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ADVANCEMENTS IN MAIZE BIOFORTIFICATION: ENHANCING NUTRITIONAL QUALITY THROUGH BREEDING

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ABSTRACT

Maize, a critical staple crop globally, is essential for human nutrition but often lacks vital micronutrients, leading to widespread deficiencies in regions where it is a primary food source. This review paper explores recent advancements in maize biofortification, a strategic approach aimed at enhancing the nutritional profile of maize through both traditional and modern biotechnological methods. It covers the integration of conventional breeding techniques with molecular genetic tools to develop maize varieties rich in essential micronutrients such as provitamin A carotenoids, iron and zinc. Key methodologies discussed include conventional selection and hybridization, genetic modification and gene editing technologies like CRISPR-Cas9. The review also examines the utilization of genetic diversity from wild relatives and landraces, the challenges of environmental variability and the socioeconomic factors influencing the adoption of biofortified varieties. Successes in biofortification are illustrated by examples like 'Orange Maize' and enhanced mineral content varieties. The paper underscores the potential of biofortification to improve global food security and public health, emphasizing the need for continued research, effective policy support, and strategies to enhance farmer and consumer acceptance. Future directions include leveraging emerging technologies and addressing broader adoption challenges to maximize the impact of biofortified maize.

Key words : Biofortification, Provitamin A Carotenoid, Iron, Zinc, Genetic modification.

Introduction

Maize, is a staple crop that sustains populations worldwide, serving as a primary source of calories for humans and as essential livestock feed. However, traditional maize strains commonly lack vital micronutrients, leading to nutritional deficiencies in regions dependent on maize as a staple food (Prasanna *et al.*, 2021). This situation underscores a critical public health issue, where populations are at increased risk of conditions like anaemia and blindness due to deficiencies in iron, zinc, and vitamin A respectively. Biofortification, the process of enhancing the nutritional profile of crops through genetic and agronomic modifications, has emerged as a strategic intervention to combat these deficiencies (Bouis and Saltzman, 2017). In recent years, significant strides have been made in maize biofortification, leveraging both conventional breeding techniques and modern biotechnological approaches to enrich nutrient content. This review paper focuses on the latest advancements in this field, detailing the integration of traditional breeding methods with molecular genetic tools to develop maize varieties enriched with essential micronutrients. The successes in biofortification are highlighted by increased levels of beta-carotene, zinc, and iron in maize, achieved through methods such as transgenic modifications and marker-assisted selection (Pixley *et al*, 2013). However, challenges persist, including the complex metabolic pathways involved in nutrient bioavailability and the socioeconomic barriers to the adoption of biofortified crops (De Steur *et al*., 2017). This review also explores the role of biofortified maize in improving global food security and health outcomes, emphasizing its potential in mitigating micronutrient malnutrition among vulnerable groups. Global importance, nutritional deficiencies associated with maize-based diets. Biofortification enhances the nutritional content of crops through agricultural practices, plant breeding, or genetic engineering. This process aims to significantly improve public health by ensuring that staple foods deliver essential minerals and vitamins. The foundational work has been laid, and with robust leadership from key institutions, biofortification has the potential to benefit one billion people by 2030.

Methods of Biofortification in maize

Conventional breeding techniques remain a cornerstone in efforts to biofortify crops, offering a time-tested route to enhancing the nutritional qualities of maize. These techniques primarily include selection, hybridization, and various evaluation processes, which together form a powerful toolkit for breeders aiming to increase the levels of specific nutrients in maize varieties. The process of selection involves identifying plants that exhibit desirable traits, such as increased nutrient levels and breeding them over successive generations. This method relies heavily on the natural genetic variation within maize populations. Phenotypic selection, the most traditional form of selection, has been utilized to enhance the visual and biochemical traits of maize that correlate with increased nutrient densities. For instance, selection for kernel color in maize has been effectively linked with enhanced carotenoid content (Burt *et al.*, 2011).

Hybridization is a method where two genetically diverse strains of maize are crossed to produce a hybrid that combines desirable traits from both parent lines. This technique has been pivotal in increasing crop yields and improving nutritional qualities. For biofortification, hybridization allows the combination of high nutrient levels from one parent with other favorable agronomic traits from another, such as disease resistance or drought tolerance. The success of hybridization in maize breeding has been well-documented, particularly in the development of high-yielding and nutrient-enriched maize hybrids (Smith, 2018). After selection and hybridization, the evaluation of hybrids under different environmental conditions is critical. This stage ensures that the desired traits are consistently expressed and that the new varieties perform well across different climatic and soil conditions. Evaluation involves both field trials and laboratory analyses to measure nutrient levels and assess the bioavailability of these nutrients. For example, field trials conducted across multiple locations have been used to confirm the stability of increased provitamin A content in maize under different growing conditions (Atlin *et al.*, 2017). While traditional breeding methods are effective, their integration with modern molecular techniques has significantly enhanced their efficiency and precision. Marker-assisted selection (MAS), for instance, uses molecular markers linked to desirable traits to facilitate the selection process. This method has been particularly useful in maize breeding for biofortification, allowing breeders to identify and select for genes that enhance micronutrient content more precisely and quickly than traditional methods alone (Babu *et al.*, 2014).

Genetic modification and gene editing

Genetic modification (GM) and gene editing represent the frontier of biotechnological interventions in the biofortification of maize. These methods allow for the precise manipulation of the maize genome to introduce or enhance traits responsible for the synthesis and accumulation of essential nutrients. Genetic modification involves the transfer of specific genes from one organism to another to confer desired traits. In maize biofortification, GM has been employed to introduce genes that code for enzymes involved in the biosynthesis pathways of vital nutrients, such as vitamin A, iron and zinc. One of the landmark achievements in this area has been the development of provitamin A-enriched ‘Golden Maize’ through the introduction of genes from bacteria and maize, which significantly enhance the carotenoid levels in kernels (Paine *et al.*, 2005). This approach has proven effective in addressing vitamin A deficiency, particularly in Sub-Saharan Africa where maize is a staple. Gene editing, particularly through CRISPR-Cas9 technology, offers a more precise tool for enhancing nutrient content. Unlike traditional GM, gene editing allows for the direct modification of the plant’s own DNA, enabling the enhancement or suppression of specific genes involved in nutrient metabolism. For instance, gene editing has been used to increase phytase activity in maize, which improves the bioavailability of phosphorus, an essential nutrient often bound in phytate form that is not readily available to humans (Li *et al.*, 2018).

The integration of GM and gene editing with conventional breeding techniques can expedite the development of biofortified maize varieties. This synergy allows for the combination of biotechnologically induced traits with those selected through traditional breeding, such as drought tolerance or disease resistance, thereby enhancing the overall agronomic viability of biofortified maize. The application of GM and gene editing in crop biofortification also brings regulatory challenges and public safety concerns. Each genetically engineered variety must undergo rigorous testing and approval processes to ensure that they are safe for consumption and the environment. Public acceptance is also crucial, as genetically modified foods often face consumer skepticism and regulatory scrutiny (Smyth, 2017). The genetic diversity inherent in wild relatives and landraces of maize represents a vast and largely untapped resource for traits associated with nutrient accumulation. These plant groups harbor alleles that can significantly enhance the nutritional quality of cultivated maize varieties. The strategies for utilizing the genetic diversity of wild relatives and landraces in maize biofortification, detailing the methods used to identify and incorporate beneficial traits into modern maize breeding programs.

Wild relatives and landraces of maize are known to possess unique genetic traits that have been lost in modern varieties due to the narrow focus of conventional breeding programs. These traits often include enhanced resistance to environmental stress and diseases, as well as improved nutritional profiles. Studies have shown that certain landraces and wild maize varieties exhibit naturally higher levels of micronutrients such as zinc and iron (Warburton *et al*., 2010). For example, certain landraces of maize have evolved under high-stress conditions, leading to the development of traits that enhance the bioavailability of nutrients such as iron and zinc (Ortiz *et al*., 2017). Research has highlighted how these genetic pools are characterized by a broader spectrum of phenotypic variability, including kernel size, color, and nutrient content, which is often higher than in refined commercial varieties (Bellon and Brush, 1994). Incorporating desirable traits from wild relatives and landraces into high-yielding commercial maize varieties typically involves complex breeding strategies. These strategies include backcrossing, where desirable traits are introgressed into modern cultivars while retaining high-yield characteristics. Marker-assisted selection (MAS) and genomic selection (GS) are increasingly used to efficiently select and retain these nutritional traits across breeding generations, minimizing the linkage drag associated with undesirable agronomic properties (Carena, 2013; Neves *et al*., 2017).

Advanced phenotyping and genotypic analysis are essential to effectively harness the genetic potential of wild relatives and landraces for nutrient biofortification. High throughput screening techniques, including genomic selection, enable the rapid assessment of genetic markers linked to desirable nutritional traits. This technological approach significantly speeds up the identification and integration of beneficial alleles into elite breeding lines (Bashir *et al*., 2019; Bazakos *et al*., 2017). Challenges and conservation issues despite the potential, using genetic material from wild relatives and landraces is not without challenges. These include the introgression of unwanted traits and the complexity of achieving stable expression of nutrient-related traits under varied environmental conditions. Furthermore, the conservation of genetic diversity in wild relatives and landraces is crucial, as these resources are threatened by genetic erosion and habitat destruction (Prescott-Allen and Prescott-Allen, 1990).

Key Nutrients Targeted for Biofortification in maize

Provitamin A Carotenoids, Importance in preventing vitamin A deficiency, breeding successes like the development of 'Orange Maize'. The provitamin A carotenoids, particularly beta-carotene, are essential micronutrients targeted for biofortification in maize due to their critical role in human health. Vitamin A deficiency (VAD) is a major public health issue in many developing countries, leading to severe health problems such as impaired vision, increased susceptibility to infections, and premature mortality. Key nutrients targeted for biofortification in maize and breeding methods illustrated in Fig. 1. This section discusses the importance of provitamin A in preventing VAD, outlines the breeding successes, and highlights the development of biofortified maize varieties like 'Orange Maize'. Diets deficient in these nutrients can lead to VAD, impacting millions of children and pregnant women in regions where maize is a staple food (West and Darnton-Hill, 2008). Breeding programs have focused on enhancing the provitamin A content in maize through both conventional breeding and genetic engineering. Traditional breeding methods have utilized naturally occurring genetic variation in maize to increase carotenoid levels. Marker-assisted selection (MAS) has been instrumental in these efforts, allowing breeders to identify and select for genetic markers associated with high carotenoid content effectively (Babu *et al*., 2013).

A significant breakthrough in the biofortification of maize was the development of 'Orange Maize,' which contains high levels of beta-carotene. This innovation was largely driven by HarvestPlus and its collaborators, who used conventional breeding techniques to incorporate genes responsible for beta-carotene synthesis from naturally high-carotenoid maize varieties. 'Orange Maize' has been shown to improve vitamin A status in children and adults, thus demonstrating its effectiveness in combating VAD in human populations (Pixley *et al*., 2013). While the development of 'Orange Maize' represents a substantial advancement, challenges remain. These include ensuring the stability of carotenoid levels under different environmental conditions, improving the bioavailability of carotenoids, and addressing consumer preferences and acceptance in target regions. Future research may focus on using gene editing tools like CRISPR to enhance the efficiency and precision of biofortification strategies (Ceballos *et al*., 2017).

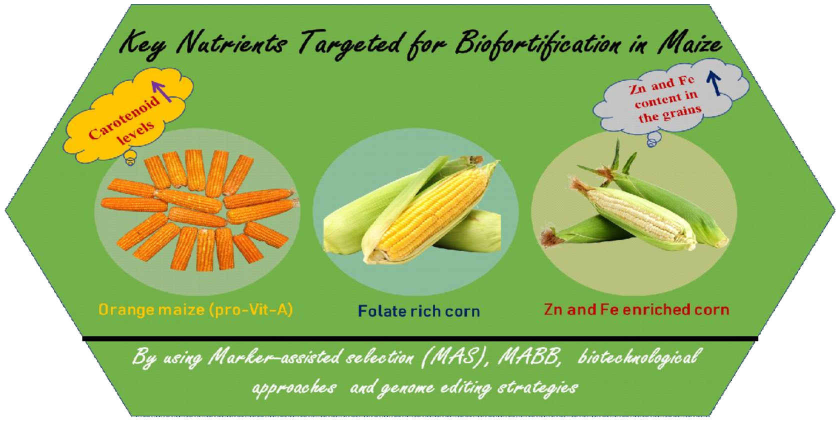

Fig. 1 : Key nutrients targeted for biofortification in maize and breeding methods.

Strategies to increase Iron and Zinc minerals and impact on public health

Iron and zinc are essential minerals critical for human health, playing pivotal roles in numerous biochemical processes. Deficiencies in these nutrients can lead to severe health issues such as anemia, impaired immune function, and stunted growth, particularly affecting children and pregnant women in developing countries. The strategies employed to enhance the iron and zinc content in maize, discusses the public health impacts of these minerals and reviews the successes and challenges in biofortification. Biofortification of maize with iron and zinc involves several strategies, including conventional breeding, genetic modification, and agronomic practices. Conventional breeding exploits natural genetic variability and selective breeding techniques to develop varieties with higher mineral content. Marker-assisted selection (MAS) has been a crucial tool in identifying and incorporating genes associated with high iron and zinc accumulation (Ortiz-Monasterio *et al*., 2007). Genetic modification offers another avenue by introducing specific genes that enhance the uptake and storage of these minerals in maize kernels (Zhao and McGrath, 2009). The effectiveness of these practices, however, often depends on the soil type, climate and other environmental factors (Cakmak, 2008).

Iron and zinc deficiencies are major public health concerns globally, with profound implications for morbidity, mortality, and overall well-being. Iron is crucial for oxygen transport in the blood, and its deficiency leads to anemia, reduced cognitive function, and decreased immunity. Zinc is essential for growth, immune function, and wound healing. Enhancing the iron and zinc content of staple crops like maize can substantially improve the nutritional status and health outcomes of populations dependent on maize as a primary food source (Gibson *et al*., 2010). One of the notable successes in this area has been the development of maize varieties with significantly enhanced levels of iron and zinc through breeding programs. For instance, the HarvestPlus program has developed maize varieties with up to double the baseline levels of zinc, which have been shown to improve zinc status in populations consuming these varieties (Bouis and Saltzman, 2017). Despite these successes, challenges remain in ensuring the stability of mineral enhancements across different environmental conditions and in integrating these efforts with broader food system approaches to improve nutrient intakes. Future directions might include the integration of crop biofortification with other public health interventions, such as dietary diversification and supplementation programs, to ensure comprehensive nutritional benefits (Saltzman *et al*., 2013).

Efforts to enhance protein quality through amino acid balance, folate biofortification. Enhancing Protein Quality through Amino Acid Balance

Maize protein is typically deficient in essential amino acids such as lysine and tryptophan, which are crucial for human health. Improving the amino acid balance in maize protein can significantly enhance its nutritional value, particularly for populations that rely heavily on maize as their primary protein source. The Quality Protein Maize (QPM) initiative is a prime example of successful breeding efforts to enhance maize protein. QPM varieties contain a naturally occurring genetic mutation that significantly increases lysine and tryptophan levels while maintaining the agronomic characteristics of conventional maize varieties (Prasanna *et al*., 2001). Recent advances in genetic engineering and molecular breeding have opened new pathways to further enhance the amino acid content of maize. For instance, transgenic approaches have been used to overexpress certain genes involved in the lysine biosynthesis pathway, leading to maize varieties with even higher lysine content than QPM (Huang *et al*., 2005).

Folate Biofortification

Folate, a B-vitamin essential for DNA synthesis and repair, is another critical nutrient that is commonly deficient in the diet of many populations. Folate deficiency is particularly concerning because it can lead to severe birth defects and anemia. Biofortification efforts aimed at increasing folate content in maize involve both traditional breeding and genetic engineering approaches. Transgenic maize lines engineered to overexpress genes involved in the folate biosynthetic pathway have shown promise, with folate levels many times higher than those found in conventional maize varieties (Diaz de la Garza *et al.*, 2007).

Challenges and Future Directions

There will be the genetic and agronomic challenges, Environmental variability, gene by environment interactions affecting nutrient levels. The pursuit of enhancing nutrient levels in maize through biofortification faces numerous genetic and agronomic challenges. One of the primary challenges in biofortification is the influence of environmental conditions on the phenotype of biofortified crops. Nutrient levels in plants are not solely determined by genetic factors but are also highly influenced by soil quality, water availability, and climatic conditions. For instance, zinc and iron content in maize can vary substantially with changes in soil mineral composition and pH levels (White and Broadley, 2009). This variability can lead to inconsistent nutrient levels in biofortified crops, complicating efforts to reliably enhance micronutrient intakes among populations.

Gene by environment (GxE) interactions occur when the environment differentially affects the expression of genetic traits across different settings. In the context of maize biofortification, GxE interactions can lead to significant variations in the expression of traits related to nutrient accumulation. For example, a gene that enhances zinc accumulation might express differently under dry versus wet conditions, leading to variable zinc content in maize grown in different regions (Bänziger and Long, 2000). Understanding and managing these interactions is crucial for the development of biofortified maize varieties that are consistently effective across diverse environments. From a breeding perspective, the integration of nutrient-enhancing traits into high-yielding maize varieties often involves complex hybridization and selection processes that must account for GxE interactions. Moreover, the need for advanced technological resources to identify and characterize these interactions can be a barrier, particularly in low-resource settings where biofortification programs are most needed (Ortiz *et al.*, 2007).

To address these challenges, future research should focus on developing more robust breeding strategies that can deliver stable nutrient profiles under a wide range of environmental conditions. This might involve the use of modern genomic tools such as genome-wide association studies (GWAS) and genomic selection (GS) to better understand and predict GxE interactions (Zhao *et al.*, 2011). Advances in precision agriculture and the development of tailored agronomic practices can also play a vital role in mitigating the impact of environmental variability. These practices include soil amendments, the use of micro-nutrient fertilizers, and irrigation management to optimize the growing conditions for biofortified crops (Cakmak, 2008). The future of nutrient enhancement in maize will likely involve a combination of advanced genetic tools like CRISPR/Cas9 for precise gene editing and traditional breeding methods to ensure that the nutritional enhancements are effectively incorporated into high-yielding, adaptable maize varieties.

Another challenge is that the acceptance and adoption by farmers and consumers, Social and economic factors influencing the uptake of biofortified varieties. The successful introduction of biofortified maize varieties into agricultural systems and food markets not only depends on the nutritional and agronomic traits of these crops, but also significantly on their acceptance and adoption by farmers and consumers. The decision by farmers to adopt biofortified varieties is influenced by several factors including the perceived benefits, cost, availability of seeds, and the agronomic performance of the varieties. Economic incentives, such as higher market demand or premium prices for biofortified crops, play a crucial role in adoption rates. However, the lack of awareness about the health benefits of biofortified crops can hinder uptake. Extension services and educational campaigns are essential in providing farmers with the knowledge and skills needed to cultivate these varieties effectively (Meenakshi *et al.*, 2010). Consumer acceptance of biofortified maize is influenced by taste, color, texture, and cultural food preferences. For example, the 'Orange Maize' enriched with provitamin A carotenoids may face acceptance challenges in regions where white maize is preferred for its taste and traditional use in recipes (De Groote *et al.*, 2014). Economic factors, including the cost of seeds and the economic status of farmers, influence the adoption of biofortified maize. In resource-poor settings, the initial cost of seeds, even if marginally higher, can be a barrier. Subsidies or financial incentives can help overcome these barriers, making biofortified seeds more accessible to smallholder farmers

(Hotz and McClafferty, 2007).

Support from governmental and non-governmental organizations is crucial in promoting the adoption of biofortified varieties. Policies that support research and development, seed distribution and marketing of biofortified crops can enhance both farmer adoption and consumer acceptance. Furthermore, integrating biofortification into national nutrition strategies can provide the necessary institutional backing to promote these crops (Saltzman *et al*., 2013). The social impacts of adopting biofortified crops, such as improved health outcomes and reduced healthcare costs, can be significant. Publicizing these benefits through community leaders and health practitioners can help shift public perception and increase acceptance (Low *et al*., 2007).

Policy and implementation like role of governmental and international policies in supporting biofortification programs. The success of biofortification programs depends significantly on the support they receive from governmental and international policies. National governments play a pivotal role in the implementation of biofortification programs. Supportive policies can include funding for agricultural research and development, subsidies for farmers growing biofortified crops and regulations that favor the marketing and distribution of these crops. For example, in countries like Zambia, national agricultural policies have been amended to include biofortification as a strategy to combat micronutrient malnutrition, leading to the widespread cultivation and consumption of provitamin A-biofortified maize (Gannon *et al*., 2014). Regulatory frameworks also play a crucial role. Approvals for the cultivation of genetically modified or conventionally bred biofortified crops can be expedited by policies designed to streamline the process, recognizing the public health benefits these crops offer. This is evident in the regulatory approaches taken by countries like Brazil, where biofortified crops have been fast-tracked through the approval process to address urgent public nutritional needs (Stein *et al*., 2007).

International organizations such as the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the Consultative Group on International Agricultural Research (CGIAR) have been instrumental in promoting biofortification globally. These entities work by setting international standards, providing a platform for collaboration, funding research and pilot projects, and facilitating knowledge transfer among countries. For instance, the HarvestPlus program, part of the CGIAR network, has played a crucial role in coordinating international efforts to breed, test, and disseminate biofortified crops across multiple countries. This program has been pivotal in integrating biofortification into the national nutrition strategies of over 30 countries (Bouis and Saltzman, 2017). Policies supporting biofortification have direct and significant impacts on public health. By making biofortified crops more available and affordable, these policies help increase the intake of essential nutrients, thereby reducing the prevalence of micronutrient deficiencies. Studies have shown that biofortified crops can improve micronutrient status and health outcomes, particularly among vulnerable populations in low-income countries (Hotz and McClafferty, 2007).

Conclusion

The journey of maize biofortification has marked significant milestones toward addressing global micronutrient deficiencies. This review has highlighted the substantial progress made in enhancing the nutritional quality of maize through both conventional breeding techniques and advanced biotechnological approaches. The development of varieties enriched with provitamin A, iron, zinc, improved protein quality and folate exemplifies the successful integration of genetic insights with agronomic practices. Moreover, the adoption and acceptance of these biofortified varieties, supported by robust governmental and international policies, underscore a global commitment to improving public health through agricultural innovation. The advancements in maize biofortification over the past decades have been driven by a combination of scientific innovation and strategic policy implementations. Techniques such as genetic modification and marker-assisted selection have revolutionized the ability to enhance specific nutrient levels in maize effectively. At the same time, traditional breeding methods continue to play a crucial role in ensuring that these enhancements are integrated into varieties that maintain high agronomic performance and consumer acceptance. The result has been the successful development and dissemination of biofortified maize varieties that are now cultivated in several countries worldwide.

Looking forward, the potential developments in genetic technologies and breeding strategies promise to further enhance the efficacy and efficiency of maize biofortification. Emerging tools like CRISPR/Cas9 gene editing offer unprecedented precision in modifying the maize genome, enabling the development of crops with optimized nutrient profiles and minimized undesirable traits. Additionally, the integration of phenotyping and genotyping data through artificial intelligence and machine learning could revolutionize breeding programs, making them more predictive and responsive to environmental variables. As biofortification moves forward, it will also be essential to address the broader socioeconomic and cultural factors that influence the adoption of biofortified crops. Strategies that encompass education, marketing, and community engagement will be crucial in ensuring that the benefits of biofortified maize reach the populations most in need. Furthermore, the ongoing evaluation of biofortified crops' health impacts will be vital in garnering continued support from policymakers, donors and the global community.

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