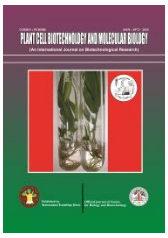

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Advancing Nutritional Quality through Genomic Approaches for Biofortification in Cereal Crops: A Review

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

ABSTRACT

Hidden hunger, characterized by micronutrient deficiencies, remains a pervasive challenge in marginalized regions worldwide, where the main source of sustenance is cereal crops. This review study examines how improving the nutritional value of cereal crops through genetic "biofortification" could help fight hidden hunger. It focuses on the biochemical and genetic underpinnings of zinc, iron, and critical amino acid accumulation. While conventional breeding efforts have made significant contributions, the complex genetic nature of mineral content in grains presents

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challenges. In response, molecular techniques such as CRISPR/Cas genome editing offer promising solutions. The paper underscores the widespread prevalence of iron and zinc deficiencies, affecting over a large population. Children under five and pregnant and lactating women in developing countries are particularly vulnerable. Biofortification, defined as the breeding of staple food crops with elevated micronutrient levels, emerges as a cost-effective and sustainable strategy to improve health in resource-poor households. It discusses the transfer of genes and quantitative trait loci (QTLs) from wild and related species to cultivated wheat, emphasizing the need for marker-assisted selection and genomic selection to accelerate breeding progress. The emergence of CRISPR/Cas genome editing techniques in recent decades has revolutionized the field of plant breeding. The paper highlights the successful application of CRISPR/Cas9 in numerous cereal crops such as rice, wheat, maize and barley, to improve crop yields and nutritional content. It also explores the potential for precise base editing and gene expression modifications. However, challenges such as transformation efficiency, specific promoters, and ethical and regulatory concerns are also mentioned and discussed. In conclusion, genetic biofortification through CRISPR/Cas-mediated genome editing presents a promising avenue for alleviating hidden hunger in cereal-dependent regions.

Keywords: CRISPR/Cas9; QTLs; biofortification; hidden hunger; conventional breeding.

1. INTRODUCTION

The global incidence of micronutrient malnutrition affects over 2 billion people, with a significant proportion of this burden borne by resource-poor households, as noted by Cashman and Vitamin [1]. This issue is particularly pressing for children of pre-school age, adolescent women and women of reproductive age, as highlighted by Bouis et al. [2]. Insufficient quantities of essential micronutrients, also called 'hidden hunger,' can lead to a range of physical, mental, social and economic challenges. These may include increased rates of illness, disability, stunted physical growth and a detrimental impact on national socio-economic development, as discussed by Ekholuenetale et al. [3]. Micronutrient deficiencies or "hidden hunger," represent a formidable health challenge on a global scale, especially in developing nations. Among these dietary deficiencies, zinc (Zn), iron (Fe), iodine(I) and vitamin A are the most common, particularly among young children and women. It's noteworthy that more than three billion people worldwide suffer from deficiencies in zinc (Zn), iron (Fe) and vitamin A [4] and these nutrient deficits are especially prevalent among certain populations [5]. Deficiency of Zinc is a prevalent risk affecting approximately 30% of the world population [6]. Anaemia, which is Iron deficiency is a widespread issue as well, impacting one-fourth of women and children worldwide. The results are cognitive skill impairment, reduced physical activity, perinatal mortality, mild mental retardation, and maternal mortality [7]. Zinc deficiency can lead to cognitive impairment, stunted growth, and a weakened immune system [8].

Recent discoveries have clarified the function of specific bacterial strains that have the ability to solubilize metals, such as zinc, making them easier for plant roots to absorb. This discovery presents an extra method of biofortification, as explained by Mumtaz et al. [9]. Introducing these microbial strains into crop seeds can improve soil fertility and crop yields by increasing the concentration of micronutrients in edible sections of the crop, as noted by Sarwar et al. [10]. Conversely, genetic biofortification involves the use of both conventional and transgenic breeding techniques to introduce genes that either modify the crop's genetic makeup using genome editing technologies to express proteins that boost micronutrient accumulation or that encourage high micronutrient accumulation into elite crop genotypes. This approach offers a sustainable solution which is also cost-effective and long-lasting solution for supplying micronutrients in the diets of malnourished populations, as discussed by Ludwig and Slamet-Loedin [11].

"Biofortification" is the procedure of increasing the essential mineral and vitamin content and their bioavailability in the edible parts of staple food crops. This can be accomplished through conventional breeding, biotechnological interventions, and the use of fertilizers. An exemplar of successful biofortification is "Golden rice," where the beta-carotene content present in rice was improved by transforming three biosynthetic pathway genes: phytoene synthase (psy), phytoene desaturase (crtI) and lycopene β-cyclase (lcy). Many countries, including Bangladesh, Brazil, China, Colombia, India, Indonesia, Malawi, Nigeria, Pakistan, Panama, Rwanda, Uganda and Zambia, have incorporated biofortification into their national health and development policies [12]. Biofortification in staple food crops has advanced through initiatives like Harvest Plus, the Grand Challenge in Global Health, the India Biofortification Programme, Scaling up Nutrition (SUN) and Global Alliance for Improved Nutrition (GAIN), among others and has garnered both global and local recognition. Biofortification is a process aimed at increasing the vitamin and mineral content in the edible parts of crops. This enhancement can be achieved through various methods, including traditional breeding, agronomic practices, and genetic engineering, as discussed by Bouis, [13]. The primary goal of biofortification is to guarantee the provision of vital micronutrients to populations with restricted access to a variety of foods. This approach seeks to reduce the occurrence of diseases and mortality associated with insufficient micronutrient intake from staple foods. Moreover, it contributes to improved food productivity, food security, and the overall quality of life, particularly among impoverished populations, as highlighted by Wakeel et al. [14].

2. CROPS UNDERGOING BIOFORTIFICATION PROCESSS

In the realm of agricultural innovation and nutritional enhancement, a diverse array of crop varieties has been meticulously developed, each with a specific focus on meeting the nutritional needs of different regions. Among these, a selection of rice, wheat, and maize varieties stands out for their unique nutrient targeting tabulated in Table 1.

2.1 Biofortification of Rice

Biofortification of rice has emerged as a critical intervention in addressing widespread micronutrient deficiencies, particularly in regions heavily reliant on rice as a staple food source. Rice is a dietary mainstay for majority of the Asian countries and a primary sustenance for over half of the population worldwide, often lacks essential micronutrients like iron and zinc. These deficiencies have led to the prevalence of various health disorders in many developing nations, underscoring the urgency of finding solutions [15]. In its raw form, rice contains these vital micronutrients, but unfortunately, they diminish notably during postharvest processing, which is essential for meeting consumer demands and enabling long-term storage of the grain without quality degradation. Processes like dehulling and debranning lead to micronutrient losses as they remove layers like the aleurone layer, pericarp, and embryonic tip [16]. This depletion has significant repercussions, especially for communities heavily dependent on rice as their primary food source, exacerbating the issue of iron and zinc deficiency. Therefore, any successful biofortification initiatives for rice must prioritize targeting the endosperm that is left behind after processing.

Table 1. Crops undergoing biofortification processs

Crop	**Variety**	**Target Nutrient**	**Nutrient Range Ppm**	**Year of Release**
Rice	DRR Dhan 45	*Zinc*	12-16	2016
Wheat	WB 02	*Zinc*	32.0	2017
		Iron	28.0-32.0	
	HPBW 01	*Zinc*	32.0	2017
		iron	28.0-32.0	
Maize	Pusa vivek QPM9	*Provitamin A*	1.0-2.0	2017
		Lysine	1.5-2.0%	
		tryptophan	0.3-0.4%	
	Pusa HM4	*lysine*	1.5-2.0%	
		tryptophan	0.3-0.4%	
	Pusa HM8	*lysine*	1.5-2.0%	
		tryptophan	0.3-0.4%	
	Pusa HM9	*Lysine*	0.3-0.4%	
		tryptophan	1.5-2.0%	

Source: ICAR, New Delhi.

The impetus to rice biofortification and combat iron and zinc deficiencies gained momentum following the "Biofortification" Index created by HarvestPlus, which found critical zinc deficiency in various Asian countries [2]. Collaborative efforts, such as those between HarvestPlus and the International Rice Research Institute (IRRI), have yielded significant progress in developing zinc biofortified rice varieties tailored to specific countries such as Bangladesh, the Philippines, India, and Indonesia. Extensive research and breeding endeavours have also been dedicated to increasing iron and carotenoid concentrations in rice varieties, with several releases till now. Notably, target values for increased grain iron and zinc in rice, aiming to meet 30% of the human Estimated Average Requirement (EAR), have been set at 13 and 28 µg/g, respectively [13]. This collective effort to biofortify rice holds immense promise in enhancing the nutritional quality of this important staple food, ultimately leading to improved health outcomes in regions where rice consumption is highest. It represents a vital step in addressing the persistent issue of micronutrient deficiencies and fostering the well-being of vulnerable populations.

The exploration of genetic variation in rice reveals promising opportunities for biofortification, especially with regard to zinc content. It is evident that there exists substantial and valuable genetic diversity in zinc content, which can be harnessed through breeding programs to create biofortified rice varieties. This emphasis on zinc is further accentuated by the relatively limited genetic variability observed for iron content, steering the focus of conventional breeding efforts predominantly towards zinc [16,17]. However, the challenge in improving iron content is compounded by the significant losses of this essential micronutrient during the polishing process, rendering selective breeding an unviable option [17]. For the enhancement of zinc content in rice, innovative breeding methods come to the forefront. Ratnasekera et al. [18] propose the utilization of the advanced backcross method, particularly for the genetic dissection of wild rice and the development of high-zinc introgression lines. This approach not only efficiently incorporates valuable genetic traits from wild rice but also offers a promising avenue for boosting zinc levels. Narrow sense heritability, with its high potential, can be exploited through single plant selection as an effective approach for nutrient quantity improvement [19]. Additionally, employing multiple crossing methods, including 3-way or 4-way crosses, or reciprocal crosses, can effectively enhance both zinc levels and yield potential in rice. Meng et al. [20] highlight the Multi-parent Advanced Generation Inter-cross (MAGIC) as a feasible method to pool genes for high zinc, creating a valuable source for selecting high-zinc lines and transgressive segregants. The concept of heterosis can also be leveraged to develop hybrids with high grain zinc content and exceptional yield potential, as satisfactory heterosis has been observed in rice [21].

However, every breeding effort must create a balance to ensure that essential agronomic characteristics, particularly high yield, are not sacrificed in the pursuit of nutrient biofortification. Calayugan et al. [19] emphasize the importance of considering yield-related traits during the selection process. Although results concerning the relationship between yield and zinc content have varied in past studies, recent research findings mostly indicate that there is no significant correlation between the two traits. This provides assurance that it is indeed possible to develop rice varieties that are both zinc-rich and high-yielding, thus meeting the dual objectives of addressing nutrient deficiencies and ensuring food security [22]. These findings underscore the potential to create rice varieties that not only nourish but also sustain communities, offering a promising path to address nutritional challenges in areas and regions where rice is a dietary staple. Rice Varieties: DRR Dhan 45 has been cultivated with a specific aim in mind – enhancing zinc content. With a target nutrient range of 12-16 ppm, it strives to address zinc deficiency among populations relying on rice as a staple.

2.2 Biofortification of Wheat

Wheat (*Triticum aestivum*) holds a vital position in global food systems, catering to roughly 20% of the dietary requirements worldwide [23,35,37-38]. This adaptable crop can thrive in diverse agro-ecosystems worldwide, which makes it one of the most widely cultivated food crops [36]. Despite being rich in calories, wheat grains are deficient in essential micronutrients like iron and zinc, and this deficiency is increased during the process of milling [20]. To address this deficiency, a target increase of +12 µg/g was set to raise grain zinc concentration from a baseline of 25 to 37 µg/g in improved wheat varieties [39]. Large-scale screening of germplasm for grain zinc content, encompassing wheat landraces and wild relatives by the International Maize and Wheat Improvement Center (CIMMYT), identified significant variability that could meet the target increments for zinc in biofortified varieties. More than 3,000 germplasm accessions were screened, revealing a zinc content range of 20 to 115 µg/g. A report from the Bangladesh Agricultural Research Institute found that average zinc concentration in wheat grain spanned from 20 to 35 µg/g [24].

Numerous high zinc genotypes with the potential to serve as parental lines in zinc breeding programs were identified, with most of them being ancestors of modern high zinc hexaploid wheat [24]. These findings offer promising avenues for enhancing the nutritional content of wheat, particularly in terms of zinc, and represent significant progress in addressing the global challenge of micronutrient deficiencies through biofortification. Wheat Varieties: WB 02, a wheat variety, concentrates on elevating zinc content, aiming for a nutrient range of 32.0 ppm. It tackles the nutritional needs of communities that rely on wheat as a dietary staple. HPBW 01, another wheat variety, takes a dual approach by targeting both zinc and iron. Its nutrient range for iron falls within the range of 28.0-32.0 ppm, while zinc is specifically enhanced to reach 32.0 ppm. This variety serves to combat the prevalence of iron and zinc deficiencies among regions where wheat is a primary food source.

2.3 Biofortification of Maize

Maize, scientifically known as *Zea mays*, holds a prominent position in the realm of "biofortification," as it is one of the most extensively targeted cereals for this purpose [34]. This is attributed to its widespread cultivation as a staple crop and its significance as a source of diverse products in regions of sub-Saharan Africa (SSA), South America, and South Asia. One of the key factors that make maize an attractive choice for "biofortification" is the vast native genetic diversity it exhibits in terms of micronutrient concentration. This genetic variability offers a solid foundation for enhancing the crop's nutritional profile through plant breeding techniques. Moreover, the adaptability of maize to a wide range of agro-ecosystems further justifies its continued inclusion in "biofortification" programs.

In SSA, maize plays a pivotal role in the daily diets of 20 countries, contributing a substantial 30% of the total calories from cereal-based foods. The reliance on maize in the region results in a significant per capita daily consumption, reaching up to 450 grams per person per day [25]. In SSA, maize is commonly consumed in various forms, such as boiled or roasted green mealies or as a thick porridge made from ground maize meal, often served with an accompanying relish. However, the challenge lies in the inherently low essential micronutrient density found in conventional maize [26], making consumers in these impoverished and marginalized regions more susceptible to various micronutrient deficiency-related health conditions.

To address this issue, substantial efforts have been made in the "biofortification" of maize, primarily led by the International Maize and Wheat Improvement Center (CIMMYT) in collaboration with HarvestPlus, an organization dedicated to improving nutrition and public health through the development of biofortified food crops. The biofortification of maize has primarily focused on enhancing protein content, specifically lysine and tryptophan, as well as increasing the concentration of zinc and pro-vitamin A in maize grains [27]. These initiatives represent significant strides in bolstering the nutritional value of maize, ultimately contributing to improved public health, particularly in regions where maize is a dietary cornerstone. The foundation for breeding Quality Protein Maize (QPM) is rooted in the existence of homologous recessive alleles of the opaque-2 gene, which typically occurs in a homozygous dominant or heterozygous state**.** When this gene is in the homozygous recessive state, it leads to the production of elevated levels of the essential amino acids tryptophan and lysine compared to regular maize varieties [28-55]. However, the initial phases of breeding QPM unveiled a challenge – the expression of the opaque-2 mutant gene resulted in a soft, chalky endosperm, which was not well-received by consumers. It was through subsequent research that a breakthrough was achieved by breeding for the presence of the mutant gene alongside its accompanying genes, resulting in the development of maize with a desirable hard endosperm, preferred by consumers.

Hence, the successful breeding of QPM necessitates a multi-faceted approach, involving the manipulation of three distinct genetic systems: (i) replacing the normal gene with the mutant gene at the opaque-2 locus, (ii) utilizing modifier genes that enhance the expression of the opaque-2 gene in relation to lysine and tryptophan content, and (iii) deploying modifier genes that induce the development of the preferred hard endosperm, ultimately delivering maize varieties that address protein quality and consumer preferences. Maize Varieties: Within the maize category, a range of varieties has emerged, each with a focus on distinct essential nutrients. Pusa Vivek QPM9 strives to increase the levels of provitamin A, lysine, and tryptophan, with nutrient ranges of 1.0-2.0 ppm for provitamin A, 1.5-2.0% for lysine, and 0.3-0.4% for tryptophan. These nutrient enhancements aim to improve the overall nutritional quality of maize, especially in regions where it plays a significant role in diets. Pusa HM4, Pusa HM8, and Pusa HM9 all target lysine and tryptophan, with nutrient ranges identical to those of Pusa Vivek QPM9. These maize varieties collectively represent a concerted effort to combat nutrient deficiencies and enrich the dietary value of maize-based diets. These crop varieties are a testament to the ongoing commitment to address nutritional deficiencies and enhance the overall nutritional quality of staple crops. By meticulously targeting essential nutrients, they contribute to improved public health and food security, particularly in regions where these crops are integral to daily life.

3. GENOME ENGINEERING USING GENOME EDITING TOOL

Genome engineering has witnessed remarkable advancements with the development of innovative tools, such as Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and the revolutionary Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system illustrated in Fig. 1 and Fig. 2. These tools enable precise manipulation of DNA sequences for various applications, including genetic research and therapeutic interventions.

a) Zinc Finger Nucleases (ZFNs): ZFNs are one of the pioneering genome engineering tools. They function through the fusion of zinc finger domains with the FokI endonuclease. Zinc finger domains are remarkable for their ability to recognize specific triplets of consecutive nucleotides in the DNA [29]. When two ZFN monomers bind to the target DNA, they position their FokI endonuclease domains in such a way that they form a dimer. This dimerization of FokI endonucleases results in the induction of Double-Strand Breaks (DSBs) in the DNA at the target site. The DNA repair processes that follow these breaks can lead to various modifications in DNA sequences, including insertions, deletions (indels), and substitutions, ultimately influencing the encoded proteins [30].

b) Transcription Activator-Like Effector Nucleases (TALENs): TALENs share similarities with ZFNs in that they induce DSBs by dimerizing their FokI nucleases. However, their unique feature lies in the use of Transcription Activator-Like Effector (TALE) repeats to recognize the target DNA site [29]. TALE repeats consist of N-terminal regions containing nuclear localization signals and C-terminal regions fused with FokI endonuclease [31]. The TALENs can be customized to recognize specific DNA sequences, making them highly adaptable for various genome engineering applications.

c) CRISPR/Cas9: The CRISPR/Cas9 system has revolutionized genome engineering due to its simplicity and versatility. Cas9, a protein, is guided by a single-guide RNA (*sgRNA*) to the target DNA sequence [32]. Importantly, the Cas9 protein contains cleavage domains, *RuvC* and *HNH*, which are guided by a Protospacer Adjacent Motif (PAM) to the target site. Once the Cas9 protein is precisely positioned, it induces DSBs at the target DNA site. The subsequent DNA repair processes, namely Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR), can lead to specific modifications in DNA sequences**.** These modifications include insertions, deletions (indels), and substitutions, which in turn affect the structure and function of the encoded proteins. ZFNs, TALENs and CRISPR/Cas9 represent cutting-edge genome engineering tools that have opened new frontiers in genetic research [33]**,** offering the potential for precise and controlled modification of DNA sequences for a wide range of applications, from basic research to the development of novel therapeutic strategies.

4. CHALLENGES FOR BIOFORTIFICATION IN CEREAL CROPS

Cereal crops, such as rice, wheat, and maize, serve as the primary source of nutrition for billions of people worldwide [40]. However, their genetic complexity and diversity pose significant challenges when it comes to enhancing their nutritional quality. Understanding and effectively harnessing the vast array of genetic variations in these staple crops is a fundamental challenge in the pursuit of biofortification. Even when researchers successfully enhance the nutrient content of cereal crops, there's a critical issue of nutrient bioavailability. Factors such as anti-nutritional compounds, absorption inhibitors, or food processing methods can limit the human body's ability to absorb these nutrients [41]. Addressing the bioavailability of enhanced nutrients is a multifaceted challenge that necessitates interdisciplinary research. An integrated approach for eradication of malnutrition including new breeding techniques, supplementation and diversification (Fig. 3).

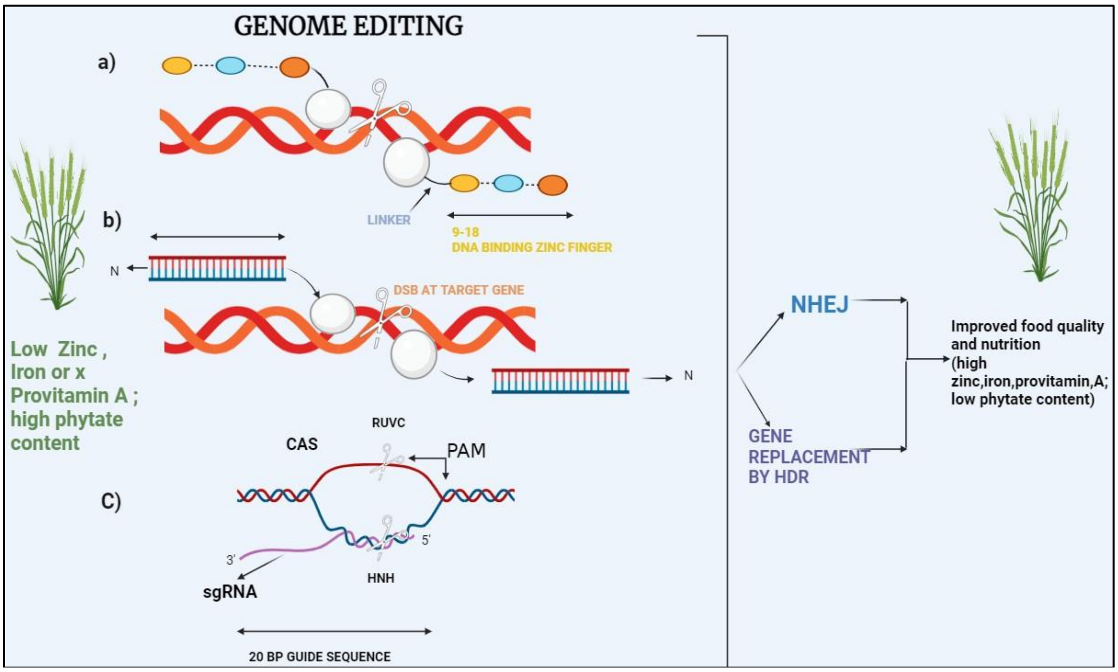

Fig. 1. Illustration of genome engineering using ZFNs, TALENs, and CRISPR/Cas9 genome editing tools

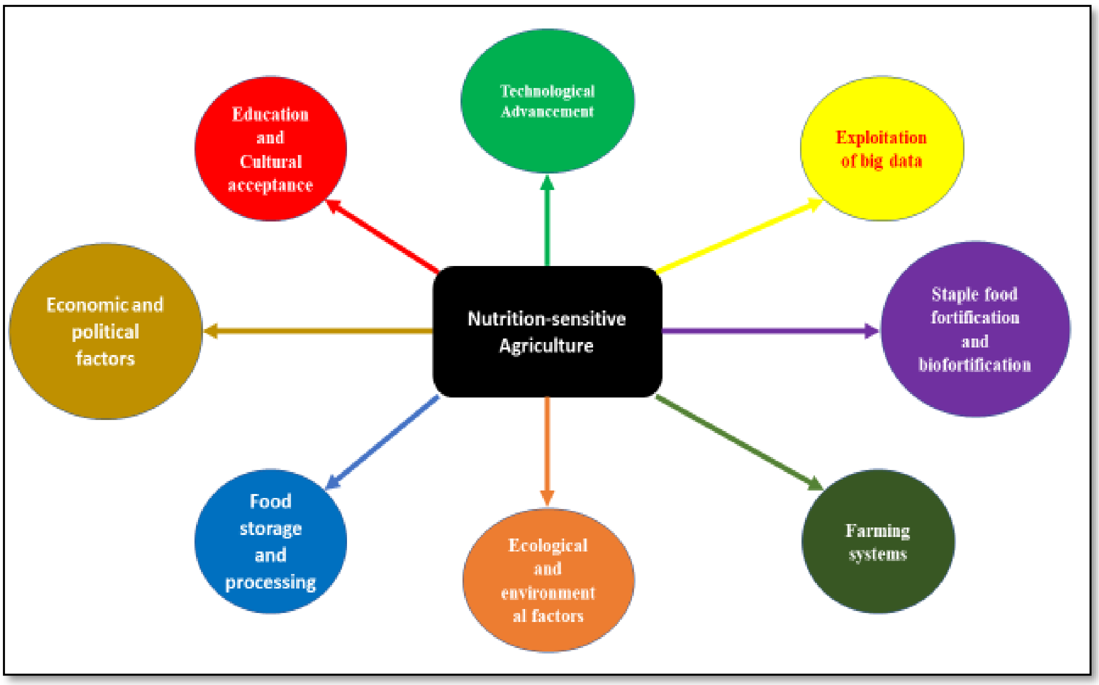

Fig. 2. The complex interplay of various factors in the development of nutrition-sensitive agriculture

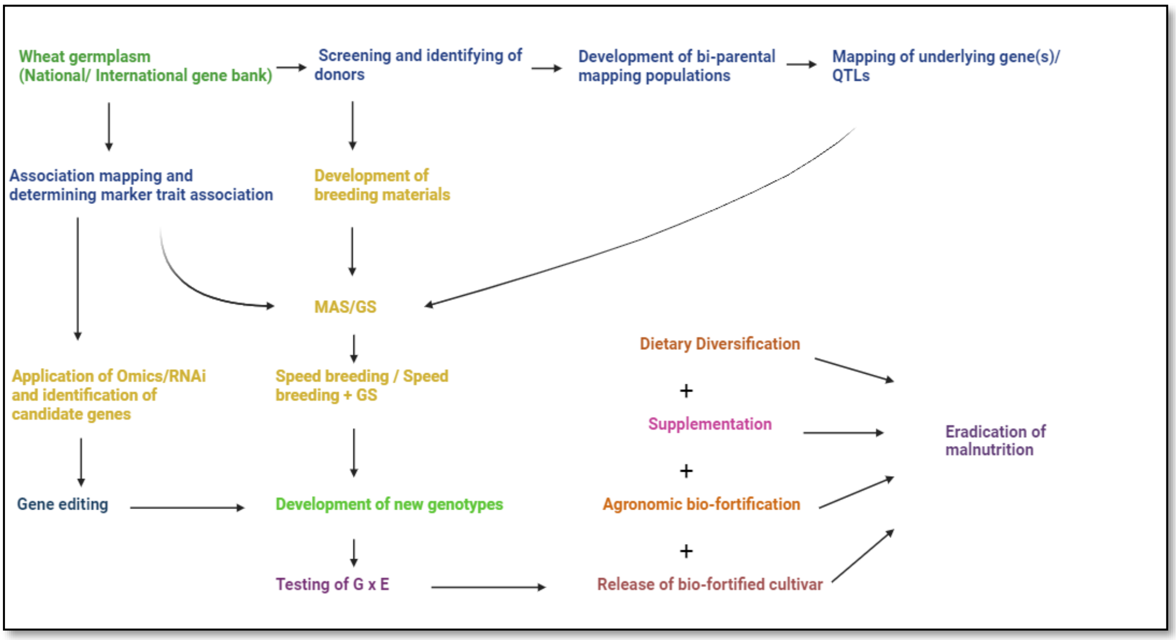

Fig. 3. An integrated approach for eradication of malnutrition including new breeding techniques, supplementation and diversification

Biofortified cereal crops may sometimes alter the taste or appearance of familiar staple foods, potentially affecting consumer acceptance. Striking the right balance between improving nutritional content and maintaining sensory qualities presents a unique challenge in biofortification efforts, as it requires considering both the science of nutrition and the preferences of consumers. The regulatory landscape surrounding genetically modified organisms (GMOs) and biofortified crops can be complex and subject to change [42]. Navigating regulatory hurdles and ensuring the safety of biofortified cereal crops is essential, as these crops may involve genetic modifications [43]. Researchers must be aware of evolving regulations and address safety concerns effectively. Enhancing the nutritional quality of cereal crops through genetic modification may have unintended consequences on the environment [44]. Researchers must consider the ecological impact of biofortified crops and work toward solutions that balance nutritional benefits with sustainability, addressing issues like land use and biodiversity. While scientific breakthroughs are critical, ensuring that biofortified cereal crops reach and benefit communities, especially in resource-limited regions, poses challenges related to scaling production and ensuring accessibility. This involves both technological advancements and socio-economic considerations.

Maintaining the stability of enhanced nutrients throughout the life cycle of the crop – from growth and harvest to storage and processing – is vital. Preventing nutrient degradation, which can occur due to various factors, is a significant challenge that needs to be addressed to ensure the nutritional impact of biofortified crops. Manipulating the genetics of cereal crops for improved nutrition may involve trade-offs with other essential crop traits, such as yield and disease resistance [45]. Researchers must find the right balance between these traits, as altering one characteristic may affect others, necessitating a careful consideration of multiple factors in biofortification efforts. Enhancing multiple nutrients simultaneously in cereal crops is more complex than single-nutrient biofortification. Researchers must address the interactions between different nutrients and ensure that the biofortified crops provide balanced nutrition without unintended consequences.

Encouraging farmers to adopt and cultivate biofortified cereal crops may require education and incentives. Understanding and overcoming the barriers to farmer adoption, which can include factors like access to seeds and training, is an essential component of successful biofortification initiatives [2]. Sustainability is a long-term concern in biofortification efforts. This includes maintaining genetic diversity in cereal crops and ensuring that biofortified varieties remain resilient and productive over time, especially in changing environmental conditions. Finally, identifying and addressing research gaps is an ongoing challenge in the field of biofortification. Continued scientific inquiry is essential to push the boundaries of what's possible in terms of improving nutritional quality in cereal crops and addressing the challenges that lie ahead. Genomic approaches in biofortification in cereals tabulated in Table 2.

5. FUTURE ASPECTS IN GENOMIC APPROACHES FOR BIOFORTIFICATION

In the realm of genomic approaches for biofortification in cereal crops, the future promises several significant advancements and challenges. One of the primary future directions lies in achieving precision. Researchers are set to refine their techniques to target and enhance specific nutrient pathways with even greater accuracy. This precision will facilitate the creation of crops that deliver nutrients more effectively, ensuring that the nutritional needs of specific populations can be met. A major focus will be on multi-nutrient biofortification. While single-nutrient biofortification has been a remarkable step forward, the future will prioritize the simultaneous enhancement of multiple nutrients in cereal crops. Developing crop varieties that provide a balanced set of essential nutrients, including vitamins, minerals, and amino acids, will be pivotal in addressing the complexities of malnutrition on a broader scale [46]. The continued evolution of gene editing technologies, exemplified by CRISPR/Cas9, will enable precise modification of specific genes responsible for nutrient content in cereal crops [47]. This will make the biofortification process more efficient and controlled, allowing for the tailored enhancement of nutritional profiles. Addressing the bioavailability of fortified nutrients in cereal crops will remain a critical focus. Researchers will explore strategies to reduce anti-nutritional factors and enhance nutrient absorption, ensuring that the enhanced nutrients are effectively utilized by the human body. Possible candidate genes associated with Fe, Zn, and vitamins tabulated in Table 3.

A more consumer-centric approach is on the horizon for biofortification efforts. Researchers recognize the significance of consumer preferences and cultural considerations and will work towards developing crops that offer improved nutrition while aligning with local tastes and culinary traditions [48]. This approach seeks to ensure greater acceptance and adoption of biofortified crops. Sustainability will be at the forefront of future biofortification initiatives. Researchers will strive to minimize the ecological footprint of biofortified crops, developing environmentally friendly and resource-efficient varieties. Sustainable practices will be crucial to

Table 2. List of genomic approaches in biofortification in cereals (rice, wheat, and maize)

Crop	Genome-editing	Nutrients	Gene	Method of transformation	Vectors used
Rice	Crispr/cas9	Carotenoid	–	Particle bombardment	–
		High amylose	SBEIIb	*Agrobacterium* mediated	*pCXUN-Cas9*
		Low phytic acid	OsITPK6		*pH_itpk6*
		Beta- carotene	Osor		–
		Amylose	Waxy	*Agrobacterium* transformation	*CRISPR/Cas9 vector*
		Sucrose efflux transporter	OsSWEET11, OsSWEET14		*pTOPO/D*
		Amylase synthase	OsU3, OsU6a, OsU6b, OsU6c		*pCAMBIA1300*
Wheat	Crispr/cas9 Crispr/cas9	Low gluten	Alpha gliadin	Biolistic transformation	*pANIC-6E destination vector*
		Fe, mg	TaVIT2	*Agrobacterium* mediated	*pBract202*
Maize	Cripsr/cas9 Cripsr/cas9	Carotenoid	Phytoene synthase		*pMD18-T*
		Low phytic acid content	Phytic acid synthesis	*Agrobacterium* transformation	*pEasy blunt vector*

Table 3. List of possible candidate genes associated with Fe, Zn, and vitamins for future application of biofortification

Crops	Candidate gene	Micronutrient
Barley	*HvHGGT*	*Vitamin E*
	HvMTP1	*Zn*
	HVPT1, HVPT2	
	HvYS1	*Fe*
	NAS, NAAT, DMAS, IDS2, andIDS3	
Maize	*crtRB1*	*Vitamin A*
	LcyE	
	Y1/Psy1	
	ZmFer1	*Fe*
	ZmYS1	
	ZmZIP	*Fe and Zn*
Rice	Crt1	*Vitamin A*
	HvNAS1	*Fe and Zn*
	MxIRT1	
	OsDMAS1	*Fe*
	OsFer2	
	OsIRO2	
	OsIRT1	
	OsNAAT1	
	OsVIT1, OsVIT2	
	OsYSL13	
	OsYSL15	
	OsYSL16	
	PSY	*Vitamin A*
Wheat	*Crt1*	*Vitamin A*
	CrtB	
	Gpc-B1	*Zn*
	OsNAS2	*Fe and Zn*
	PvFERRITIN	
	TaFer1, TaFer2	*Fe*

ensure the long-term viability of biofortification efforts. As biofortification matures as a field, there will be concerted efforts to streamline regulations and establish clear guidelines for the development and deployment of genetically modified biofortified crops [49]. This will facilitate faster adoption and implementation, ensuring that biofortified crops reach the populations that need them.

Engaging local communities and farmers in the biofortification process will be a pivotal aspect of future efforts. Building awareness, providing training, and ensuring equitable access to biofortified seeds will be central to the success of biofortification programs, particularly in resource-limited regions. Data-driven approaches using big data and advanced analytics will play a substantial role in optimizing genomic approaches for biofortification [50]. This will help in identifying the most effective genetic targets and cultivation practices, facilitating evidence-based decision-making in biofortification research and implementation. Global collaboration is set to increase as scientists, governments, non-governmental organizations, and the private sector work together on a global scale [51]. This international cooperation will facilitate the exchange of knowledge, resources, and best practices, accelerating the pace of biofortification research and deployment.

Given the ongoing impact of climate change on agriculture, the development of biofortified cereal crops that are resilient to changing climate conditions will be a priority [52]. These crops should perform well under a variety of environmental stresses, ensuring that biofortification efforts are sustainable and adaptable. Ultimately, the goal is to achieve nutritional security for all. In the future, research will focus on integrating biofortified crops into broader food and nutrition security strategies. This will ensure that these crops reach and benefit vulnerable populations globally, contributing to improved global health and food security.

6. CONCLUSION

Hidden hunger, characterized by micronutrient deficiencies, remains a persistent challenge, particularly in marginalized regions where cereal crops serve as the primary source of nutrition. While conventional breeding efforts have made significant strides, the complex genetic nature of mineral content in grains presents ongoing challenges. However, recent advances in molecular techniques, such as CRISPR/Cas9 genome editing, offer a promising solution to this global issue. The prevalence of iron and zinc deficiencies, affecting a substantial portion of the global population, especially children under five and pregnant/lactating women in developing countries, underscores the urgency of addressing this problem. Biofortification, which involves enhancing the micronutrient content of staple food crops, emerges as a cost-effective and sustainable strategy to improve the health and well-being of resource-poor households. This review paper has shed light on the transfer of genes and quantitative trait loci (QTLs) from wild and related species to cultivated wheat, emphasizing the importance of marker-assisted selection and genomic selection to expedite breeding progress. Furthermore, it has discussed the revolutionary impact of CRISPR/Cas9 genome editing techniques in plant breeding, highlighting their successful application in various cereal crops, including rice, wheat, maize, and barley, not only for improving crop yields but also for enhancing nutritional content. Issues like transformation efficiency, the selection of specific promoters, and ethical and regulatory concerns must be addressed for the widespread adoption of these techniques. Genetic biofortification through genome editing holds the promise of alleviating hidden hunger in regions heavily reliant on cereals for sustenance. The potential to enhance the nutritional content of these staple crops offers hope for a future where vulnerable populations have improved access to essential micronutrients, ultimately contributing to better health and nutrition outcomes globally. As the field of genetic biofortification continues to evolve, collaboration among scientists, policymakers, and communities will be essential to maximize the benefits of this ground-breaking approach and combat hidden hunger more effectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cashman KD. Vitamin D deficiency: defining, prevalence, causes, and strategies of addressing. Calcified tissue international. 2020;106(1):14-29.
2. Bouis H, Birol E, Boy E, Gannon BM, Haas JD, Low J, Mehta S, Michaux K, Mudyahoto B, Pfeiffer W, Qaim M. Food biofortification: reaping the benefits of science to overcome hidden hunger. InOctober webinar on The Need for Agricultural Innovation to Sustainably Feed the World by 2050 2020 (No. 69). Council for Agricultural Science and Technology (CAST).
3. Ekholuenetale M, Tudeme G, Onikan A, Ekholuenetale CE. Socioeconomic inequalities in hidden hunger, undernutrition, and overweight among under-five children in 35 sub-Saharan Africa countries. Journal of the Egyptian Public Health Association. 2020; 95:1-5.
4. Roy C, Kumar S, Ranjan RD, Kumhar SR, Govindan V. Genomic approaches for improving grain zinc and iron content in wheat. Frontiers in Genetics. 2022;13: 1045955.
5. Beal T, White JM, Arsenault JE, Okronipa H, Hinnouho GM, Morris SS. Comprehensive Nutrient Gap Assessment (CONGA): A method for identifying the public health significance of nutrient gaps. Nutrition reviews. 2021;79 (Supplement_1):4-15.
6. NairK M, Choudhury DR. Zinc nutrition in health and diseases. Journal of SAT Agricultural. 2013;11:1–6.
7. Stoltzfus RJ, Mullany L, Black RE. Iron deficiency anaemia. Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors. 2004;1:163-209.
8. Cakmak I. Enrichment of fertilizers with zinc: An excellent investment for humanity and crop production in India. Journal of trace elements in medicine and biology. 2009;23(4):281-9.
9. Mumtaz MZ, Ahmad M, Jamil M, Hussain T. Zinc solubilizing Bacillus spp. potential

candidates for biofortification in maize. Microbiological research. 2017;202:51-60.

10. Sarwar N, Akhtar M, Kamran MA, Imran M, Riaz MA, Kamran K, Hussain S. Selenium biofortification in food crops: Key mechanisms and future perspectives. Journal of Food Composition and Analysis. 2020;93:103615.
11. Liang Z, Zhang K, Chen K, Gao C. Targeted mutagenesis in Zea mays using TALENs and the CRISPR/Cas system. Journal of Genetics and Genomics. 2014; 41(2):63-8.
12. Virk PS, Andersson MS, Arcos J, Govindaraj M, Pfeiffer WH. Transition from targeted breeding to mainstreaming of biofortification traits in crop improvement programs. Frontiers in Plant Science. 2021;12:703990.
13. Bouis HE. Biofortification: an agricultural tool to address mineral and vitamin deficiencies. InFood fortification in a globalized world. 2018;69-81.
14. Wakeel A, Farooq M, Bashir K, Ozturk L. Micronutrient malnutrition and biofortification: recent advances and future perspectives. Plant micronutrient use efficiency. 2018:225-43.
15. Bashir K, Nozoye T, Nagasaka S, Rasheed S, Miyauchi N, Seki M, Nakanishi H, Nishizawa NK. Paralogs and mutants show that one DMA synthase functions in iron homeostasis in rice. Journal of Experimental Botany. 2017;68(7):1785-95.
16. Ndolo VU. Characterisation of chemical components in manually isolated aleurone and associated layers from maize, wheat and barley kernels; 2013.
17. Hunt JM. Mobilizing Nutritional Improvements as a Regional Public Good. Regional Public Goods. 2003:375.
18. Ratnasekera D, Tennakoon A, Sandamal S, Wijerathna P. Perception of Advanced Techniques in Conservation and Utilization of Wild Genetic Resources of Rice. InPlant Genetic Resources, Inventory, Collection and Conservation. 2022;463-504.
19. Calayugan MI, Swamy BM, Nha CT, Palanog AD, Biswas PS, Descalsota-Empleo GI, Min YM, Inabangan-Asilo MA. Zinc-biofortified rice: A sustainable food-based product for fighting zinc malnutrition. InRice improvement: Physiological, molecular breeding and genetic perspectives. 2021;449-470.
20. Meng L, Zhao X, Ponce K, Ye G, Leung H. QTL mapping for agronomic traits using multi-parent advanced generation inter-cross (MAGIC) populations derived from diverse elite indica rice lines. Field Crops Research. 2016;189:19-42.
21. Naik SR, Singh SK, Singh DK, Khaire AR, Korada M, Habde S, Majhi PK. Estimation of heterosis for yield related traits and grain zinc in rice (*Oryza sativa* L.). Electronic Journal of Plant Breeding. 2021; 12(4):1227-35.
22. Anusha G, Rao DS, Jaldhani V, Beulah P, Neeraja CN, Gireesh C, Anantha MS, Suneetha K, Santhosha R, Prasad AH, Sundaram RM. Grain Fe and Zn content, heterosis, combining ability and its association with grain yield in irrigated and aerobic rice. Scientific reports. 2021;11 (1):10579.
23. Mohammadi-joo S, Mirasi A, Saeidi-Aboeshaghi R, Amiri M. Evaluation of bread wheat (*Triticum aestivum* L.) genotypes based on resistance indices under field conditions. 2015;6(2): 331-337.
24. Das S, Chaki AK, Hossain A. Breeding and agronomic approaches for the biofortification of zinc in wheat (*Triticum aestivum* L.) to combat zinc deficiency in millions of a population: A Bangladesh perspective. Acta Agrobotanica. 2019; 72 (2).
25. Ekpa O, Palacios-Rojas N, Kruseman G, Fogliano V, Linnemann AR. Sub-Saharan African maize-based foods-processing practices, challenges and opportunities. Food Reviews International. 2019;35(7) :609-39.
26. Cakmak I, Kutman UÁ. Agronomic biofortification of cereals with zinc: a review. European journal of soil science. 2018;69(1):172-80.
27. Goredema-Matongera N, Ndhlela T, Magorokosho C, Kamutando CN, van Biljon A, Labuschagne M. Multinutrient biofortification of maize (*Zea mays* L.) in Africa: current status, opportunities and limitations. Nutrients. 2021;13(3):1039.
28. Maibvisira NA, Gasura E, Maphosa M, Nyakurwa CS. Genetic basis and the current breeding efforts for quality protein maize in Southern Africa. African Crop Science Journal. 2018;26(4):529-41.
29. Shamshirgaran Y, Liu J, Sumer H, Verma PJ, Taheri-Ghahfarokhi A. Tools for efficient genome editing; ZFN, TALEN, and CRISPR. Applications of genome modulation and editing. 2022:29-46.

30. Savino S, Desmet T, Franceus J. Insertions and deletions in protein evolution and engineering. Biotechnology Advances. 2022;60:108010.
31. Zhang J, Zhang H, Botella JR, Zhu JK. Generation of new glutinous rice by CRISPR/Cas9-targeted mutagenesis of the Waxy gene in elite rice varieties. Journal of integrative plant biology. 2018; 60(5):369.
32. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346(6213): 1258096.
33. Santra CR. A Mini Review on CRISPR-Cas: The Cutting Edge of DNA Editing. American Journal of Applied Bio-Technology Research. 2021;2(2): 91-114.
34. Nisar S, Rashid Z, Touseef A, Kumar R, Nissa SU, Faheem J, Angrez A, Sabina N, Shabeena M, Tanveer A, Amal S. Productivity of fodder maize (*Zea mays* L.) SFM-1 under varied sowing dates and nitrogen levels. International Journal of Bio-resource and Stress Management. 2024;15(Jan, 1):01-12.
35. Rathore M, Yellanki Pravalika RK, Tutlani A, Aggarwal N. Enhancing seed quality and insect management in wheat (*Triticum aestivum* L.) through optimization of storage treatments with natural and chemical compounds. Plant Archives. 2024;24(1):26-36.
36. Paudel P, Pandey MK, Subedi M, Paudel P, Kumar R. Genomic approaches for improving drought tolerance in wheat (*Triticum aestivum* L.): A Comprehensive Review. Plant Archives. 2024;24(1):1289-300.
37. Reddy B, Kumar B, Kumar R, Thota H. Analysis of Heterotic Potential for Yield and Its Contributing Traits in Wheat (*Triticum aestivum* L.). International Journal of Environment and Climate Change. 2023;13(9):388-400.
38. Santhoshini A, Dubey N, Avinashe HA, Thonta R, Kumar R. Inheritance Studies in Segregating Population of Bread Wheat (*Triticum aestivum* L.). International Journal of Environment and Climate Change. 2023;13(9):277-87.
39. Aggarwal N, Rathore M, Kumar, R, Tutlani A, Kumari S and Yellanki P. Identification of Induced Chemical Mutants for Improving Yield and Its Attributing Traits in Wheat (*Triticum aestivum* L.) Against Changing Climate. Agricultural Science Digest. 2024; 1-8.
    DOI: 10.18805/ag.D-5959
40. Awika JM. Major cereal grains production and use around the world. InAdvances in cereal science: implications to food processing and health promotion. 2011;1-13.
41. Thakur A, Sharma V, Thakur A. An overview of anti-nutritional factors in food. Int. J. Chem. Stud. 2019;7(1):2472-9.
42. Turnbull C, Lillemo M, Hvoslef-Eide TA. Global regulation of genetically modified crops amid the gene edited crop boom–a review. Frontiers in Plant Science. 2021; 12:630396.
43. Goredema-Matongera N, Ndhlela T, Magorokosho C, Kamutando CN, van Biljon A, Labuschagne M. Multinutrient biofortification of maize (*Zea mays* L.) in Africa: current status, opportunities and limitations. Nutrients. 2021;13(3):1039.
44. Cellini F, Chesson A, Colquhoun I, Constable A, Davies HV, Engel KH, Gatehouse AM, Kärenlampi S, Kok EJ, Leguay JJ, Lehesranta S. Unintended effects and their detection in genetically modified crops. Food and Chemical Toxicology. 2004;42(7):1089-125.
45. Bailey-Serres J, Parker JE, Ainsworth EA, Oldroyd GE, Schroeder JI. Genetic strategies for improving crop yields. Nature. 2019;575(7781):109-18.
46. Poole N, Donovan J, Erenstein O. Agri-nutrition research: Revisiting the contribution of maize and wheat to human nutrition and health. Food Policy. 2021; 100:101976.
47. Tiwari M, Kumar Trivedi P, Pandey A. Emerging tools and paradigm shift of gene editing in cereals, fruits, and horticultural crops for enhancing nutritional value and food security. Food and Energy Security. 2021;10(1):e258.
48. Pawera L, Khomsan A, Zuhud EA, Hunter D, Ickowitz A, Polesny Z. Wild food plants and trends in their use: From knowledge and perceptions to drivers of change in West Sumatra, Indonesia. Foods. 2020;9 (9):1240.
49. De La Torre-Roche R, Cantu J, Tamez C, Zuverza-Mena N, Hamdi H, Adisa IO, Elmer W, Gardea-Torresdey J, White JC. Seed biofortification by engineered nanomaterials: a pathway to alleviate malnutrition?. Journal of Agricultural and Food Chemistry. 2020;68(44):12189-202.

50. Peramaiyan P, Craufurd P, Kumar V, Seelan LP, McDonald AJ, Kishore A, Singh S. Agronomic biofortification of zinc in rice for diminishing malnutrition in South Asia. Sustainability. 2022;14(13): 7747.
51. MacDonald C, Hilton B, Dove R. Integrating biofortified crops into community development programs. African Journal of Food, Agriculture, Nutrition and Development. 2017;17(2):12063-77.
52. Satyavathi CT, Ambawat S, Khandelwal V, Srivastava RK. Pearl millet: a climate-resilient nutricereal for mitigating hidden hunger and provide nutritional security. Frontiers in Plant Science. 2021;12: 659938.
53. Aswini M. S., Kiran, Lenka B, Shaniware YA, Pandey P, Dash AP, Haokip SW. The Role of Genetics and Plant Breeding for Crop Improvement: Current Progress and Future Prospects. Int. J. Plant Soil Sci. [Internet]. 2023 Sep. 19 [cited 2024 May 22];35(20):190-202. Available:https://journalijpss.com/index.php/IJPSS/article/view/3798
54. Kumar KP, Walia P. Speed Breeding in Cereal Crops: Accelerating Genetic Improvement for Rapid Agricultural Advancement. J. Exp. Agric. Int. [Internet]. 2024 May 11 [cited 2024 May 22];46 (6):465-77. Available:https://journaljeai.com/index.php/JEAI/article/view/2498
55. Zhang Y, Massel K, Godwin ID, Gao C. Applications and potential of genome editing in crop improvement. Genome biology. 2018;19(1):210.

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