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APPLICATIONS OF THE CRISPR/CAS9 TECHNIQUE IN MAIZE AND WHEAT BREEDING

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Abstract

Maize and wheat are some of the world's most important food crops, so their breeding programs are important for global food security. Genome editing techniques (the latest advancement in genetics) are not replacements for conventional breeding techniques, they are new methods and innovative, which promote agricultural crop breeding programs and offers unprecedented solutions to food insecurity. Among these techniques, CRISPR-Cas9 is considered a more effective tool for genome editing due to its low cost and simplicity. This paper summarizes recent applications of the CRISPR/Cas9 technique in maize and wheat breeding. The implementation of this technique allows the production of non-transgenic crops with high yield under different environmental stresses promoting sustainable agriculture.

***Key words**: CRISPR/Cas9, genome editing, maize, wheat*

INTRODUCTION

The world's population rapidly increased and continues to do so, estimating that in 2050 it will reach 10 billion, therefore a 60-100% increase in world food production will be necessary (Dhankher and Foyer, 2018), while the agricultural area on per capita is decreasing every year (Ritchie and Roser, 2019). This situation is aggravated by climate changes that affect agricultural systems and food security. Therefore, obtaining new crop varieties (with high yield and resistant to biotic and abiotic stress) is the main objective of plant breeding (Bonea, 2020). Maize and wheat are some of the most important crops used as food, feed and industrial raw material that ensure food security for billions of people (Ștefan and Constantinescu, 2011; Constantinescu and Olaru, 2017; Bonea, 2020; Constantinescu et al., 2021). Due to their worldwide importance, these crops have become a target for genetic improvement. According to the Food and Agriculture Organization, in 2020/2021 of the total global production of maize (1483.2 million tonnes), 871.1 million tonnes were utilized as feed, 393.6 million tonnes to other non-food uses and 223 million tonnes as food, and of the total global production of wheat (776.7 million tonnes), 148 million tonnes were utilized as feed, 88.9 million tonnes to other non-food uses and 525.5 million tonnes as food (FAO, 2022).

Farmers and plant breeders have developed varieties of various agricultural plants through conventional technologies, such as selection and propagation of plants with useful traits. Genetic modification of plants (transgenesis) is a modern breeding technique used to improve agricultural crops which consists in modifying the genome of an organism, as a result of introducing new genes (transgenes), or as a result of changing the expression of one/some genes already present in the cell (Bonea, 2013; Urechean and Bonea, 2017; Bonea, 2021). In 1996, genetically modified crops occupied only 1.7 million hectares, and in 2018 it reached an area of 191.7 million hectares (Bonea, 2022). However, these genetically modified crops have been controversial for various reasons (agricultural policies, insufficient information, public concerns, etc) (Ezezika et al., 2012). Also, concerns regarding the use of certain chemicals (e.g. glyphosate) in combination with herbicide-tolerant genetically modified plants, or the transmission of antibiotic resistance genes, have led to the adoption of very strict regulations on genetically modified plants, making it difficult to produce varieties suitable for current threats (Zaidi et al., 2020).

Recently, new plant breeding technologies have emerged that include genome editing and differ from genetic modification.

According to scientists, gene/genome editing is not "genetic modification" because the method of introducing DNA changes is no different from changes that can occur during conventional reproduction or in nature (Pacher and Puchta, 2017). For example, CRISPR-Cas can be used for precise genetic manipulation without inserting exogenous DNA, such as antibiotic resistance genes, thus eliminating the fear that foreign DNA may be present in the final product (He and Zhao, 2020).

This paper presents a summary of recent CRISPR/Cas9 technique applications in the maize and wheat breeding.

GENOME EDITING

Genome editing includes a series of molecular techniques that allow the induction of directed (targeted) changes in the genomes of organisms.

Four major classes of sequence-specific nucleases (SSNs) are used for genome editing: Meganucleases (MNs); Zinc-Finger Nucleases (ZFNs); TALENs (Transcription Activator-Like Effector Nucleases) and proteins Cas9. These nucleases can be engineered to bind and cleave a specific nucleic acid sequence, introducing double-strand breaks (DSBs) at or near the target site (Pickar-Oliver and Gersbach, 2019).

Figure 1 shows the advantages and disadvantages for each of these genome editing techniques.

CRISPR/CAS9 TECHNIQUE FOR CROP IMPROVEMENT

Clustered Regularly Interspersed Short Palindromic Repeats/CRISPR- associated protein 9 (CRISPR/Cas9) system has two main components: the Cas9 protein which produces DSBs (double-strand break) at a targeted site and a single guide RNA (sgRNA) that identifies a specific DNA sequence thus, when changing the design of sgRNA, numerous desired sites can be targeted (Jinek et al., 2012; Kim and Kim, 2014).

Figure 2 shows the mechanism of genome editing using CRISPR/Cas9 involving DNA unwinding by sgRNA, cutting of gene by Cas9, genome analysis, sgRNA cloning, plants transformation and selection, regeneration, genomic DNA extraction and sequence analysis to confirm the results. Therefore, this process needs no foreign element for editing (Rasheed et al., 2021).

The CRISPR/Cas9 technique is a more versatile genome editing technique that has many advantages: low cost, good adaptability, time efficiency, the ability to instantly direct the reproduction of multiple genes, it is also more cost-effective and simpler compared to ZFNs, TELENTs and MNs (Hsu et al., 2014; Braatz et al., 2017).

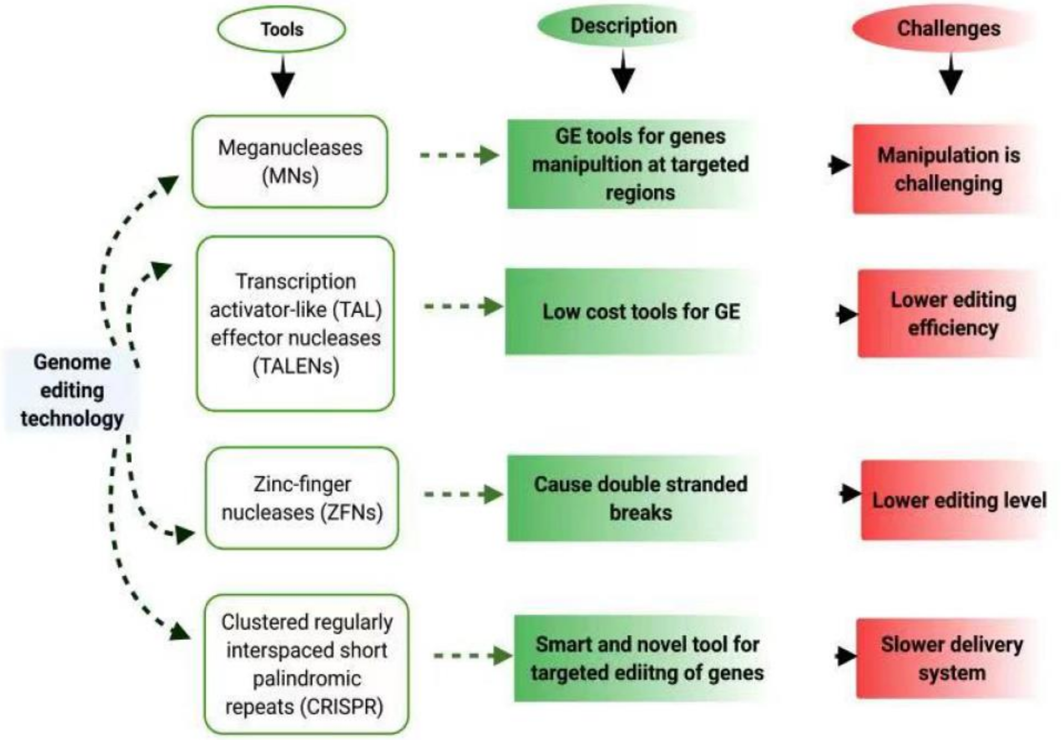

Figure 1. A comparison of genome editing tools regarding their efficiency and limitations (Source: Rasheed et al., 2021)

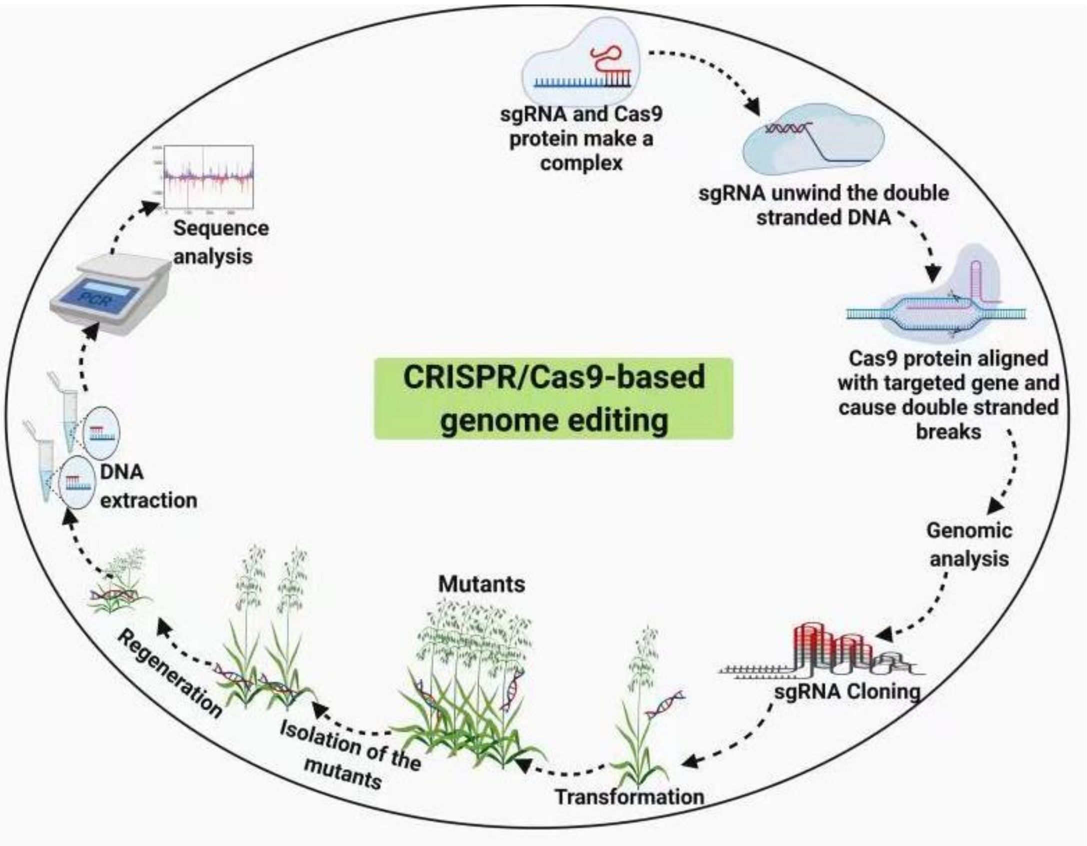

Figure 2. Mechanism of genome editing using CRISPR/Cas9 (Source: Rasheed et al., 2021)

Due to these advantages, CRISPR/Cas9 has been used to improve some traits in many monocot and dicot crops, such as yield, quality, and resistance to biotic and abiotic stresses (Ma and Liu, 2016).

Creating a new approach to manipulating the genomes of living organisms, genome editing has revolutionized the biological sciences.

Many techniques have been developed to improve some traits to crops, but CRISPR/Cas9 is the most useful and latest method (Rasheed et al., 2021).

According to Martin-Laffon et al. (2019), the total number of patents on the CRISPR-Cas9 landscape until 2019 was of 1052, in agriculture was of 374, in industry was of 192 and in medical was of 614.

Zion Market Research firm projected that the CRISPR-Cas9 gene editing market will reach US$4.271 billion by 2024, a compound annual growth rate (CAGR) of 36.8%, and Grand View Research firm anticipates the global market for gene editing will reach $8.1 billion by 2025 (CRS, 2018).

CRISPR/Cas, is a critical gene editing tool that was developed in 2013 by two scientists named Jennifer Duodna and Emmanuelle Charpenteir, who demonstrated that CRISPR can be used to modify human genes outside the body (Jinek et al., 2012; Doudna and Charpentier, 2014).

This technique is currently used for various aspects in plant breeding such as yield and quality, disease resistance, drought tolerance.

Table 1 shows some recent applications in maize and wheat breeding.

Table 1. Examples of recent applications of the CRISPR/Cas9 technique to maize and wheat breeding

Species	Trait improved	Technique	Gene(s) edited	References
Maize	grain yield	CRISPR/Cas9	*Waxy*	Gao et al. (2020)
	grain yield	CRISPR/Cas9	*CLE*	Liu et al. (2021)
	drought tolerance	CRISPR/Cas9	*ARGOS8*	Shi et al. (2017)
	herbicide tolerance	CRISPR/Cas9	*ZmALS1, ZmALS2*	Nuccio et al. (2021)
	salinity tolerance	CRISPR/Cas9	*ZmHKT1*	Zhang et al. (2018)
	reduced phytic acid	TALENs, CRISPR/Cas9	*ZmPDS, ZmIPK1, ZmIPK, ZmMRP4*	Liang et al. (2014)
	reduced zein content	CRISPR/Cas9	*PRL*	Qi et al. (2016)
	thermosensitive male-sterile	CRISPR/Cas9	TMS5	Li et al. (2017)
Wheat	grain yield (seed size and thousand grain weight)	CRISPR/Cas9	*GW2, LPX-1,MLO*	Wang et al., 2018
	grain yield	CRISPR/Cas9	*GASR7*	Zhang et al. (2016)
	low gluten wheat for reduced allergenicity	CRISPR/Cas9	*Alpha-gliadin array, Gli-2 locus*	Sanchez-Leon et al. (2018)
	powdery mildew resistance	CRISPR/Cas9	*EDR1*	Zhang et al. (2017)
	grain quality in hardness, starch composition and dough colour	CRISPR/Cas9	*pinb, waxy, ppo and psy*	Zhang et al. (2021)
	increased phosphorus uptake	CRISPR/Cas9	*PHO2*	Ouyang et al. (2016)
	Increased nutritional quality (biofortification)	CRISPR/Cas9	*VIT*	Connorton et al. (2017)
	increased abiotic stress	CRISPR/Cas9	*DREB, ERF*	Kim et al. (2018)

INTERNATIONAL REGULATION

The rapid development of these new plant breeding techniques has led to the emergence of several aspects related to the state of regulation of plants obtained by genome editing.

According to many scientists, the modifications made by CRISPR are no different from natural or conventional breeding, and thus CRISPR-edited varieties should not be subject to the same regulations as GMOs (Pacher and Puchta, 2017).

However, many countries still rely on the same GMO regulations, and others are reviewing and developing new regulations for GMOs and genome editing products.

Australia, Japan and USA have decided not to regulate the genome-edited products. Brazil, Argentina and Chile have partial regulations but largely do not regulate genome editing products. Canada, Mexico, New Zealand, India, Malaysia, South Africa, Thailand and EU regulate both GMOs and genome-editing products, but here there is debate and re-evaluation to arrive at the best solution (Prasetya and Nugroho, 2021).

In July 2018, the Court of Justice of the European Union ruled that gene-edited crops must be regulated as genetically modified organisms.

CONCLUSIONS

World population increase, along with climate change, has exacerbated the problem of food shortages. Obtaining new crop varieties through conventional breeding methods can be a lengthy process that cannot provide the rapid progress needed to ensure food security and tackle climate change.

Genome editing technologies are new and innovative tools of great importance for plant breeding, the key elements of their efficiency being the precision and the significant reduction of the time to obtain the final product.

In recent years, the CRISPR/Cas9 technique has been widely used in maize and wheat breeding to develop some essential agronomic traits and to combat abiotic and biotic stress.

The success of obtaining new crop varieties through this technique will largely depend on the regulations for the use of such plants.

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