017
Cotton	*Rep, βC1*	Resistance to cotton leaf curl virus (CLCuV)	Khan *et al.,* 2020
Wheat	*TaDREB2*	Dehydration response protein	Kim *et al.,* 2018
Soybean	*GmFT2*	Flowering	Cai *et al.,* 2018
Tomato	*SIMAPK3*	Drought tolerance	Wang *et al.,* 2017
Lettuce	*NcED4*	Increased seed germination	Bertier *et al.,* 2018
Wheat	*TaERF3*	Ethylene responsive factor	Kim *et al.,* 2018
Soybean	*Rj4*	Root nodulation	Tang *et al.,* 2016
Tomato	*SlIAA9*	Improved the leaf shape and provided seedless fruits	Ueta *et al.,* 2017

Many other desirable characters are improved using CRISPR/Cas genome editing. Knockout mutant of *TaERF3* explained it as ethylene responsive factor in wheat (Kim *et al.,* 2018). Knockout of *Rj4* in soybean provided root nodulation with many strains (Tang *et al.,* 2016). Targeted mutations in *SlIAA9* genes of tomato improved the leaf shape and provided seedless fruits (Ueta *et al.,* 2017).

CONCLUSION

CRISPR/Cas is a magnificent tool which must be used along the conventional breeding methods for desirable results. It must be a part of conventional methods but not an alternative. It can be used to improve the desirable characters and to create desirable variation. This precise tool helps to improvise the quantity, quality, resistance and tolerance to many biotic and abiotic stresses, storage ability, earliness and many other traits and crop improvement objectives. It also helps to decrease the hunger and malnutrition and can be considered as one of the best tools that contribute food security. It is a precise mutagenesis technique which have an important role in crop improvement and genome editing programmes.

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How to cite this article: Yashwanth, Sadu, Chaitanya, Sri Sai and Darwankar, Mayur S. (2021). Role of Clustered Regularly Interspaced Short Palindromic Repeats in Crop Improvement - A Review. *Biological Forum – An International Journal*, **13**(1): 26-29.