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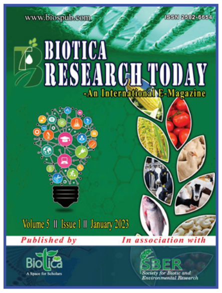

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CRISPR/Cas: A Novel Genetic Tool for Crop Quality Improvement

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Abstract

Climate change and demographic growth have become a major concern for the global thinkers, leading to food insecurity in terms of both quality and quantity. Since the beginning of crop improvement, yield has been the primary driver. But now, to feed the population a healthy diet, new methodologies have to be designed in addition to conventional methods. In this regard, CRISPR/Cas is a genome editing tool which changes base sequences at specific location (gene of interest) to carryout desirable point mutations that may give improvement in the quality related traits.

Introduction

The rapidly increasing world population has led to a substantial rise in the demand for staple crops. There has been estimate that by 2050, the world population will be around 9.6 billion and 60% increase in demand for the staple food crops. Since green revolution, major focus has been on yield improvement ignoring the importance of the quality. But crop quality has been a matter of concern since it is directly associated with human health by making availability of multiple nutrients such as proteins, fibres, vitamins, minerals, and bioactive compounds (Slavin and Lloyd, 2012). In the Global Hunger Index Report 2022 (Grebmer *et al.,* 2022) India ranked at 107th out of 127 countries deciphering need of improvement of nutritional profile of the crops along with the significant improvement in the yield. Improvement in quality through traditional crop breeding methods require more labour and resources and often time consuming. So, genome editing through use of novel technology, clustered regularly interspaced short palindromic repeat (CRISPR/Cas) have potential to create predictable and inheritable mutations in specific regions with the minimum chances of off-site mutations (Lino *et al.,* 2018). Hence, by the use of this technology we can accelerate the pace of plant breeding in the direction of quality improvement.

Basic CRISPR/Cas9 Structure

The CRISPR/Cas9 locus consists of three elements, namely a tracer RNA at the 50 end, a Cas protein-encoding gene, and a CRISPR locus at the 30 end, which includes a lead sequence, a 23-50 base pairs repeat sequence, and a spacer sequence (Figure 1).

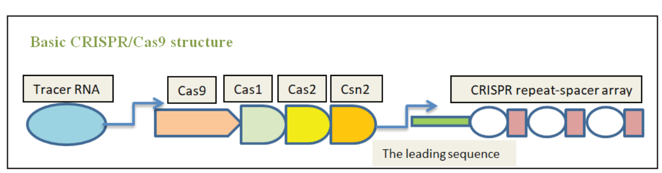

Figure 1: Basic CRISPR/Cas9 structure

Workflow of the Technology

Step 1: Selection of desired gene and designing of spacers for these genes

Step 2: Preparation of transformation carrier

Step 3: Transformation of plant cells

Step 4: Identification of edited lines in T_0 by NGS

Step 5: Selection of null plants having edited genes in T_1 and confirm by NGS in T_2

Step 6: Evaluation of expression of target genes in the edited homozygous lines

Step 7: Use of null lines for breeding new variety

Quality Improvement by using CRISPR/Cas Gene-Editing Technology

As living of standards is increasing day by day in this modern era, the demand for high-quality crops is growing. Crop quality depends upon both external and internal traits and has profound influence on the market value of crops. Physical attributes, like size, colour, texture and fragrance are important determinates affecting crop quality. The composition of nutrients (proteins, starch and lipids) and bioactive substances (carotenoids, lycopene, γ-aminobutyric acid and flavonoids) influence the internal qualityrelated traits (Liu *et al.,* 2021). Recent advancement in quality improvement by the application of CRISPR/Cas9 gene-editing technology has resulted in production of grains having superior nutritional, physical, textural and cooking quality (Table 1).

Table 1: List of Crops Improved for different Quality Parameters by CRISPR/Cas

Application	Crop	Target gene	Associated trait
Physical and appearance quality	Wheat	TaGW7	Grain shape
	Maize	Psy1	Seed colour
	Rice	GW2, GW5, TGW6	Grain length and width
	Tomato	OVATE, Fas, Fw2.2	Fruit size, oval fruit shape
Texture, palatability quality	Banana	MaACO1	Long shelf life
	Rice	OsGBSSI	Low amylose content
	Barley	HvGBSSIa	Low amylose content
Nutritional quality	Rapeseed	BnITPK	Low phytic acid content
	Wheat	α-gliadin genes	Low gluten content

Conclusion

In the domain of crop breeding and functional genomics, the CRISPR/Cas9 system has been utilized as a versatile tool. It induces target specific gene modulations that has made possible to develop numerous new varieties having desired characteristics. In spite of this, gene modulation is still at a stage of deciphering the genomic function and regulatory mechanisms. The commercialization of gene-edited crops still has far to go. Despite the challenges that need to be resolved, it is believed that this will be more widely used in future and will play an important role in improvement of crop quality.

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