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Genome Editing in Maize

Shourat Fatma[1], Pankaj Pandey[1], Shreya Mehindiratta[1], Neha[1], Abhishek Kumar Jha[1], Komal Rani[1], Alla Singh[2], Mamta Gupta[2], Shambhu Krishan Lal[3], Bhupender Kumar[1] and *Krishan Kumar[1]

[1]ICAR-Indian Institute of Maize Research, Delhi Unit, PUSA Campus, New Delhi, India
[2]ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana, India
[3]ICAR-Indian Institute of Agricultural Biotechnology, Ranchi, Jharkhand, India
*Corresponding Author's email: krishan.iimr@gmail.com

Maize (*Zea mays* L.) is one of the most produced grain crops globally. It has been used in biofuel production, as raw materials for various industries, and as livestock feed. By 2050, there will be 10 billion people on the planet, and while food production must be on par with this rate of increase, the ever-growing effects of climate change pose an unprecedented threat to modern agriculture. To reduce future losses from the increased frequency, severity, and duration of stresses linked to global climate change, it is necessary to adopt improved agricultural practices and continuously develop new maize cultivars aimed at better genetic adaptation. Transformation and genome editing are scientific approaches required for maize improvement, along with tissue culture and somatic embryogenesis for the transformation biology of maize. Traditional maize breeding through genetic crosses takes 8–10 generations to introduce desirable traits, requiring extensive screening and large populations. This method is labor-intensive, time-consuming, and costly, with unpredictable outcomes. It also faces challenges like linkage drag, where separating closely linked desirable and undesirable traits is difficult due to limited recombination events. Multiple gene mutations can occur simultaneously thanks to recent developments in genome editing techniques based on CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR/associated proteins) technology. Furthermore, the potential for genetically engineering complex traits across germplasms utilized in plant breeding programs is increased when genome editing and haploid induction are combined.

A long-term impact on global agriculture, food security, and plant research, genotype-independent maize transformation, and genome editing are the way of the future for the advancements in maize. In this article, we have discussed the current genome editing techniques in maize, its application in improving various traits, ethical considerations, and future breakthroughs in the coming years for genome editing in maize.

Key Genome editing techniques

Traditional methods of maize gene editing include irradiation (gamma rays) and chemical mutagens (ethyl methanesulfonate), which induce random mutations during DNA repair. The major disadvantage of these approaches is that they are imprecise, leading to uncontrolled genetic changes with both positive and negative effects. Transposon tagging is another method used for gene discovery, but it is time-consuming, costly, and causes random mutations, making it impractical for large-scale studies. According to the fundamentals of targeted genome editing, nucleases must be designed to induce a double-strand break (DSB) in the DNA at the target site, which most frequently results in mutation because of either endogenous repair mechanisms (non-homologous end joining, NHEJ) or the use of an externally added homologous DNA repair template (Homologous directed repair, HDR). Using this principle of genome editing, zinc finger nucleases (ZFNs), transcription activator-

like effector nucleases (TALENs) and clustered regularly interspaced palindromic repeats (CRISPR)-CRISPR-associated (Cas)-CRISPR-Cas systems tools have been in use.

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas), zinc-finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs) are examples of site-specific nucleases. Artificial proteins called ZFNs and TALENs are made up of a particular DNA-binding domain and a DNA cleavage domain. These methods introduce double-strand breaks (DSBs) at specific locations within the DNA. Two mechanisms - homology direct repair (HDR) and non-homologous end joining (NHEJ), a form of error-prone repair, are used to instantly repair the DSBs. By introducing single-nucleotide alterations or introducing exogenous DNA elements into specified genomic regions, researchers have been able to disrupt certain genes with the aid of these molecular repair pathways. The most recent method of genome alteration is the CRISPR-Cas system, which is made up of CRISPR and Cas proteins.

The Cas9 effector protein, which causes double-strand DNA breaks (DSBs), the crRNA array, which identifies target sequences, and the trans-activating CRISPR RNA (tracrRNA), which enables crRNA-guided DNA cleavage and crRNA processing, make up the class 2 CRISPR/Cas9 system. The CRISPR/Cas9 system was further simplified by the fusing of the crRNA and tracrRNA to create the single-guide RNA (sgRNA), which made it a flexible tool for genome engineering. Usually, a 20-bp DNA target is cloned into the sgRNA scaffold, which is translated as a ~96-nucleotide sgRNA, right upstream of 5'-NGG protospacer adjacent motifs (PAMs) within a desired gene. To prevent possible off-target changes, it is crucial to choose a distinct 20-bp target within a given genome. Blunt-end DSBs are produced at the 3-bp upstream of the PAM by the Cas9/sgRNA complex. The DSB repair processes can result in desired gene changes. By introducing short indels at the DSBs, nonhomologous end-joining (NHEJ) repair can produce highly effective targeted alterations. Alternatively, by offering a repair template containing desired sequences, the homology-directed recombination (HDR) mechanism might induce particular gene changes. CRISPR/Cas9 has been successfully adapted for generating targeted mutations in maize.

Applications in Maize Improvement

CRISPR/Cas systems have emerged as flexible genome-editing technologies with a broad range of potential uses over other tools like TALENs and ZFNs because of their simplicity, effectiveness, affordability, and capacity to target numerous genes. Owing to these distinctive qualities, it has been quickly used in plants and could be a useful remedy for a number of issues in plant breeding. This method has been used to edit a variety of crops, including rice, maize, wheat, soybean, barley, sorghum, potato, tomato, flax, rapeseed, camelina, cotton, cucumber, lettuce, grapes, grapefruit, apple, oranges, and watermelon. The creation of null alleles, or gene knockouts, is primarily accomplished by introducing small indels that cause frame-shift mutations.

One of the main concerns in crop breeding is yield. A tiller-spreading phenotype was produced in rice when the *LAZY1* gene was knocked out using CRISPR/Cas9, which in some cases boosted crop production. The *Gn1a*, *DEP1*, and *GS3* genes of the rice cultivar Zhonghua11 were mutated using the CRISPR/Cas9 system, resulting in mutants with increased grain size, dense erect panicles, and increased grain quantity. Grain Weight 2 (GW2) is a crucial gene in cereal crops that causes wheat's grain weight and protein content to rise when it is disturbed. Similarly, such key regulatory genes can be edited in maize also to enhance kernel yield.

Maize is one of three cereal grains that account for almost half of all dietary calories consumed worldwide. Nowadays, public agencies and major agricultural seed firms use maize breeding initiatives as their "engine room." Through the Drought-Tolerant Maize for Africa Project (http://dtma.cimmyt.org/), the International Maize and Wheat Improvement Center (CIMMYT) has conducted a substantial conventional breeding program to produce optimal lines. However, compared to efforts made to generate new maize varieties in the

tropics, more money has been spent over a longer period cultivating new varieties for temperate climates.

Progress in data science, large-scale data processing and administration, and the development of innovative breeding techniques have all coincided with advancements and contributions to maize breeding projects. Breeding programs are improved by using strong predictive data for trait selection, which is made possible by genomics and computational biology, which offer a solid foundation for the production of novel varietals through trait identification. In reality, the emerging layers of genomic technologies provide a growing body of knowledge that, when combined with the potential of genome editing, offers the opportunity to explore new lines of inquiry in functional genomics and accelerate the development of new cultivars with advantageous characteristics. This circumstance will serve as the foundation for almost all upcoming improvements to maize. The scientific milestones of the development of genome editing techniques in maize are given in Table 1.

Challenges and Future Directions

Genome editing in maize offers promising advancements, but several hurdles remain. Technically, the maize genome is large and highly repetitive, making assembly and annotation challenging. Even with advanced sequencing technologies, achieving gapless assembly and accurate gene annotation requires integrating multiple data types, adding complexity. Ethically, concerns around genetically modified maize persist. While some see it as a solution to food security, others fear biodiversity disruption and corporate monopolization, limiting accessibility for small farmers. Regulatory differences further complicate global adoption, with strict policies in some regions slowing progress. Economically, the high cost of genome sequencing and annotation remains a barrier. Technologies like Nanopore sequencing require substantial financial investment, restricting widespread use. Overcoming these challenges is key to ensuring responsible and accessible advancements in maize genome editing.

The future of genome editing holds immense potential but also comes with critical challenges. As technology advances, ensuring ethical and regulatory oversight will be essential. Striking a balance between scientific progress and responsible application will shape how genome editing is integrated into medicine, agriculture, and biotechnology. In healthcare, CRISPR and other gene-editing tools could revolutionize treatments for genetic disorders, cancer, and even viral infections. However, the risks of unintended mutations and long-term consequences must be thoroughly assessed before clinical applications become widespread. In agriculture, genome editing can improve crop yield, resistance to diseases, and climate adaptability, but concerns regarding ecological balance and corporate control over food production remain. While genome editing has the power to reshape various fields, its future will depend on how responsibly it is developed and implemented.

Table 1. Scientific Milestones in the Development of Genome Editing Techniques in Maize

Year	Breakthrough	Description	Reference
2016	CRISPR/Cas9-Mediated Gene Editing in Maize	Successful application of CRISPR/Cas9 for targeted gene editing in maize, demonstrating its potential for crop improvement.	Svitashev et al., 2016
2017	Base editing in maize	Introduction of a CRISPR-Cas9 cytidine deaminase fusion enabled targeted conversion of cytosine to thymine in maize, allowing precise single-base changes without double-stranded DNA breaks.	Zong et al., 2017
2020	Prime editing in maize	Development of prime editing technology in maize, allowing for precise genome modifications without creating double-stranded breaks.	Lin et al., 2020
2023	Optimized Prime Editing in Maize	Development of an optimized prime editing system (ePE5max) that efficiently generated heritable mutations conferring herbicide resistance in maize, overcoming previous efficiency obstacles.	Qiao et al., 2023

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