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REVIEW PAPER

Zinc biofortification of maize: Progress and challenges

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Abstract One of the most common health issues affecting women and children in developing nations is zinc deficiency. Zinc is the most widespread micronutrient deficient in soils. The low zinc levels in the soil resulted in reduced yields and crop plants with low nutritional value. Malnutrition can be treated with various strategies, but biofortification is the most effective, practical, long-lasting, and socially acceptable approach. Since maize is an important crop cultivated in areas where zinc nutritional deficiency is common, it makes sense to target this crop for zinc biofortification. Agronomic and genetic methods could be used to biofortify maize with zinc. Agronomic ways for biofortification include seed priming, foliage, and soil applications; however, the effects of applying these treatments individually or in combination vary concerning Zn enrichment are: (1) The genetic strategies for zinc biofortification increases zinc accessibility or increasing zinc levels in kernels. (2) Eliminating anti-nutritional elements or increasing bioavailability contributing factors. This can be done via conventional breeding, genome editing, or transgenic approaches. This review summarizes the status and prospect of zinc biofortification in maize.

Keywords: Agronomic approach · Biofortification · Conventional Breeding · Genetic approach · Maize

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Introduction

Zinc is a crucial micronutrient for human wellness (Zinc, 2005). Due to its many vital functions for human health, zinc is also known as the "metal of life" (Kaur *et al.*, 2014). Zinc deficiency ranks fifth among health risk variables contributing to diseases in developing nations (Maret and Sandstead, 2006). Its deficiency affects nearly one-third of the individuals on the entire globe (Hotz and Brown, 2004), while over fifty percent of pregnant women and youngsters in developing nations exhibit a zinc deficiency. Besides humans, it is an essential micronutrient for the growth and development of plants. It is crucial for several enzyme-mediated reactions, metabolic functions, redox processes, plant hormone metabolism, encouraging the growth of plant reproductive systems, enhancing plant immunity to pathogen infection, and enhancing plant defense against stress.

The primary cause for zinc deficiencies in humans is the consumption of cereal-based diets, such as those based on maize, rice, and wheat (Pfeiffer and McClafferty, 2007). The zinc deficiency in grains can be addressed by biofortification. Biofortification is the process of using agronomic assistance or genetic approaches to enhance the accessibility of vital nutrients in the consumable parts of crop plants. It is a potent strategy combining conventional breeding, chemical implementation, and genetic engineering to improve the content of vitamins, minerals, and micronutrients. Currently employed methods for Zn biofortification include breeding for high zinc genotype, agronomic biofortification, and transgenic/genetic-engineering-based techniques, (Jha *et al.*, 2020; Garg *et al.*, 2018). Agronomic biofortification entails implementing Zn fertilizers in the soil or straight to the crops, to improve the amount and bioaccessibility of Zn in food crops (Adu *et al.*, 2018). By exploiting the genetic variations among genotypes and wild relatives of the same crops, biofortification through breeding seeks to increase the content and bioaccessibility of zinc in high-yielding cultivars (Al-Khayri *et al.*, 2016; Marques *et al.*, 2021). As plant breeding might not be able to biofortify some crops due to a dearth in genetic variation among them, in such cases, the transgenic methods are to be employed by inserting appropriate genes into crop plants to produce desired nutritional traits (here high zinc) (Garg *et al.*, 2018). These three strategies have been used mostly to biofortify cereals, legumes, oilseeds, vegetables, and fruits (Garg *et al.*, 2018). Given the significance of zinc for the well-being of humans, this review precedes the overview of the development and advancement of zinc biofortified maize genotypes. Since maize is one of the most prevalent edible crops in areas with common zinc nutritional deficiencies, it is ideal for biofortification.

Key approaches for biofortification

There are three key approaches for the biofortification of crops namely conventional breeding, agronomic, and genetic engineering/transgenic approaches.

1. Conventional breeding approach

Biofortification is a sustainable, economical way to improve the nutritional quality of crops through conventional breeding. For this approach, sufficient genotypic variation is a prerequisite for breeding programs to be effective. The natural variations are identified within maize germplasm to identify maize lines with high zinc levels in the kernels and then such lines are utilized in the breeding programs using the selection and crossing technique. For instance, Harvest Plus in collaboration with the International Institute of Tropical Agriculture (IITA, Africa) and International Maize and Wheat Improvement Center (CIMMYT) has breeding programs on developing maize with high zinc levels along with traits like drought tolerance and disease resistance using methods like phenotypic selection and crossing (CIMMYT, 2020). The high-zinc hybrids and open-pollinated varieties of maize developed under the Harvest Plus project can potentially supply up to 70% of daily zinc requirements and thereby help in reducing zinc insufficiency in countries where maize is a key staple food. (https://www.harvestplus.org/crop/zinc-maize/). To date, a few mapping studies mapped several genomic regions i.e., quantitative trait loci (QTL) for Zn accumulation/content (Table 1).

2. Agronomic approach

Agronomic biofortification is a technique that aims to increase the concentration and bioavailability of micronutrients in food crops. This is achieved by applying micronutrient-rich fertilizers, soil amendments, and plant

Table 1. The details of major quantitative trait loci (QTLs) for Zn accumulation/content and associated traits in maize

S.No.	Mapping populations	No. of QTLs	Chromosome no.	Traits	LOD range	Marker type	References
1.	Ye478 × Wu312 RIL	3	2,10	Zinc score	3.9-19.8	SSR	Xu *et al.* (2021)
	population	6	2,3,5	Shoot and root dry weights	3-6.3		
		4	1,2,10	Root-to-shoot ratio	3.1-8.9		
2.	$F_{2:3}$ 178/P53	4	2,5,10	Zinc content	3.01-5.58	SSR	Jin *et al.* (2013)
3.	B84 × Os6-2	2	3, 4	Biofortification trait	5.19-5.21	SSR & SNP	Šimic *et al.* (2012)
4.	$F_{2:3}$ populations derived from the crosses Mu6 × SDM	6	1,2,3,7,9	Zn concentration in kernel and cob	2.58-6.84	SSR	Qin *et al.* (2012)
	$F_{2:3}$ populations derived from the crosses Mo17 × SDM	6	1,7,9,10		2.56-11.78		
5.	Double haploid (DH) population: DH8/DH40	4	2, 3, 4, 6, 7, 10	Zn in maize seed	2.15-5.23	SSR	Zhou *et al.* (2010)
	DH86/S137	6			2.03-6.56		
6.	RIL	22	2,3,4,5,6,7,8,9	Maize grain Zn concentration	3.01-31.94	SSR	Zhang *et al.* (2017)

optimization practices. Increasing the nutritional state of maize by opting for agronomic practices is a quick and easy approach (Bhardwaj *et al.*, 2022). The main methods include foliar application with diluted fertilizer sprays, soilless cultivation, nutri-priming, and soil allocation of micronutrient fertilizer to help crops to absorb nutrients. By following these methods, crops can absorb more nutrients, resulting in an increased concentration of micronutrients. The main objective is to optimize agricultural production while minimizing environmental harm and ensuring sustainability.

Soil application

Soil application of micronutrients aids in restoring micronutrients in the soil where a crop or plant is cultivated. This method is utilized by crops highly susceptible to micronutrient deficiencies (Martens and Westermann, 1991). Enhancing the zinc fertility of the soil enables plants to absorb more zinc and transfer it into grains and other parts of plants. In soil, the most common mineral fertilizers containing zinc are zinc oxide, zinc chelates, and zinc sulfate. Zn fertilization of the soil (50 kg ha^{-1} of Zinc sulfate) has been shown to improve the production of maize grains significantly (Obaid *et al.*, 2022). Anwar *et al.* (2022) demonstrated that treatment of $ZnSO_4$ and $FeSO_4$ alone or in combination increased grain quality as well as the yield characteristics of the maize crop.

Foliar application

Applying one or more essential plant mineral nutrients to the aerial portions of plants through foliar spraying is known as foliar fertilization. By reducing nutrient loss and enabling nutrients to be directly absorbed by plants, foliar micronutrient feeding has been shown to raise the zinc content of grain (Hassan *et al.*, 2021; Johnson *et al.*, 2005). Zn-administered foliar application is phloem-mobile and actively translocate into maize kernels while growing, thereby, enhancing its bioavailability (Haslett *et al.*, 2001; Erenoglu *et al.*, 2011). Saleem *et al.* (2016) evaluated the response of maize hybrid towards zinc and iron fertilizers via soil and foliar application and observed increased zinc and iron content in maize kernels compared to control plants.

Nutipriming/seed priming

Seed priming is the process of treating seeds with various solutions or nutrients before they sow Osmo-priming and hydro-priming techniques are frequently employed. When seeds are treated with zinc-rich solutions, there is an increase in the concentration of zinc in the mature corn kernels. Priming of seed with $ZnSO_4$ has been reported in a significant increase in corn yield (Maqbool and Beshir, 2019). Attributes like genotype, crop kind, nutrient priming duration, priming solution osmotic potential, and environmental conditions impact this method's efficiency. Previously, the effect of adding zinc sulfate to maize was investigated in vitro, on-station, and in on-farm trials which reported increased zinc content in maize as well as a significant increase in grain yield in maize. Recently, micronutrient seed priming has been demonstrated to improve maize early seedling growth in micronutrient-deficient soil (Nciizah *et al.*, 2020).

3. Genetic Engineering-based approach

By implementing the principles of plant breeding and biotechnology, genetic engineering introduces novel agronomic or nutritional traits to a particular crop (Melash *et al.*, 2016). Biofortification using the genetic engineering approach involves recognizing and introducing appropriate genes into crops to enhance their nutritional qualities (Garg *et al.*, 2018). The desired foreign genes that might result in zinc biofortification in maize can be obtained from bacteria, fungi, and other microorganisms. Additionally, introducing pathways from bacteria and other organisms into crops can help explore substitute pathways for metabolic engineering (Newell-McGloughlin, 2008). By these means, crops can be genetically modified to improve zinc absorption, transportation, and concentration by incorporating useful genes (Singh *et al.*, 2016; Mir *et al.*, 2020). For instance, overexpression of the *HvNAS1* gene (encoding for nicotianamine synthase enzyme) in rice has been shown to increase Zn content by 1.5-fold in grain (Masuda *et al.*, 2009). Similarly, higher expression of *AtNAS1* and *OsNAS2* genes was also shown to increase Zn and Fe contents (Singh *et al.*, 2017). NAS genes are responsible for nicotianamine (NA) production, a chelator that binds to zinc and iron and aids in their regulation. Since the genes that encode for NAS are differentially regulated by Zn and Fe treatment in several plant species, overexpression of maize orthologue would likely lead to higher zinc accumulation in kernels.

Phytic acid (PA), inositol 1,2,3,4,5,6-hexakisphosphate, is a natural product present in maize seeds, which represents about 75% of the total seed phosphorus. However, PA is an anti-nutritional compound, as it cannot be digested by humans/monogastric animals due to the absence of phytase enzymes and can cause environmental pollution (Malik and Maqbool, 2020; Zhou and Erdman, 1995). It acts as a chelator of divalent cations of Iron (Fe^{+2}), zinc (Zn^{+2}), calcium (Ca^{+2}), and Magnesium (Mg^{+2}) hence reducing the bioavailability of these important micronutrients (Iqbal *et al.*, 1994; Gibson *et al.*, 2010., Ibrahim *et al.*, 2022). Thus, in order to enhance the level/bioavailability of Zn^{+2} and Fe^{+2} in maize kernels, it is important to reduce the antinutrient (PA) content of maize seeds. Inositol pentakisphosphate 2-kinase 1 (*IPK1*) gene has been reported to be an important gene that catalyzes the last step of the multistep PA biosynthesis pathway. Therefore, a CRISPR/Cas9-based genome-editing approach has great potential and could be utilized by specifically targeting the *IPK1* to avoid other lethal phenotypes. Recently, in wheat, disruption of the *IPK1* gene has been shown to reduce the phytic acid content in the seeds, which in turn, resulted in the high bioavailability of Fe and Zn (Ibrahim *et al.*, 2022). Considering the same, disruption of most downstream enzymes i.e., *IPK1* of PA metabolic pathways would be a plausible strategy for maize biofortification without affecting the plant inositol metabolism, upstream processes, and other related pathways.

Challenges in producing nutritionally-enriched crops

Producing crops that are enriched with nutrients is a complex process, and there are several challenges involved in it. Some of the most significant challenges are as follows:

1. Phenotypic evaluation for zinc biofortification of maize

Zinc content in the kernel is not a stable trait i.e., lots of variation is there due to environment. The same genotype may produce kernels with significant differences in zinc content if grown under two different locations or soil types or climatic conditions. Thus, uniformity and stability of traits is a big challenge. Breeding initiatives for zinc biofortification require careful consideration of different stages of breeding, considering any physical or genetic contamination may compromise the genetic basis of desirable traits. The maintenance of the isolation block is necessary for trial administration of the fields during line development, maintenance of lines, and the multiplication of seeds. Therefore, having an efficient personnel or laboratory for zinc content assessment is crucial for zinc biofortification programs (Gupta *et al.*, 2015a).

Genetic markers associated with zinc accumulation should be identified by combining physical traits and genetic information to increase the speed of breeding for zinc-biofortified maize. (Andersson *et al.*, 2017). Visual prediction of zinc enrichment is not technically possible because Zn biofortification of maize does not result in apparent alterations in grain color. This makes it difficult to convince farmers, sellers, buyers, processors, traders, or any other parties involved in the marketing chain to pay a premium price for a nutrient with invisible value-added benefits (Maqbool and Beshir, 2018). Hence, determining the amount of zinc in maize grains requires quantitative measurement techniques like atomic absorption spectroscopy, inductively coupled plasma mass spectrometry (Pfeiffer and McClafferty, 2007), or colorimetric assays. These methods often require specialized and costly equipment, trained personnel, and standardized protocols to ensure precise and reproducible results (Goredema-Matongera *et al.*, 2021; Maqbool and Beshir, 2018; Andersson *et al.*, 2017). Further, maintaining consistency across different research studies or breeding programs can be difficult as a result of differences in experimental conditions and approaches used.

2. Recessive nature of genes

Recessive genes play a major role in enhancing the nutritional value of plants via genetic manipulation. The majority of genes that enhance the nutritional quality of biofortified maize are recessive (Gupta *et al.*, 2015a; Gupta *et al.*, 2015b). Contamination from normal, non-biofortified maize pollen may cause xenia, which is higher closer to the borders and less in the middle of the fields. Pollen contamination does not eliminate nutritional traits but may be significantly reduced. The contamination level in biofortified cultivars is influenced by different factors, such as the distance between normal maize and biofortified cultivars, the timing of their flowering, the direction of the wind during pollination, and the competitiveness of the normal and biofortified pollen for fertilization (Vivek *et al.,* 2008). Therefore, it is necessary to carry out studies to determine the effects of pollen contamination on Zn biofortified maize.

Biofortified maize's acceptability/consumer preferences for biofortified maize

For biofortification initiatives to be successful, farmers and consumers must be willing to accept biofortified crops (Mir *et al.*, 2020). Consumer acceptance of zinc biofortified crops is limited because people lack knowledge and comprehension of the advantages of biofortification. The lack of supportive policies, incentives, or infrastructure for promoting and disseminating zinc biofortified maize can hinder acceptance. This challenge can be addressed via effective communication campaigns, collaborating with stakeholders, and engaging local communities are necessary and highlighting the positive outcome on health and well-being. However, there is still hope for Zn biofortified maize as it does not alter the genotype-related pigment, and thus, genotypes with higher approval for Zn-enriched maize may still exist. Various other platforms, such as agricultural shows, field days, seed fairs, and media, can be utilized to promote Zn-enhanced maize (Groote *et al.*, 2010).

Policies

Policy regulations related to agriculture policies, food safety, and biofortification significantly impact the development, commercialization, and adoption of zinc-biofortified maize hybrids. Regulatory agencies usually have strict requirements for the approval of genetically modified organisms (GMOs) or novel plant varieties. Increasing public awareness of the dietary advantages of biofortification, particularly among women, can facilitate the rapid approval of biofortified crops. Zinc biofortified maize could be instrumental in enhancing the nutritional status of people. The efforts should be made by policymakers like the introduction of biofortified maize in the PDS (Public Distribution System) to popularize zinc biofortified maize in India. Zinc and iron biofortified maize crops can help alleviate hidden hunger and it is essential to educate farmers and consumers about such crops (Okwuonu *et al.*, 2021). Conflicting priorities among government agencies may impede comprehensive strategies for promoting zinc biofortification of maize.

Future Prospects

Maize biofortification holds great promise as a long-term and affordable solution to combat nutritional deficiencies despite other methods. Although conventional breeding is the most acceptable approach however lack of sufficient diversity in germplasm is a major limitation of this approach. Therefore, genetic engineering-based approaches (transgenic and genome editing) may play a pivotal role in developing zinc-biofortified maize. Since transgenic or genetically modified crops have lesser consumer acceptance, genomic edited (SDN I and SDN II type) zinc biofortified maize should be the most suitable and viable crop. Being transgene-free such genome-edited maize has a chance of gaining wider public acceptance (Kumar *et al.,* 2020).

Conclusion

Malnutrition and starvation are global issues affecting both developed and developing countries. Biofortification is a practical and cost-effective approach to combat malnutrition. It involves techniques such as mineral-based fertilizers, transgenic crops, and plant breeding to address micronutrient deficiencies. However, even after producing biofortified crops, socio-political and economic issues must be addressed to encourage their production and consumption. To successfully address hidden hunger, a coordinated approach involving political leaders, agriculturalists, genetic engineers, nutritionists, and educators is essential. One of the primary shortcomings of biofortification is the lack of public acceptance, particularly for transgenic crops. Therefore, greater education and marketing ventures are necessary to increase understanding and promote consumer consumption. Further transgene-free genome-edited maize has the potential to address the major issues associated with GM or transgenic crops. Therefore, genome editing tools should be deployed for developing Zn biofortified maize plants. Another major challenge is to maintain high levels of zinc in biofortified crops once they are released to farmers. Factors such as the mixing of biofortified and non-biofortified seeds, poor agronomic practices, and biotic and abiotic stress can reduce nutrient concentration. Therefore, continuous monitoring of zinc biofortified crops is necessary to mitigate perturbations in zinc concentration. By addressing the challenges and implementing a coordinated global strategy, zinc biofortification may offer an applicable solution to improve global nutritional well-being as well as reduce zinc malnutrition.

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