

Development Trend of Genetic Improvement of High Protein Maize

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Abstract. Global maize production has maintained significant growth in recent years, with total output reaching approximately 1.2 billion tons by 2024. As China's primary food and feed crop, maize holds a core strategic position in the national food security framework. High-protein maize plays a pivotal role in enhancing crop nutritional capacity and global food supply security. Given China's heavy reliance on international markets for protein raw materials, developing and promoting high-protein maize varieties has become a critical approach to alleviating protein resource shortages. Research indicates that high-protein maize not only effectively improves feed self-sufficiency rates and reduces soybean meal supplementation ratios, but also mitigates nitrogen emissions in livestock farming at the source, significantly contributing to building a green and sustainable agricultural economy. This paper systematically examines the nutritional properties of high-protein maize, particularly its applications in human diets and livestock breeding, while providing a comprehensive review of its breeding progress and current research status. It was further analyzed on challenges in the promotion and application of high-protein maize, such as insufficient germplasm resource exploration and the lack of high-quality specialized varieties. The paper proposes solutions including strengthening germplasm innovation and optimizing breeding technology systems, aiming to provide theoretical references for the industrial development and utilization of high-protein maize in China.

Keywords: high protein; maize; genetic improvement; development trend.

1. Introduction

With the continuous optimization of dietary structure among Chinese residents, the proportion of animal-derived food consumption has significantly increased from 7% to 28%, driving strong demand for high-quality feed protein^[1]. As the top crop in China by total production, maize has become the core energy feedstock for livestock development due to its outstanding environmental adaptability. However, China's feed protein supply faces structural challenges, with approximately 80% of soybean meal relying on imports. Given the current high international protein raw material prices, genetically improving maize to increase its protein content and partially replace soybean meal has become a crucial strategic direction for the feed industry to alleviate cost pressures and ensure supply chain security. Studies showed that a 4% increase in maize's crude protein content could reduce China's annual soybean meal import dependence by about 3,000 tons, while each 1% increase in crude protein content achieves direct cost savings in feed formulations^[2]. Therefore, developing high-protein maize is not only about technological breakthroughs but also holds practical economic significance for reducing livestock production costs and enhancing the industry's risk resilience. Although China ranks among the world's top maize producers, there remains room for improvement in yield per unit area. With rising living standards, higher demands are placed on maize yield and nutritional quality. Under these circumstances, high-protein maize breeding has become a key focus.

2. Global History and Current Status of High-Protein maize Breeding

Maize kernels are rich in various nutrients, including starch, protein, and trace elements, with protein accounting for 10% and alcohol-soluble protein (ASP) constituting 60%-70% of the total protein content. In 1914, Osborne et al. first discovered that mature maize kernels lacked lysine and tryptophan, essential amino acids required by the human body^[3]. This deficiency rendered maize unsuitable as feed or staple food, necessitating optimization of its protein amino acid composition. It was not until the 1920s that Singleton and Jones identified the opaque-2 (O2) mutant and mapped the

corresponding gene^[4]. Subsequently, Mertz et al. demonstrated in 1964 that O2 mutants altered the appearance and amino acid profile of maize endosperm, increasing lysine and tryptophan content by over 70%^[5]. This groundbreaking discovery provided a novel approach to addressing the fundamental issue of suboptimal protein quality in conventional maize. Subsequent studies identified other high-lysine endosperm mutants, though their applications remained limited. Overall, O2 homozygotes represent the optimal choice for high-lysine maize breeding. However, early varieties developed from O2 mutants exhibited severe defects such as opaque kernels, soft texture, low yield, susceptibility to diseases and pests, and mechanical damage, leading to their widespread discontinuation in production and the termination of numerous breeding programs. Faced with such challenges, international agricultural research institutions represented by the International Center for Maize and Wheat Improvement (CIMMYT) adopted an alternative approach. In 1971, Surinder Vasal's team utilized the o2 mutant and its modified genes, employing the modified gene accumulation method to successfully "repair" the o2 gene-induced soft endosperm into a hard or semi-hard transparent endosperm with high lysine content. This overcame the drawbacks of low yield and poor disease resistance, and the new maize variety was named Quality Protein Maize^[6](QPM). Subsequently, countries such as the United States, the Soviet Union, and Romania continued to advance the breeding and application of QPM.

3. High-protein maize breeding in China

Since the 1990s, China has successfully developed a series of high-protein maize varieties through the integration of traditional hybridization and molecular breeding techniques. For instance, the "Shandan 17" variety, developed by Northwest A&F University in 1996, boasts a crude protein content exceeding 11% and exhibits excellent agronomic traits such as early maturity and stress resistance^[7]. In 2003, the Chinese Academy of Sciences utilized the opaque-2 (O2) gene combined with endosperm modification genes into high-protein maize variety "Zhongdan 9409," with a crude protein content surpassing 10%^[8]. Subsequently, varieties such as "Jiyu 10" (2005, crude protein 9.37%) and "Wunuo 3" (2017, crude protein 12.3%) were successively developed, maintaining high yield and disease resistance while significantly enhancing protein content.

However, modern maize has encountered a trade-off dilemma between yield and quality during long-term domestication and genetic improvement. The crude protein content of its wild ancestor, teosinte, can reach 30%, while that of modern hybrids was only 5%-10%^[9]. Since the 1930s, the crude protein content of maize has declined at a rate of approximately 0.3% per decade^[10]. Currently, the crude protein content of maize kernels is about 7%-9%, with approximately 70% being alcohol-soluble protein (ASP). ASP severely lacks essential amino acids such as lysine and tryptophan, leading to nutritional imbalance. Many studies have confirmed that crude protein content in maize was a quantitative trait controlled by multiple genes, with high protein levels exhibiting partial dominance. The traditional approach to increasing lysine content involves reducing ASP and increasing non-ASP proportions. However, it was noteworthy that when crude protein content increased from 10.9% to 25.4%, the incremental portion was still primarily ASP^[11].

To overcome this bottleneck, Chinese scientists have actively utilized cutting-edge technologies such as genetic modification. For instance, introducing the high-lysine gene sb401 from potatoes into maize can simultaneously increase both total protein and lysine content^[12]. In 2022, the discovery of the key gene THP9^[13] marked a significant breakthrough—introducing this gene can elevate the protein content of maize kernels by approximately 35% and substantially enhance nitrogen levels across all plant tissues, providing novel genetic resources for breeding "high-yield, high-protein, and high-nutrient" maize.

In summary, through years of systematic research, China has established a relatively comprehensive high-protein maize breeding technology system, developed a range of varieties that balance both quality and yield. In the future, with the in-depth exploration and application of key genes such as THP9, it was expected to further break the negative correlation between yield and protein content, propelling maize genetic breeding into a new phase of precision design.

4. Recent advances on genetic improvement in high protein maize

4.1. Molecular genetic approaches to developing quality protein maize

In recent years, the development of high-quality protein maize (QPM) has entered into a precision design phase centered on molecular genetics. The early breakthrough was the discovery of the recessive mutant gene *opac2* (*o2*), which significantly reduces the synthesis of lysine-deficient zein proteins, thereby increasing grain lysine content. However, the *o2* mutation alone resulted in soft grains, susceptibility to pests and diseases, and low yields, limiting its application. Consequently, the current focus of QPM research has shifted from identifying *o2* to its "modification." Modern molecular genetic approaches aim to identify and introduce a series of modified genes (or quantitative trait loci) that do not alter the high lysine characteristics induced by *o2* but can restore grain hardness, glassy texture, and stress resistance by regulating the expression of other zein proteins. Studies utilized high-throughput molecular markers for genome-wide association analysis and linkage map construction to precisely located these modified loci. On this basis, marker-assisted selection and genome-wide selection have become mainstream breeding tools, enabling the rapid and efficient integration of complex *o2* backgrounds with multiple modified genes, significantly shortening the QPM breeding cycle and overcoming the "linkage disequilibrium" issue in traditional backcross breeding. This direction marked a fundamental shift in QPM breeding from phenotypic selection to genotypic selection, provided a reliable technical pathway for achieving the perfect combination of nutritional and agronomic traits^[14].

4.2. Genetic analysis of quality protein maize (QPM)

From a genetic analysis perspective, QPM represents a paradigm of maize where nutritional quality and physical traits have been successfully balanced through genetic means. Advances in this field have systematically revealed the complex genetic architecture underlying this phenomenon. The core gene *o2*, as a transcription factor, exhibits mutations that directly lead to extensive reprogramming of downstream zein gene expression, which forms the basis for lysine accumulation. However, the characteristics of QPM constitute a comprehensive trait controlled by both major and minor genes, the *o2* mutation itself has adverse pleiotropic effects, such as the softening of corn kernels and reduced yields. The breakthrough of QPM lies in the fact that breeders, by introduced the *o2* modifier gene, have restored the hard endosperm phenotype of the kernels while retaining the high-lysine trait. Genetic analysis demonstrated that grain modification phenotypes (hardness, luster) were regulated by multiple loci, which were closely associated with genes encoding proteins such as 27-kD γ -zein. Among these, the gene encoding the 27-kD γ -zein was identified as a key regulatory factor located on chromosome 7. Functional studies confirmed that enhanced expression of this gene can fill the structural gaps in the proteome caused by the *o2* mutation, thereby restoring grain hardness—when the 27-kD and 50-kD γ -zein genes were simultaneously silenced via RNA interference, the hard endosperm phenotype of QPM was almost completely eliminated, and the number of proteomes decreased by more than 90%. Suboptimal dose experiments further demonstrated that the dose-dependent effect of γ -zein plays a critical role in endosperm modification. Enhanced expression of these genes can compensate for the proteomic structural gaps caused by *o2* mutations, thereby restoring hardness. Additionally, the study focused on additional QTLs influencing essential amino acid composition, total protein content, and yield-related traits. Through QTL mapping and allele mining, scientists were constructing a more comprehensive genetic network map of QPM. This included identifying natural *o2*-modifying allele variations in tropical and subtropical local germplasms to expand the genetic basis. Current genetic analyses not only served MAS but also elucidated the expression patterns and interaction mechanisms of modified genes under different genetic backgrounds, laid a solid theoretical foundation for breeding next-generation QPM varieties with high-yield potential adapted to diverse ecological environments^[15].

4.3. Zein isoforms associated with high protein quality traits for targeted improvement in maize-based nutrition

The fine resolution of maize zein isoforms has opened new dimensions for targeted improvement of protein quality. Research has recognized that an increase in total protein content did not necessarily imply an improvement in nutritional value, with the key lying in protein composition. In maize grain proteins, zeins account for up to 60% of the total protein content, but they are generally deficient in lysine and tryptophan. Consequently, the focus of research has shifted to systematically elucidating the specific functions of different zein isoforms and their associations with final nutritional quality through proteomics and reverse genetics approaches. Studies have revealed that not all zeins were detrimental to nutrition. For instance, high expression of certain γ -zeins was crucial for maintaining the hardness of QPM grains, while reducing the expression of specific isoforms (such as some α -zeins) may relatively increase the composition of lysine-rich non-zein proteins (e.g., globulins, albumins) without affecting grain structure. This has refined breeding objectives from the traditional "increasing lysine" to "regulating specific zein expression profiles." Through gene editing techniques or the use of natural mutants, precise knockout or expression regulation of zein gene family members can be achieved, enabling targeted design of grain protein composition. This approach guides metabolic fluxes toward pathways for synthesizing high-quality proteins, providing precise tools for more thorough and efficient nutritional fortification^[16].

4.4. THP9 enhances seed protein content and nitrogen-use efficiency in maize

The discovery of the THP9 gene represented a milestone breakthrough in high-protein maize breeding, closely linked the two critical traits of "high protein" and "nitrogen efficiency." This study cloned the THP9 allele from the wild ancestor of maize, teosinte, and was introduced it into modern maize inbred lines. As the wild ancestor of maize, teosinte can achieve a seed protein content of up to 30% without the application of nitrogen fertilizer—three times that of modern cultivated maize—yet this high-protein trait was gradually lost during the long process of domestication. Using a strategy combining third-generation sequencing with 3D genomics, researchers successfully decoded the highly complex wild maize genome and, using map-based cloning, precisely localized the major QTL—THP9—to chromosome 9. The results demonstrated that THP9 significantly increased grain protein content (with particularly notable enhancement under low nitrogen conditions) without sacrificing yield, and even improved nitrogen utilization efficiency. The mechanism lies in THP9 encoding an asparagine synthetase4(ASN4), which plays a central role in nitrogen assimilation and recycling. In the B73 inbred line, THP9-B73 exhibits mis-splicing of the transcript due to the deletion of the 10th intron, resulting in low ASN4 expression; however, the introduction of the wild-type maize gene THP9-T significantly increases ASN4 expression levels. It likely enhances the mobilization and redistribution of nitrogen (in the form of asparagine) from vegetative organs such as leaves to developing grains, ensuring sufficient nitrogen sources for protein synthesis during grain filling under overall limited nitrogen supply. Experimental data show that after introducing THP9-T into B73, seed protein content increased by approximately 35%, while nitrogen content in roots, stems, and leaves increased by 54%, 94%, and 18%, respectively. This discovery holded dual revolutionary significance: first, it broke the commonly held genetic negative correlation between protein content and yield, proved that dual-advantage genes can be obtained by exploring wild resources. Second, it placed protein nutrition improvement within a broader framework of resource-efficient utilization and agricultural sustainability. Even when nitrogen fertiliser application is reduced, plants carrying THP9-T can still effectively maintain their biomass and nitrogen content levels.. New varieties developed using the THP9 allele can maintain high grain protein output while reducing nitrogen fertilizer application, offering significant potential for lowering production costs, mitigating environmental pollution, and addressing food security challenges under climate change. This represented a new paradigm in high-protein maize breeding^[9].

4.5. Spatial transcriptomics uncover sucrose post-phloem transport during maize kernel development

The application of spatial transcriptomics technology has provided unprecedented spatiotemporal resolution insights into the microcompartmentalized metabolic processes underlying maize grain development and high-protein formation, which were unattainable with traditional omics technologies. The study precisely delineates the detailed pathways and gene expression profiles of sucrose (the primary form of photosynthetic products) after its unloading from the grain parenchyma, as it is transported and distributed to various tissues (such as the embryo, endosperm basal transfer cells, amylopectin endosperm, and aleurone layer). The research revealed that the transport and distribution of sucrose exhibit high spatial heterogeneity and temporal dynamics, which directly influence cell proliferation, starch accumulation, and protein synthesis in different regions. For high-protein maize research, the implications of this technology were that the final protein content and quality of grains depend not only on the metabolic potential determined by genetic background but also on the efficiency and spatiotemporal coordination of nutrient (carbon and nitrogen sources) delivery to protein-synthetic active regions (endosperm). Spatial transcriptomics data indicated that genes involved in nitrogen metabolism (such as asparaginase and glutaminase) were co-expressed with starch synthesis genes in the endosperm adjacent region, suggested that the close spatial coupling of carbon and nitrogen metabolism was crucial for the efficient synthesis of storage compounds. This study offered breeders a novel perspective: in addition to modifying genes encoding storage proteins, optimizing the spatiotemporal expression patterns of key transport proteins and enzymes involved in grain internal nutrient transport, distribution, and metabolism can more efficiently convert photosynthetic products and nitrogen into high-quality proteins, thereby achieving synergistic improvements in grain nutritional quality^[17].

5. Summary and Outlook

As a novel high-quality protein source, high-protein maize can be widely used to replace conventional maize in feed formulations, significantly reducing the consumption of industrially synthesized protein feeds such as lysine and soybeans. This will effectively alleviate China's protein resource shortage. Additionally, the continuous advancement of socioeconomic and scientific technologies has driven growing public demand for healthy diets. With increasing awareness of the importance of balanced nutrition, functional foods are gaining popularity. Correspondingly, high-quality protein maize, characterized by higher protein utilization efficiency and rich in essential amino acids, was being increasingly chosen by consumers. This holds profound significance for regions with protein deficiencies. In summary, genetic improvement of high-protein maize is embracing various new opportunities. Although China has made significant progress in breeding high-quality protein maize in recent years, challenges remain such as limited germplasm resources, high breeding costs, and lengthy cycles. For instance, institutions like the International Center for Maize and Wheat Improvement and Pioneer Corporation in the United States have developed varieties with significantly increased lysine and tryptophan content, achieving total protein levels of 10%-12% and superior amino acid balance. However, most high-protein maize varieties in China still primarily rely on increased zein content, with limited improvements in lysine and tryptophan levels, falling short of QPM standards. To accelerate the development of high-quality protein maize, new methods and strategies must be adopted. Strengthening research on germplasm resource expansion and utilization, extensively collecting and introducing domestic and international germplasm resources, and conducting identification and application are essential. We will intensify the exploration and utilization of high-protein gene resources from wild teosinte, local varieties, and tropical germplasm. By actively participating in international breeding networks, we aim to enhance the global competitiveness of China's maize seed industry. With the deepening understanding of key gene regulatory mechanisms and the integration of biotechnology with intelligent technologies, we are confident that achieving a synergistic leap in maize nutritional quality and productivity will become

a reality. This advancement will make a whole new world of possibilities and greater contributions to food security and sustainable agricultural development, both in China and worldwide.

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