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Revolutionizing Maize Production: Breakthrough Technologies and Methods for Developing Transgenic and Quality Protein Varieties

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Abstract

Maize is a multi-purpose crop known to be affected by multiple stresses, with intensified impact due to climate change which may pose a threat to global food security. Genetic Engineering provide solution for incorporating resistance and quality related transgenes may provide sustainable yield and overcome hidden hunger under various environmental conditions. Improving the transformation efficiency to achieve various breeding goals of maize is crucial. In this regard, recent articles about plant transformation technologies for the development of maize transgenic lines was reviewed. New breakthrough technologies with improved transgenic efficiency, identification of transgenes to develop biofortified maize varieties provide new resilience for food security. Genetic modification could be induced by protoplast transformation and particle bombardment. Furthermore,

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Agrobacterium-mediated transformation, targeted genome editing and nanoparticle delivery could enhance maize transformation methods. A thorough understanding of maize genetics, specifically the role of the Opaque-2 gene in protein quality, is essential for producing high-quality protein maize. Breeding programs need to choose optimal parents with sought-after traits and evaluate genetic variation to enhance crop production. The progress in maize transformation technology has the potential to increase maize production, productivity, and quality, which will be advantageous for both farmers and consumers.

Keywords: Genetic modification, Protoplast, Particle Bombardment, *Agrobacterium-mediated*, nanoparticles, Biofortified

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Introduction

Maize is a widely grown cereal crop, ranking third in importance after wheat and rice. It serves as a vital source of food for both humans and livestock (Shavanov 2021). Approximately 15% of protein requirements and 19% of calorie needs from plant sources are met by consuming maize (Hossain et al. 2019; Kaul et al. 2019). In developing countries, maize plays a crucial role in providing dietary proteins to a large population, as evidenced by its use in meeting the protein needs of millions of people globally (Hossain et al. 2019). Moreover, in developing nations, 78% of maize is used as feed for livestock (Ekpa et al. 2018). Maize plays a role of a source of calories in low areas, such as Africa. People threatened by protein and essential amino acid inadequacies consume 17–60 percent of their daily protein from maize (Muleya et al. 2022). It is possible to raise the protein content by up to 18 percent by introducing extra prolamine fractions into the maize endosperm (Sethi et al. 2021).

The growing global population and the rise in animal-based food consumption have resulted in a greater demand for maize grains. However, the production, productivity, and quality of maize and sunflower are hindered by a variety of biotic and abiotic stresses, especially on limited cultivable land **(**Gul et al. 2024; Gul et al. 2021; Kumar 2020; Shehzad et al. 2021). Genetic modification of maize to introduce specific genes has become a common technique to create transgenic cultivars with enhanced features (Yassitepe et al. 2021). The 1996 introduction of transgenic maize cultivars in the US marked a major advance in plant genetic engineering. Maize has since become the primary focus for genetic engineering, with the largest number of commercialized transgenic events among all crops (Cabrera-Ponce et al. 2019). Therefore, transformation techniques for creating transgenic maize have emerged as a leading technology for enhancing this crop's genetic potential (Pellegrino et al. 2018).

Advancements in Protoplast-to-Plant Transformation for Transgenic Maize Development

This method involves transferring the desired DNA into protoplasts through the application of electric pulses. In 19th centuries, scientists directly electrochemically added a transgene to Black Mexican Sweet maize protoplasts. In this 1986 procedure, no effective ways to regenerate plants fully transformed into plants existed. However,

Yassitepe et al. (2021) created the first fully grown transgenic maize plants. A lot of plant materials were tried for transgenic maize development in the 1980s, however, only a number of them were able to grow full plants from protoplasts. Gene delivery protoplasts were best obtained from immature embryos. Yassitepe et al. (2021) electroporated isolated protoplasts from embryogenic cell suspension culture of the inbred line A188 in 1988. In the above-mentioned electroporation, another method moved DNA of interest into protoplasts from 188 using PEG. From He/89 germplasm, this method made fertile transgenic maize plants (Ušák 2020).

Development of a More Efficient and Precise Particle Bombardment

The global adoption of transgenic maize

In various countries globally, 30 nations (including the European Union) have approved a combined 143 genetically modified maize events for cultivation or commercial purposes (Bonea et al. 2022). These events fall into six primary categories: herbicide resistance (121 events), insect resistance (115 events), enhanced product quality (12 events), pollination control system (6 events), and tolerance to abiotic stress (4 events) (Malenica et al. 2021). Frequently, multiple events will occur simultaneously. In 2022, transgenic maize varieties were planted on 66.2 million hectares, which accounted for 92% of the world's total maize land of 197 million hectares in Figure 1 (Bekere 2022).

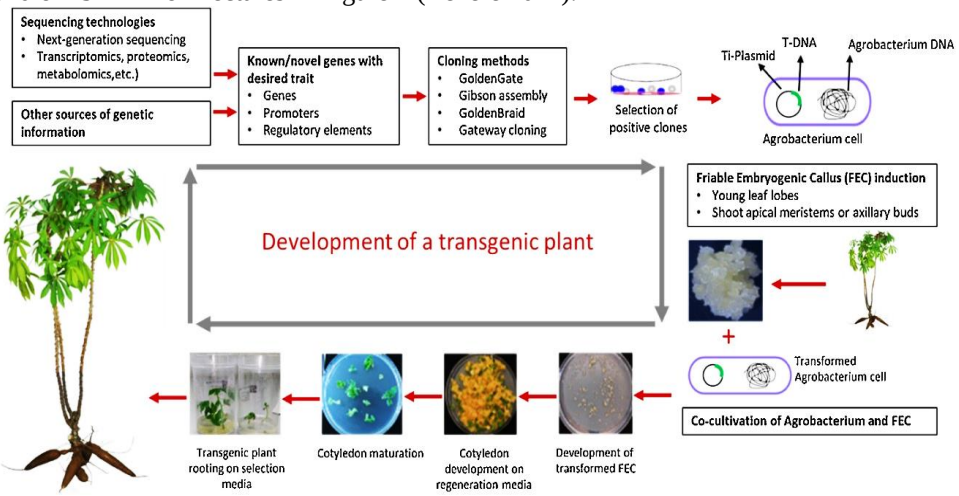

Figure 1. Development of transgenic plant

Advancements in Agrobacterium-Mediated Plant Transformation

Agrobacterium tumefaciens, a soil pathogen, can genetically change plants. This technique has been widely used to alter dicot plants by inserting vast portions of DNA with little genome modifications, resulting in high-quality transgenic plants. Further studies showed that this approach might change maize (Andorf et al. 2019). Harman and Uphoff (2019) found that changing shoots yielded favorable benefits (Anand et al. 2019) super-binary vector with maize transformation vir genes was the breakthrough. Over time, pre-heating, using copper and silver ions during co-cultivation, and extending the procedure to 7 days

have boosted transformation efficacy. Sonication-Assisted Agrobacterium-Mediated Transformation (SAAT) addresses the shortcomings of the earlier method. Sonication waves with Agrobacterium increase DNA transport in several plant species (Tripathi & Shukla 2024).

Advancements in CRISPR-Cas Genome Editing Techniques for Plant Breeding

Recently, there have been significant advancements in plant CRISPR-Cas genome editing (CGE). In 2013, three teams showcased successful CGE methods aimed at genes in rice, wheat, Nicotiana benthamiana, and Arabidopsis thaliana. Delivery of CRISPR-Cas via conventional techniques such as Agrobacterium or biolistic poses challenges due to resistance to transformation in numerous crop varieties (Kuluev et al. 2019). Multiple successful methods exist for transferring CRISPR-Cas machinery into germplasm with crossing challenges (Ali et al. 2023). The cross elite recipient inbred lines with pollen from a stably transformed line containing a CRISPR-Cas construct. Yassitepe et al. (2021) DTM modifies the target gene by directly inserting it into a specific allele using trans-acting CRISPR-Cas (Figures 1A-C). Das et al. (2022) said that sperm cells have the ability to transport Cas RNPs into high-quality egg cells during gametophytic expression or generate Cas and sgRNA in the zygote after gamete fusion in zygotic expression; as shown in Figure 1D. Various types of promoters are required to control the expression of cassettes in CRISPR-Cas systems (Hsieh-Feng & Yang 2020). Therefore, by crossing, plants without CRISPR-Cas are obtained with both the original genetic background and the targeted gene homozygous (Figures 2A to E). The introgression breeding procedure still involves backcrossing to completely restore the original genotype, but this approach lessens the need for marker-assisted backcrossing and minimizes linkage drag across fewer generations.

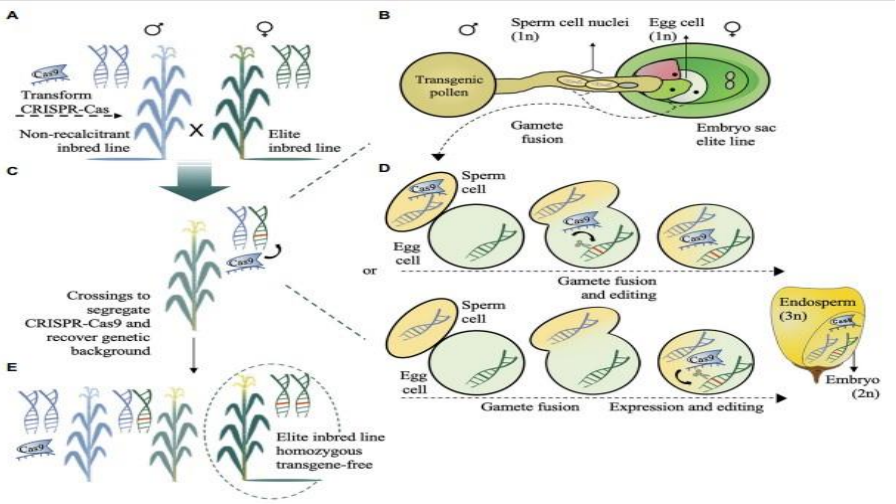

Figure 2(A) A non-resistant genetically pure line is given the CRISPR-Cas system for trans editing. (B) The non-resistant line and the high-quality maize line reproduce by using the CRISPR-Cas tool to aim at particular genes. (C) The elite line target gene is modified by trans-acting CRISPR-Cas. (D) The sperm cell's receptor transports RNPs to the egg cell, resulting in hybrid-altered embryos through gametophytic or zygotic expression. The

additional crossing is used to obtain CRISPR-Cas-free plants with the same elite line genetic background and homozygous for the desired mutation following trans editing.

Technique for Transformation in Maize

Tungsten or gold particles coated with plasmid DNA are utilized to deliver the target DNA into the cell wall. These rapidly moving particles are directed towards specific tissues of maize plants, such as cell suspension cultures, calluses derived from Type I or Type II cells, and organogenic calluses from embryos, seedlings, or shoot meristem cultures that are still immature (Kausch et a. 2020). Many individuals utilize corn for genetic modification through particle bombardment. It is more effective at generating fertile transgenic events from embryogenic callus compared to protoplast transformation (Bertini et al. 2019). The cry1Ab gene was successfully transformed using this method on immature embryos (Lv et al. 2021). Plant regeneration through direct gene transfer by plant bombardment (Figure 3). Over time, enhancements have been made to the biolistic transformation method. The development of a robust protocol for Hi-II genotype was achieved by McCaw et al. (2021), and it was observed that pre-culturing immature embryos before particle bombardment can increase survival and transformation efficiency (Ahmed et al. 2018; Narra et al. 2018). Several studies have also demonstrated the effectiveness of microprojectile bombardment on maize (Cabrera-Ponce et al. 2019; Sarangi et al. 2019; Zobrist et al. 2021).

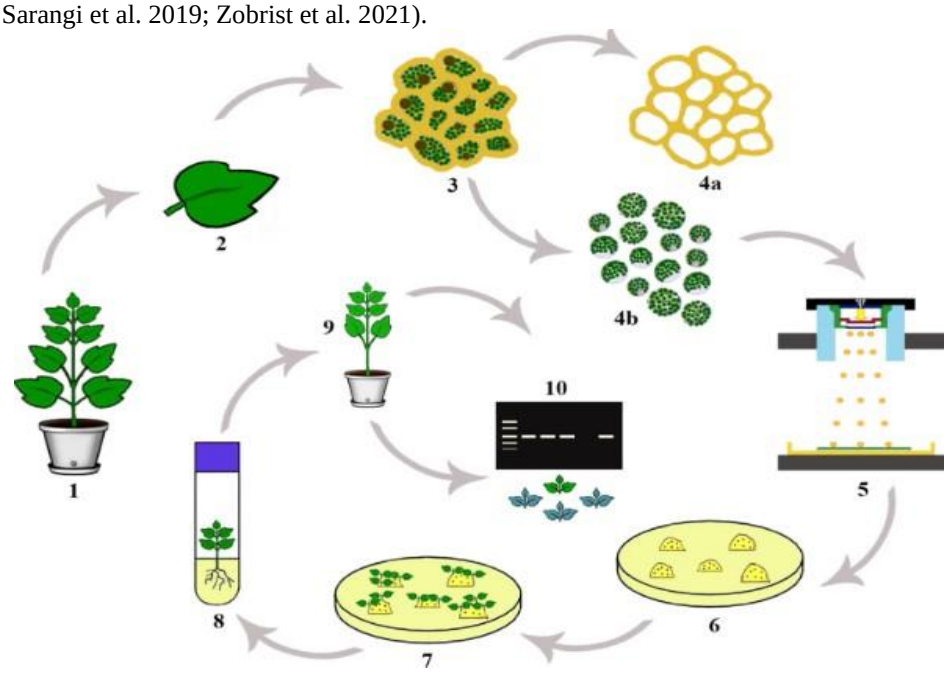

Figure 3. Plant regeneration through direct gene transfer (1) Mother plant (2) Leaf (3) Isolated leaf cells (4a & b) Cell wall removed protoplasts (5) Gene transfer using PBF (6) Callus induction (7) Organogenesis (8) Rooting (9) A young plantlet (10) Molecular and histo-chemical analysis.

Furthermore, a technique was created for effectively converting organogenic calli **(Efferth, 2019)**, and shoot meristem culture was employed to transform challenging

inbred lines such as B73 and PHTE4 **(Cabrera-Ponce et al. 2019)**. It should be noted that this is the primary cause of most of the deregulated events in maize (Table 1).

Table 1. Traditional techniques for producing of transgenic maize crops

Serial no.	Traditional techniques of transformation	Development of commercial deregulation
1	utilizing chemical agents to facilitate the introduction of protoplasts and subsequently supporting the healing procedure	T14, T25
2	Electroporation involves creating temporary openings in cell membranes	MS3, MS6
3	Employing microparticles for bombarding plant cells.	676,678, 680, Bt11TC1507
4	Whiskers assists in altering plants.	DAS40278
5	Transformation of plants using Agrobacterium tumefaciens	32,138,327,233,121
6	Aerosol Beam Injection	HCEM485

Typically, a plant gene cassette and additional genetic components in a bacterial plasmid serve as vehicles for transformation. Transgene production requires only the gene cassette, not the plasmid. This indicates that methods such as particle bombardment, which do not require manipulation or incorporation of T-DNA, do not require the vector backbone (Baskar, Kuppuraj, Samynathan, & Sathishkumar, 2019; Singh, Singh, Singh, & Singh, 2022). Vectors and plant transformation constructs are primarily utilized for technical purposes rather than out of necessity for experiments. Kausch et al. (2019) devised an approach to launch particles without the need for vector sequences to address this issue. Once the plasmid is removed, the linear cassette containing only the gene of interest is utilized for transformation (Weninger et al. 2018).

The Muhuri et al. (2021) findings indicated that this simple cassette effectively incorporated and demonstrated the transgene. Additionally, the transgenic plants produced had simpler integration patterns and fewer copies compared to the entire constructs. Low et al. (2018) similarly utilized this approach to transfer multiple genes into plants, resulting in increased transgene expression, individual genetic insertions, and consistent inheritance. This contradicts the notion that particle bombardment leads to large, numerous occurrences that are prone to becoming unsteady and still. The flexibility and accuracy of the "clean DNA transformation" technology, as demonstrated by Low et al. (2018); Basso, Arraes, Grossi-de-Sa, Moreira, and Grossi-de-Sa (2020), indicate notable advancements in the field. This recent discovery enables the creation of marker-free transgenic plants with multiple genes. It is crucial for regulatory purposes to ensure the production of high-quality transgenic events that have clean integration sites and do not disrupt native genes. It would be terrific if enhancements could be made to transformation tools to facilitate the retrieval of top-notch events (Malode et al. 2022).

Comparison of CIS genesis and intrageneric strategies in genetically modified crops

The aim of cis genesis is to incorporate a gene from the donor plant's pool of sexually compatible genes, including its promoter and terminator, in its original orientation (Dudziak et al. 2019). On the flip side, intra genesis allows for the utilization of genes from the recipient plant's gene pool in either a forward or reverse orientation. The promoter

and terminator can be obtained from the recipient plant as well (Tian et al. 2022). Both techniques avoid using artificial markers, and in order to obtain marker-free plants, alternative methods such as clean DNA transformation using particle bombardment may be necessary. However, advanced versions of cis genic or intragenic maize are not currently widely available.

Challenges in Targeted Genome Editing Techniques

Genome editing, known as targeted genome editing, has the potential to address issues with transgenic techniques. Zinc-finger nucleases (ZFNs), Transcription activator-like effector nucleases (TALENs), and CRISPR/Cas are employed for accurate genome modifications (Saraswat et al. 2023). ZFNs and TALENs were developed through genetic engineering. They attach to and stick to DNA, forming locations with double strand breaks (DSBs). Non-homologous end joining (NHEJ) and homology-directed repair (HDR) have the ability to fix these breaks by incorporating external DNA or modifying individual nucleotides (Shams et al. 2022). The more recent CRISPR/Cas system modifies genetic material by utilizing CRISPR and Cas proteins. This technique is effective for rice, wheat, sorghum, tomato, and tobacco. It could also be effective with corn. Scientists are researching methods for transmitting guide RNA (gRNA) molecules (Galizi et al. 2020). They are working on plans to enhance genome editing techniques. It is crucial to keep in mind that targeted genome editing does not involve incorporating genes from other species (Zhang 2019). This indicates that the outcomes resemble natural genetic changes. Farmers benefit because it simplifies the process of locating and delivering products (Kheybari et al. 2021).

Advancing Maize Transformation through Nanoparticle-Mediated Delivery

In the past few years, advancements in technology have decreased the reliance on specific genotypes for maize transformation, particularly through genome editing (Kausch et al. 2019). These sophisticated tools can now be utilized in academic and industrial settings, leading to improved productivity. The use of nanoparticles for delivery shows potential as a technique that does not rely on tissue culture. NPs have the benefits of being small, coming in various forms, and having versatile binding capabilities (Gauba et al. 2023). According to Banerjee et al. (2019), animal cell membranes can only allow materials up to 500nm, plant cell walls have a width ranging from 5-20nm and are the primary hindrance to material transport. Carbon nanotubes (CNTs), nanoparticles (NPs) measuring up to 20nm in size, are capable of bypassing entry methods that cause harm to cells. SWCNTs measuring 12nm or less have the ability to deliver biomolecules to healthy plant leaf cells and shield them from nuclease breakdown, as demonstrated by (Kaymaz et al. 2023). Nanoparticles can be utilized for genetic modification, gene expression, and pharmacological therapies by transporting DNA, RNA, proteins, RNPs, and small chemicals (Chen et al. 2020).

The Importance of Opaque 2 in Enhancing Protein Quality in Maize

In the United States, researchers discovered that the gene Opaque-2 controls the production of α zein protein in maize. This causes the endosperm to become less transparent and more tender. When the gene is homozygous, there is a significant increase in lysine and tryptophan levels. Opaque-2 triggers transcription by binding to the promoter region of the α zein protein. It consists of 454 amino acids and shares similarities with animal transcriptional activators (Sultana et al. 2019). Other maize loci regulate the

synthesis of Zein protein. The recessive homozygous individual shows a 60% decrease in zein protein levels, particularly in the 22 KD component, due to the presence of the opaque 2 mutant allele (Tamvar et al. 2019). This enhances levels of lysine and tryptophan. Studies indicate that Opaque-2 activates gene transcription for the 22kD zein protein on chromosome 7, whereas the genes for the protein are located on chromosome 4 (Khan, Sheteiwy et al. 2019). Opaque-2 is directed to the nucleus by cues for nuclear localization. This localization has been confirmed in maize and tobacco using the Opaque-2 gene. Splitting the protein into three parts and merging it with a reporter protein revealed two parts in the nucleus, thus confirming its localization (Zhou et al. 2021). Onion cells temporarily changed with the Opaque-2 gene exhibit nuclear localization as well.

Modifying gene action of Opaque 2 protein

The presence of zein protein and starch granules is responsible for the firm consistency of the endosperm in typical maize varieties. However, the introduction of opaque-2 genes causes numerous changes, resulting in a smoother core and reduced zein protein levels (Darrah et al. 2019; Li et al. 2021). In the end, this results in post-harvest losses, as illustrated in Figure 4. It is crucial to use genetic systems that can neutralize the multiple effects of the opaque 2 mutation in order to produce high-quality protein maize.

Figure 4.

Exploration of Genetic Variability and Selection of Optimal Parent Selection in Breeding Programs

Many breeding projects utilize a variety of traditional and non-traditional methods to enhance crop yield. Selecting parents with desired traits and important agro-morphological characteristics that impact yield is crucial for this task (Shrestha et al. 2023). These characteristics could either have a beneficial or detrimental impact on the desired outcome. The choice of selection method is based on the characteristic, population type, and geographical setting (Singamsetti et al. 2021). Selecting parents solely based on direct criteria may not be effective, as agromorphological traits could be interconnected in ways that lead to the choice of traits that negatively impact yield or are susceptible to external influences (Simmons et al. 2021). As a result of this, breeders must examine the genetic diversity of various genotypes prior to beginning the breeding process. While hybridization and mutation can create variation, it is important to still examine the genetic material, especially when breeding hybrids from genetically distinct parents. Principal component analysis is frequently used to assess diversity, and it can assist in identifying prospective parents by demonstrating the overall impact of diversity on the process (White et al. 2020). For instance, Amegbor (2020) examined 40 QPM and non-QPM lines, employing molecular, biochemical, and morphological techniques to identify the most suitable lines for converting regular lines into QPM. They achieved this through the use of principal component and cluster analysis.

Conclusion

Maize has essential nutrients and nourishes people and animals around the globe. biotic and abiotic stressors decrease the yield, productivity, and quality of maize, requiring genetic modifications. Transformation procedures are crucial for enhancing the genetic potential of maize, ultimately benefiting both farmers and consumers with improved traits in transgenic cultivars. Proven methods for genetically modifying maize include protoplast-to-plant transformation, particle bombardment, and agrobacterium-mediated transformation. These tactics aid in producing transgenic plants without markers and achieving top-notch transgenic results. Newly created technologies such as CRISPR-Cas genome editing, targeted genome editing, and nanoparticle-mediated delivery could enhance maize transformation. Nevertheless, breeding operations need to address challenges such as choosing the optimal parents and reducing the negative effects of the Opaque-2 gene. These advancements in maize transformation are crucial for meeting the needs of worldwide food security and demand for maize grain.

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