Mahalingam Govindaraj
Velu Govindan
Natalia Palacios *Editors*

Breeding Zinc Crops for Better Human Health

Springer

Biofortified Cereals Increase Dietary Zinc Intake: Wheat and Maize as Case Studies

Swarnim Gupta and Nicola M. Lowe

Introduction

Micronutrient deficiency, also known as hidden hunger, is a major global concern. It compromises immune systems, delays child growth and development and has a debilitating effect on human potential (Bailey et al. 2015; Tulchinsky 2010). Globally, iron and zinc deficiencies are the most widespread mineral micronutrient malnutrition and these often occur concurrently (Sandstead 2000). Recent estimates derived from 24 nationally representative surveys indicate that over half of preschool-aged children and two-thirds of non-pregnant women of reproductive age (WRA) are deficient in at least one core micronutrient (iron, zinc, and folate) (Stevens et al. 2022). Although no region of the world is unaffected from this burden, including high-income countries, the burden is considerably higher in low- and middle-income countries (LMICs). Regionally, South Asia together with sub-Saharan Africa, East Asia and the Pacific is home for three-quarters of preschool-aged children with hidden hunger. Over half (57%) of non-pregnant women of reproductive age with micronutrient deficiencies live in East Asia and the Pacific or South Asia. However, in the UK and USA, it is estimated that 43% and 32% respectively of WRA are deficient in at least one core micronutrient (Stevens et al. 2022).

Hidden hunger is also challenging the agricultural and nutritional research communities because of the ever-rising global population and expanding food demand. The agricultural interventions to boost food quality by improving the nutritive value of edible crops appear to be one of the viable solutions. Biofortification is one of the promising alternatives to alleviate mineral micronutrient deficiency. It involves increasing the nutrient levels in edible plants during the growth period through conventional breeding, mineral fertilization and transgenic approaches either alone or synergistically (Saltzman et al. 2013). While transgenic crops with high nutrient

S. Gupta · N. M. Lowe (✉)
Centre for Global Development, University of Central Lancashire, Preston, UK
e-mail: Sgupta6@uclan.ac.uk; nmlowe@uclan.ac.uk

M. Govindaraj et al. (eds.), *Breeding Zinc Crops for Better Human Health*,
https://doi.org/10.1007/978-3-031-84342-6_7

content, such as golden rice, have not been well received by both consumers and regulatory bodies, programs such as 'HarvestPlus' are exploiting the widely acceptable conventional breeding techniques to enhance the micronutrient content of commonly consumed staples around the world. As a result, biofortified crops like wheat, maize, rice, banana, cassava, potato, capable of assimilating higher concentrations of micronutrients such as zinc, iron and vitamin A have been released in LMICs to benefit a large section of the population who subsist on low cost staple-based diets (Lockyer et al. 2018).

This chapter offers insights not only into the efficacy and effectiveness of studies conducted on zinc biofortified cereals, specifically focusing on wheat and maize, but also their acceptability to producers and consumers which is key to their successful scale-up. Furthermore, the chapter delves into the strengths and weaknesses of this approach to address zinc deficiency, in comparison to supplementation and food fortification, taking the recent contributions and further perspectives from research into consideration.

The Problem of Zinc Deficiency in LMICs

Zinc is indispensable for all biological systems. It is needed for vital functions at cellular and subcellular levels that can be categorized under catalytic, structural, and regulatory roles. Zinc is a component of more than 300 human enzymes and many other proteins and has function in optimal nucleic acid and protein metabolism, cell growth and differentiation, as well cell-mediated immunity (King et al. 2015). Functional consequences of zinc deficiency encompass compromised physical growth, immune capability, reproductive function and neurobehavioural development (King et al. 2015; Caulfield and Black 2004; Brown et al. 2001; Prasad 2013). The impact disproportionally affects settings with low intakes of absorbable zinc resulting in high rates of stunting, increased child morbidity and mortality, and adverse maternal health and pregnancy outcomes. In LMICs, zinc deficiency is responsible for up to 4.4% childhood death and 1.2% of the burden of disease (3.8% in children 6 months to 5 years) (Fischer Walker et al. 2009).

Recent estimates of the prevalence of zinc deficiency among young children and non-pregnant WRA reported it to be >20% for most of the LMICs regardless of the population sub-groups as assessed using most widely used indicator serum/plasma zinc concentrations (PZC) (Gupta et al. 2020). The prevalence of zinc deficiency was as high as 84% in women and 67% in young children in Cameroon and Cambodia, respectively based on population level surveys (Stevens et al. 2022) (Fig. 1). Although data on PZC in men (reported by only four LMICs) were limited, the consistently high prevalence rates, approximately 66% in Malawi, 77% in Kenya, 42.6% in Mexico, and 31% in the Philippines, suggest that zinc deficiency is not confined solely to children and WRA (Gupta et al. 2020).

In LMICs, low dietary diversity coupled with a reliance on low zinc, high phytate foods are the primary contributors to zinc deficiency. Based on inadequate zinc in

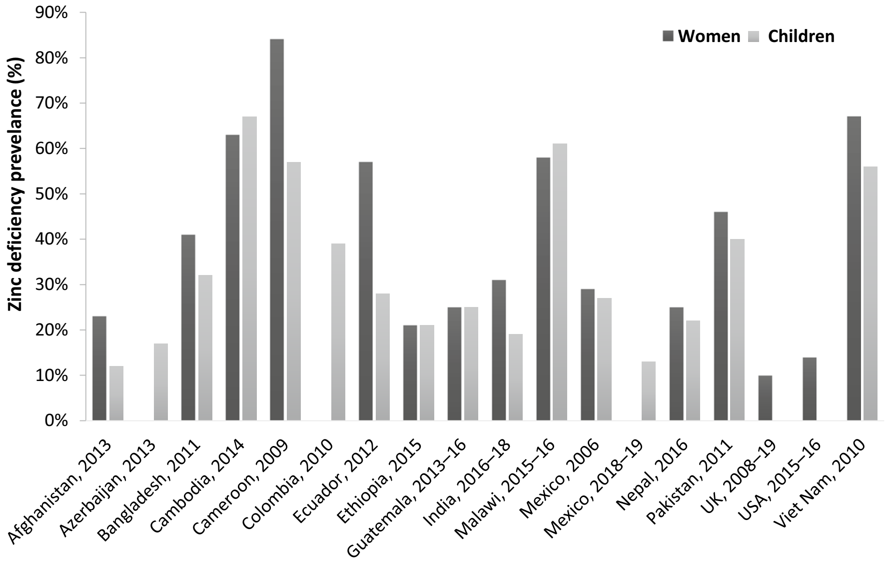

Fig. 1 Prevalence of zinc deficiency among preschool-aged children and women of reproductive age worldwide. Data Source: Stevens et al. (2022). Year indicates the year of the survey

the diet, World Health Organization (WHO) estimates zinc deficiency affects 31% of the global population, with prevalence rates varying from 4% to 73% in different regions (Caulfield and Black 2004). Specifically, prevalence is low (4–7%) in North America and Europe, while it is high in North Africa and the Eastern Mediterranean (25–52%), South and Central America (68%), and in South and Southeast Asia (34–73%). Regardless of the method used to assess zinc status, the situation regarding zinc deficiency in LMICs is a cause for concern.

Zinc Biofortification of Cereals: An Approach with Outstanding Potential for Ameliorating the Problem

Various strategies, such as supplementation, fortification, dietary diversification, and biofortification, have been suggested to improve zinc intake (Gupta et al. 2020; Gibson and Ferguson 1998). The extent of benefits that can be derived from these strategies depends on the context and the resources available for their implementation.

Zinc supplementation in the form of tablets or syrups can rapidly increase zinc intake and address deficiencies in individuals with limited access to diverse diets. It is particularly useful in acute cases of zinc deficiency. However, challenges such as cost, distribution logistics, and long-term compliance can hinder sustained impact and scalability. Despite the known benefits of zinc supplementation on zinc nutriture and health, supplementation in LMICs is primarily limited to being an adjunct therapy for managing diarrhoea in children due to logistical and financial constraints (World Health Organization (WHO) 2006). The coverage of zinc supplementation as an auxiliary therapy for diarrhoea remains low due to insufficient scaling efforts (Black 2019). This approach of therapeutic zinc supplementation is suboptimal for preventing zinc deficiency in children since they can only access supplemental zinc after falling ill, provided their caregivers actively seek diarrhoea treatment (Nasrin et al. 2013). Further, the reachability of any supplementation program to remote or marginalized populations is limited.

Fortification involves adding micronutrients such as zinc to commonly consumed food items such as cereals, flour, or condiments during processing or to food immediately prior to consumption (e.g. multiple micronutrient powders) (Lowe 2021). It can be applied for widespread nutrient deficiency mitigation, either through mass fortification or by targeting vulnerable groups including children and pregnant women. Although fortification has been successful in addressing deficiencies of nutrients such as iodine, zinc fortification faces specific challenges. The bioavailability of fortified zinc can be influenced by food processing and interactions with other dietary components (Lönnerdal 2000). Achieving optimal levels of zinc fortification while maintaining bioavailability can be complex. More importantly, fortification programs require well-established food processing industries and regulatory frameworks which are generally missing in LMICs (Gibson and Ferguson 1998). Food fortification tends to favour urban areas, where the distribution infrastructure for fortified products is more developed compared to rural regions and a possibility of creating a demand for fortification exists due to higher socioeconomic status and greater health literacy levels (Lowe 2021).

Low dietary diversity has been found to be associated with low micronutrient status including zinc (Wiafe et al. 2023). Promoting dietary diversity and or diet modifications encourages individuals to consume a variety of foods that are naturally rich in zinc over a sustained period (Gibson and Anderson 2009). This is highly suited for the needs of LMICs because it does not rely on a constant financial support/infrastructure, which is the case with supplementation and fortification. This approach entails both enhancing zinc intake as well as its absorbability, in contrast to fortification that addresses only intake. While dietary diversity is beneficial for overall health and can provide a comprehensive range of essential nutrients, including zinc, with minimal risk of antagonistic interactions, however, it may be limited by factors such as economic constraints, cultural preferences, and seasonal availability of certain foods. Although promising, the promotion of food-based strategies remains in the early stages of development in LMICs. Programmatic experience with the promotion of home processing techniques to increase absorbable zinc in diet is limited (Brown et al. 2004). Information on locally available, low-cost, culturally acceptable zinc-rich foods and identification of best approach to promote their consumption by those who are at risk of zinc deficiency is required for developing such programmes (Gupta et al. 2020).

Biofortification involves enhancing the nutritional content of in the edible parts crops by increasing the concentration of essential minerals and vitamins. Zinc biofortification specifically focuses on improving the zinc content of mainly staple crops such as rice, wheat, maize, and sorghum, which are major dietary sources for many populations through advanced biotechnology (transgenic) techniques, conventional breeding techniques, agronomic methods (adding zinc fertilizer), or combinations of the latter two (Praharaj et al. 2021).

The transgenic approach, also referred to as genetic modification, involves the insertion of genes necessary for the accumulation of a specific micronutrient in a crop where it would not naturally occur. This technique presents exciting opportunities not only for significantly increasing the nutrient content but also for enhancing its bioavailability. Prominent crops such as rice, wheat, and maize have been genetically modified to enhance their zinc content. Moreover, genetic engineering can also enhance bioavailability by reducing inhibitors or potentially improving the production of enhancers. It has been feasible to increase zinc levels in the edible germ by exploiting this method (Balk et al. 2019). However, transgenic crops face limited acceptance by consumers and regulatory bodies despite their benefits and time-saving advantages over traditional breeding (Kumar et al. 2020; Cui and Shoemaker 2018).

There are several approaches to achieving agronomic biofortification, which include applying zinc fertilizers to soil, leaves, or priming seeds (Praharaj et al. 2021; Bhardwaj et al. 2022). This method is particularly successful in regions where mineral fertilizers are employed, and zinc is added during the manufacturing or distribution process. Importantly, this approach circumvents any limitations posed by low zinc levels in the soil, ensuring optimal zinc accumulation in grains.

Agronomic biofortification with zinc has demonstrated its effectiveness in increasing zinc concentration in crops and offers additional benefits, such as improved yields, even in diverse soil and environmental conditions (Cakmak and Kutman 2018). Furthermore, the utilization of nano-fertilizers for zinc biofortification provides advantages by enhancing the efficiency of micronutrient application, reducing nutrient waste, and minimizing environmental contamination (Dapkekar et al. 2018).

Conventional breeding is a widely utilized method for producing biofortified crop varieties, including those with the capacity to accumulate high amounts of zinc. This process involves crossing parent lines with high nutrient content with recipient lines possessing desirable agronomic traits over multiple generations (Garg et al. 2018). As a result, biofortified crops such as zinc-enriched wheat, rice, and iron-zinc enriched lentils have been successfully developed and released in various countries (HarvestPlus 2023). Table 1 summarizes the zinc-rich cereal varieties that have been released to date.

There are several reasons why zinc biofortification of cereals holds exceptional promise in tackling zinc deficiency particularly in a resource limited setting:

Accessibility and Affordability: Cereal crops, especially wheat, rice, and maize, are widely consumed by large populations, making them an ideal vehicle for

Table 1 Zinc-rich cereal varieties released across the world

Country	Crop	Variety released
Bangladesh	Wheat	BARI Gom -33
	Rice	BRRI Dhan62, BRRI Dhan64, BRRI Dhan72, BRRI Dhan74, BU Aromatic Hybrid Dhan-1, Binadhan 20, BU Aromatic Dhan-2, BRRI Dhan84, BRRI dhan100, BRRI dhan102
Bolivia	Wheat	INIAF Okinawa
	Rice	CIAT BIO-44 +Zinc
Brazil	Wheat	BRS 331
Colombia	Rice	Fedearroz BIOZn 035
	Maize	BIO-MZn01, SGBIOH2, SGBIOH6
El Salvador	Maize	CENTA Porrillo 2020
	Rice	CENTA A-Nutremas
India	Wheat	BHU-3, Zn-Shakti, BHU-1, BHU-5, WB-02, HPBW01, BHU-25, BHU-31, HUW 711, HI 8777 (DURUM), MACS 4028 (DURUM), PBW 757, HI 1633, PBW 771, MACS 4058, DBW 332
	Rice	DRR Dhan 49
	Sorghum	Parbhani Shakti
Indonesia	Rice	INPARI IR Nutri Zinc, Inpara 11 Siam HiZInc, Inpara 12 Mayas
Mexico	Wheat	Nohely F2018
Nepal	Wheat	Zinc Gahun1, Himgange, Panchakoshi, Zinc Gahun2, Zinc wheat 3, Borlaug 100
Nicaragua	Rice	INTA Las Minas
	Maize	Fortinica, INTA-Nutremas
Pakistan	Wheat	Zincol-2016, Akabar-2019, Nawab-21, TARNAB-REHBAR, TARNAB-GANDUM-I
Honduras	Maize	DICTA B02, DICTA B03
Guatemala	Maize	ICTA HB-18ACP+Zn, ICTA B-15ACP+Zn, Fortaleza 17

Data Source: Harvest Plus webpage for Database of Biofortified Crops Released (HarvestPlus 2022)

delivering increased zinc intake to vulnerable communities. Biofortified crops can be easily integrated into existing agricultural practices and local food systems, ensuring accessibility and affordability for the target populations.

Non-disruption of Usual Dietary Behaviours: In contrast to dietary diversification approaches, biofortification generally requires no change in consumer behaviour because it has minimal impact on the sensory attributes of the crops involved. This aspect enhances the acceptability and sustainability of biofortification as an effective intervention for combating zinc deficiency.

Sustained Impact: Once biofortified varieties are introduced and adopted into mainstream seed markets, they can consistently contribute to combatting zinc deficiency. After the successful development of the biofortified plant, its seeds can be widely distributed and continually cultivated by farmers year after year. Following the initial investment in the breeding program, ongoing costs are minimal, although support may be necessary to ensure optimal fertilizer application to maximize the crop's zinc content in regions where soil zinc levels are low. Unlike interventions relying on supplementation or fortified foods, which may encounter challenges with long-term implementation and compliance, biofortified crops offer a continuous source of zinc in the regular diet.

Nutrient Synergy: Cereals are often consumed alongside other staple foods, including legumes, vegetables, and animal products. By increasing the zinc content in cereals, the overall dietary zinc intake can be improved, as these foods are often consumed together, leading to a synergistic effect on nutritional status. Attempts to biofortify wheat have also demonstrated an increase not only in zinc content but also in minerals such as iron and selenium (Lowe et al. 2020; Gupta et al. 2022a).

Improved Agronomic Traits: Biofortification programs also consider agronomic traits, such as yield, disease resistance, and climate resilience, in addition to nutritional enhancement. This ensures that the biofortified crop varieties are not only high in zinc but also perform well in terms of productivity and resilience to environmental stresses, benefiting farmers and encouraging wider adoption.

Comprehensive Approach: Biofortification enables the targeted delivery of nutrients to populations at risk of zinc deficiency, including communities with limited access to diverse diets and those heavily reliant on staple cereals within their local grain production, processing, and consumption systems. This strategy also extends its coverage to populations who may be difficult to reach through supplementation programs. Moreover, it addresses nutritional gaps within specific vulnerable groups, such as WRA and young children, who are particularly susceptible to zinc deficiency. In cases where the equitable household distribution of other zinc-rich foods may not be feasible, this approach remains crucial. Recent data have also highlighted that even adolescent and adult males in LMICs may also be zinc deficient (Gupta et al. 2020). Therefore, this approach bridges the gap between the typical dietary intake and the recommended levels for all population groups without raising concerns about excess consumption.

Analysing Strategies to Improve Zinc Intakes: A Fortification and Biofortification Case in Pakistan

Supplementation is effective for targeted interventions but is impractical for widespread use due to cost and distribution challenges. Achieving dietary diversification remains a distant and complex long-term goal, necessitating a major transformation of agricultural and food systems and the reduction of global inequalities through international political commitment and commercial incentives. In such a situation, only food fortification and biofortification provide practical solutions to address hidden hunger at the population level in the medium term to long term.

In Pakistan, micronutrient deficiencies impact urban and rural populations spanning all geographic regions and income brackets, hence there is a need for a comprehensive, population-wide strategy to effectively address this widespread challenge (Government of Pakistan and United Nations Children's Fund (UNICEF) 2023). It is worthwhile to explore the strengths and weaknesses of these two approaches, especially in the context of Pakistan, where fortification and biofortification initiatives were independently initiated but implemented concurrently in 2016. The Food Fortification Program (FFP), supported by UK Aid, aimed to address vitamin A, iron, and zinc deficiencies, particularly among women and children. An independent consortium evaluated the FFP after 5 years (e-Pact Consortium 2021). Concurrently, Zincol-2016, a biofortified wheat variety was released in collaboration with the HarvestPlus program and the Pakistan National Agriculture Research Centre. A biofortification project, BiZiFED research program, funded by UKRI Global Challenges Research Fund, was initiated to generate data on the effectiveness, acceptability and feasibility of Zincol-2016 (Lowe et al. 2020, 2018; Ohly et al. 2019).

In 1965, Pakistan introduced mandatory fortification of oil and ghee with Vitamin A, but by 2011, national survey revealed persistently high deficiencies in pregnant women (Bhutta et al. 2011). In 2016, a 5-year FFP was launched to combat deficiencies in vitamin A, iron, and zinc, focusing on women and children, and expected to reach 150 million people (e-Pact Consortium 2019). To address specific deficiencies, the FFP utilized different vehicles for fortification. Vitamin A and D were added to oil and ghee, which are commonly used in meal preparation and had the potential for broad distribution, promoting equity. For iron, folate, and zinc, the program turned to fortification of wheat flour, a staple used in various forms of bread consumed daily throughout the year. The approach revolved around enhancing the availability of fortified food items, generating consumer interest, and establishing a favourable setting for food fortification. This all-encompassing strategy entailed providing technical support to local and provincial government agencies, forming partnerships with industry stakeholders, and advocating for the cause to both policymakers and the public.

The final evaluation of the oil and ghee fortification program showed significant progress in enhancing fortification standards and increasing the number of registered mills. This led to the mills achieving adequate levels of vitamins A and D and being on track to meet the annual production target of 2.5 million metric tonnes of fortified oil/ghee by 2021 (e-Pact Consortium 2021). Unlike oil and ghee, the fortification of wheat flour with iron, folate, and zinc was carried out by commercial flour mills on a voluntary basis. In 2020, the FFP faced COVID-related challenges and a wheat shortage affecting prices. Despite this, it improved premix access and micro feeder installation, enabling more mills to fortify in the future if incentives align. However, it fell short of its goal to provide 1.5 million metric tons of fortified wheat flour annually, with fortified flour comprising less than half of the mills' total production (e-Pact Consortium 2021). This approach required a strong public demand for fortified products to incentivize millers and retailers to adopt the fortification strategy and increase supply. The government's control over wheat prices in Pakistan did not offer a compelling economic incentive for voluntary fortification, as producers cannot pass the cost on to consumers. Further, this strategy raises concerns about equity, as the poorest individuals might not have access to these premium products. A potential solution could involve government subsidies to offset the cost of premix and fortification, although this might conflict with the original sustainability goal of fortified wheat flour production. Alternatively, the introduction of legislation for mandatory flour fortification could be considered, although this process is intricate and time-consuming in Pakistan due to the decentralization of decision-making to provincial governments. Additionally, the choice to focus on large commercial roller mills posed a challenge to the flour fortification program's scalability. This is because the mills process only a portion of the wheat flour consumed in Pakistan, estimated to be between 40 to 60% of household wheat flour procurement (Ansari et al. 2018). The rest of the household flour comes from wheat grain kept for self-consumption by farmers or received as in-kind payment by farm laborers, which is milled in numerous small local mills called "chakkis" found in both urban and rural areas across the country.

In 2016, Pakistan introduced Zincol-2016, its first zinc biofortified wheat variety. The BiZiFED program, initiated in 2017, assessed the viability of using this biofortified wheat to combat zinc deficiency on a population scale. The program comprised an efficacy trial from 2017 to 2019 (Lowe et al. 2018) and an effectiveness trial from 2019 to 2021 (Lowe et al. 2020), aiming to study health outcomes in women, adolescent girls, and children, assess crop performance under various conditions, and identify barriers and enablers for scaling up adoption. The study showed that Zincol-2016 had a significantly higher zinc content compared to the Galaxy control, resulting in an increased daily zinc intake for participants (Gupta et al. 2022a; Lowe et al. 2021). Even when local farmers grew Zincol-2016 under real-world conditions with some technical support for zinc fertilizer application, the grain maintained satisfactory zinc levels (Gupta et al. 2022a). Importantly, it was found that the enhanced zinc content in Zincol-2016 did not lead to higher phytate levels, which meant that the bioavailability of zinc was comparable and had the potential for improved absorption compared to the non-biofortified variety (unpublished). Although the study did not demonstrate significant increments in height/weight based anthropometric measurements in adolescent girls and young children after consuming biofortified wheat for six months, there were signs of improved health outcomes related to upper respiratory tract infections toward the end of the intervention period (Gupta et al. 2022b; Gupta et al. 2023) as well as a modest increase in head circumference in children, favouring the biofortified group (unpublished). Notably, existing biomarkers lack the sensitivity to detect subtle changes in dietary zinc intake although one novel biomarker appeared to have captured this. Further details regarding the randomized controlled trials' findings are elaborated in the subsequent sections. Consumer acceptability of biofortified flour discussions revealed that community members and elders appreciated the potential health benefits of biofortified flour (Mahboob et al. 2022). Despite concerns about potentially higher prices compared to standard flour, there was a willingness among consumers to pay a bit extra for the health benefits it offered. Numerous farmers opted to persist in cultivating Zincol-2016, due to its favourable yield and nutritional benefits, as elaborated in section "Acceptability of Zinc-Biofortified Cereals: Consumer Perception and Regulatory Considerations" of this chapter. Nonetheless, they conveyed a requirement for government subsidies to help mitigate the expenses associated with fertilizers needed to enhance the zinc content in wheat grain (Ceballos-Rasgado et al. 2022).

Overall, establishing a supportive environment through policies and programs is crucial for scaling up both wheat flour fortification and biofortification in Pakistan. Both approaches rely on collaborations within the food value chain. However, when considering scalability, biofortification shows promise. According to the latest HarvestPlus report from 2022, the market share of zinc biofortified wheat in Pakistan is expected to reach 20% of the certified seed sector in 2022, benefiting over 1.4 million households growing these varieties (HarvestPlus 2022).

Zinc Content in Traditional vs. Biofortified Crops: What the Data Shows?

The world's primary cereal crops, including maize, rice, and wheat, are cultivated across extensive areas globally. Their combined annual yield, reaching approximately 2.8 billion tons of grain according to Food and Agriculture Organization Statistical Database (FAOSTAT), highlights their paramount significance (Food and Agriculture Organization of United Nation 2023). These crops play an integral role in diets, societies, and economies worldwide, especially in densely populated developing regions. The global demand for all three cereals is steadily increasing, a trend expected to persist until the middle of this century. Consequently, these major cereals offer pivotal opportunities for improving nutritional outcomes.

Wheat

Globally, about 20% of calories come from wheat. In some countries, it is more than 70%. Thus, increasing zinc levels in wheat grain could deliver more zinc to people whose diet relies directly or indirectly on wheat-based food and could help mitigate zinc deficiency. In Asian and African countries, along with international organizations such as International Maize and Wheat Improvement Center (CIMMYT), International Center for Agricultural Research in the Dry Areas (ICARDA), HarvestPlus, are actively developing bio-fortified wheat varieties (Wani et al. 2022). Their collaboration with national research institutes has resulted in the successful development of zinc rich wheat varieties in countries including India, Pakistan, Nepal, Bangladesh, and others Latin American countries, as outlined in Table 1. Zinc biofortification of wheat has already gained momentum in India with at least 16 high zinc varieties released over the past 7 years.

Pakistan has also introduced five zinc-biofortified wheat varieties since 2016 (Table 1).

The year 2020 marked a significant moment in the release of biofortified wheat varieties. A collaboration between CIMMYT and the Nepal Agricultural Research Council (NARC) resulted in a notable achievement—the introduction of six new wheat varieties in Nepal during that year (see Table 1). Five of these varieties were derived from crosses with wild relatives and contained 20–40% more zinc and iron content compared to local crops. These new varieties not only excelled in terms of yield but also demonstrated enhanced disease resistance in comparison to existing types.

The zinc content of biofortified wheat varieties released worldwide is summarized in Table 2. On an aggregate level, biofortified wheat contains approximately 50% more zinc as compared with non-biofortified varieties.

Maize

Increasing zinc levels in maize grain holds the potential to provide greater zinc intake to people whose diets rely directly or indirectly on maize-derived foods, offering a promising solution to mitigate zinc deficiency, particularly in Africa and South America. Guatemala has taken the lead by releasing zinc-enhanced maize hybrids. Notably, the ICTA HB-18 variety has a 15% higher zinc content compared to other commercially available varieties. Additionally, tortillas produced from ICTA B-15 exhibit an increase in zinc content of up to 60% compared to tortillas made from other commercial varieties (Maqbool and Beshir 2019).

In addition to Guatemala, the CIMMYT has achieved notable success in the development and introduction of zinc-enriched maize varieties in countries including Honduras, Colombia, Nicaragua. According to the HarvestPlus database, a total of 11 high-zinc maize varieties have been launched thus far, containing an additional 13 mg per kg of zinc compared to non-biofortified varieties (Table 2).

Other Cereals

Rice is the world's most vital crop, with over half of the global population heavily reliant on it for sustenance. This dependency makes high zinc rice varieties a crucial intervention in combating zinc deficiency, particularly in regions where daily rice consumption is prevalent. Remarkably, more than half of the 18 high zinc rice lines released thus far were introduced in Bangladesh, a country known for its high per capita rice consumption (144.5 kg/year) (Saha et al. 2021). What is even more

Table 2 Zinc content of biofortified cereal varieties released worldwide

Biofortified crop	Variety name	Release year	Zinc content (ppm)	Comparison with traditional varieties (ppm)
Zinc wheat	Nohely F2018	2018	31	37 vs. 25
	BRS 331	2012	37.3	
	INIAF Okinawa	2019	32	
	Zincol-2016	2016	37	
	Akhbar-2019	2020	37	
	Nawab-21	2021	37	
	TARNAB-REHBAR	2023	34	
	TARNAB-GANDUM-I	2023	36	
	Zinc Gahun-1	2020	38	
	Himgange	2020	54	
	Panchakoshi	2020	39.4	
	Zinc Gahun-2	2020	39.4	
	Zinc wheat-3	2020	48	
	Borlaug100	2020	31	
	BHU-3	2014	30.5	
	Zn-Shakti	2014	34.2	
	BHU-1	2013	34.8	
	BHU-5	2013	29.5	
	WB-02	2017	31	
	HPBW01	2017	31	
	BHU-25	2018	31	
	BHU-31	2018	39.5	
	HUW 711	2019	31	
	HI 8777 (DURUM)	2018	43.6	
	MACS 4028 (DURUM)	2018	40.3	
	PBW 757	2018	42.3	
	HI 1633	2020	41.1	
	PBW 771	2020	41.4	
	MACS 4058	2020	37.8	
	DBW 332	2021	40.6	
	BARI-Gom33	2017	33	
Zinc maize	Fortinica	2018	34.9	33 vs. 20[a]
	INTA-Nutremas	2018	35	
	DICTA B02	2017	34.5	
	DICTA B03	2017	35.1	
	ICTA HB-18ACP+Zn	2018	31	
	ICTA B-15ACP+Zn	2018	30	
	Fortaleza 17	2020	32	
	CENTA Porrillo 2020	2020	32	
	BIO-MZn01	2018	34.5	
	SGBIOH2	2019	33	
	SGBIOH6	2020	32	

(continued)

Table 2 (continued)

Biofortified crop	Variety name	Release year	Zinc content (ppm)	Comparison with traditional varieties (ppm)
Zinc rice	INTA Las Minas	2020	22	25 vs. 16
	CENTA A-Nutremas	2019	22.8	
	Fedearroz BIOZn 035	2021	26	
	CIAT BIO-44 +Zinc	2019	22	
	INPARI IR Nutri Zinc	2018	25	
	Inpara 11 Siam HiZInc	2022	33.9	
	Inpara 12 Mayas	2022	29.8	
	DRR Dhan 49	2018	25.2	
	BRRI Dhan62	2013	20	
	BRRI Dhan64	2014	24	
	BRRI Dhan72	2015	23	
	BRRI Dhan74	2015	24.2	
	BU Aromatic Hybrid Dhan-1	2016	22	
	Binadhan 20	2017	27.5	
	BU Aromatic Dhan-2	2016	22	
	BRRI Dhan84	2017	27.6	
	BRRI dhan100	2021	25	
	BRRI dhan102	2022	25.5	

Data Source: HarvestPlus Database of Biofortified Crops Released (HarvestPlus 2022)
[a]Zinc content for traditional variety is adopted from Prasanna et al. (2019)

remarkable is that these releases (all except two) occurred within a short span of just four years, highlighting the concentrated efforts towards addressing zinc deficiency in the region. Overall, high zinc rice can provide around 50% more zinc as compared to traditional rice but some varieties such as INPARA developed and released in Indonesia can contain up to twice as much as zinc in the non-biofortified rice (Sitaresmi et al. 2023). Since 2019, Latin American countries including Nicaragua, El Salvador, Colombia, Plurinational State of Bolivia have also released their first biofortified zinc rice.

In 2018, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) launched India's inaugural biofortified sorghum variety, 'Parbhani Shakti' (ICSR 14001), distinguished by its elevated iron and zinc levels compared to regular sorghum. Subsequent efforts have been directed towards expanding its production and dissemination in the sorghum-consuming regions of central India. This recently introduced variant has an average grain concentration of 45 ppm (parts per million) of iron and 32 ppm of zinc, surpassing conventional varieties that typically contain 30 ppm of iron and 20 ppm of zinc, respectively (Gaikwad et al. 2020; Kumar et al. 2018). Notably, it also offers a higher protein content at 11.9%, compared to the typical 10% found in most sorghum types, and a lower phytate content (4.14 mg/100 g) as opposed to the usual 7.0 mg/100 g, thereby enhancing nutrient absorption.

Acceptability of Zinc-Biofortified Cereals: Consumer Perception and Regulatory Considerations

Biofortified cereals enriched with essential micronutrients such as zinc have garnered increasing attention as potential solutions to malnutrition and nutrient deficiencies, particularly in regions where staple foods constitute a major part of the diet. However, their success hinges not only on scientific efficacy but also on the acceptance of these biofortified varieties by consumers and the endorsement of regulatory bodies (Bouis and Saltzman 2017). The earliest and most prominent biofortified crop, Golden Rice, created in 2000 is an example (Dubock 2017). Despite the open availability of licensing for Golden Rice (transgenic) and its derivatives, along with firmly documented nutritional benefits, their on-farm utilization has been impeded until now. This hindrance is due to cautiousness surrounding public health and environmental issues, coupled with substantial adverse publicity from anti-biotechnology interest groups (Listman et al. 2019). In this section, we will focus on the consumer acceptability of biofortified products resulting from selective breeding and/or mineral application, which are generally more acceptable compared to the transgenic approach.

Consumer acceptance of zinc-biofortified cereals is a crucial determinant of their viability. While enhancing the nutritional content of staple crops is an endeavour with far-reaching benefits, it's essential to gauge how these modified varieties resonate with local preferences and cultural norms. Factors such as taste, texture, appearance, and cooking methods can significantly influence consumer adoption. Several studies have provided insights into this aspect. Sensory evaluations, focus groups discussions (FGDs), and surveys have been conducted to assess the palatability and sensory qualities of zinc-biofortified cereals (Woods et al. 2020; Rizwan et al. 2021; Gannon et al. 2019; Mahboob et al. 2020, 2022; Talsma et al. 2017). These studies have shown that while consumers prioritize taste, they are often willing to embrace the health benefits of biofortified options if the changes in taste and appearance are minimal.

A mixed-methods study was conducted alongside a cluster-randomized controlled effectiveness (BiZiFED2) trial in the Peshawar region, Pakistan from November 2020 to July 2021. This study involved semi-structured FGDs with farmers who grew Zincol-2016 wheat for the trial. Additionally, a year after the study was completed, a survey was conducted with 686 farmers in Punjab province, Pakistan's main wheat-growing region, to ascertain if they had grown biofortified Zincol-2016 variety again in the subsequent season. The findings revealed that 47% of participants continued cultivating Zincol-2016 wheat after the trial had ended. Motivations included seed availability, high grain yields, disease resistance, improved flour quality, and nutritional benefits. Farmers appreciated the flour taste and texture and consumed it at home. Qualitative analysis from focus groups identified that technical and financial support, better grain quality, and health advantages promoted scaling up, while challenges encompassed unfamiliarity with biofortification, production costs, and external threats such as COVID-19 pandemic (Ceballos-Rasgado et al. 2022).

In order to mainstream zinc-biofortified cereals into food systems and dietary practices, it is imperative to systematically consider consumer preferences, cultural intricacies, and regulatory demands. Equally vital is the provision of essential resources and training for cultivators. This endeavour necessitates continuous collaboration among researchers, policymakers, food industries, and communities, encompassing both producers and consumers, as they collectively address the multifaceted challenges in this pursuit.

Biofortified Cereals Increase Zinc Intake: Evidence Available from Efficacy and Effectiveness Trials

Several studies have shown that the zinc content of staple crops can be enhanced through conventional breeding or the application of minerals and can lead to several other desirable traits (Lockyer et al. 2018; Cakmak and Kutman 2018; Rashid et al. 2019; Nestel et al. 2006). However, there has been limited research to confirm the translation of this increase in zinc content to benefits for human health. Nonetheless, these studies do indicate a successful incremental increase in zinc intake when consuming biofortified cereals over non-biofortified cultivars.

The studies conducted on biofortified cereals, including modest-scale investigations into the efficacy of conventionally bred biofortified cereals, revealed a distinct increase in zinc intake ranging from 21% to 169% over the control cereal (non-biofortified), depending on the population subgroup. Out of the nine studies listed in Table 3, six tested the efficacy of zinc biofortified wheat (Gupta et al. 2022a; Lowe et al. 2021; Sazawal et al. 2018; Rosado et al. 2009; Signorell et al. 2019, 2023), while one each tested maize (Chomba et al. 2015) and rice (Jongstra et al. 2022). Only one study explored the usefulness of high-iron and high-zinc millet among Indian children (<2 years old) (Mehta et al. 2022). Consuming biofortified pearl millet provided 1.5 mg of daily zinc, compared to the 0.5 mg provided by the control. This scrutiny indicates that when included as a dietary cornerstone, children receive nearly 40% of their zinc requirements from biofortified pearl millet alone. Despite this increased zinc intake over nine months, high-iron and high-zinc pearl millet did not significantly improve zinc biomarkers or growth compared to the control.

Although a bio-efficacy study specifically for maize has not been conducted at the time of writing, the absorption of zinc from consuming high-zinc maize was investigated by Chomba and co-workers (Chomba et al. 2015). Their study demonstrated that the total daily zinc intake from biofortified maize (5.0 mg) was significantly higher ($P < 0.001$) than that from the control maize (2.3 mg) among young rural Zambian children. While the group found no significant difference in the fractional absorption of zinc between the control maize (0.28 mg) and the biofortified maize (0.22 mg), the daily absorption of zinc from the biofortified maize (1.1 mg) was higher ($P < 0.001$) than that from the control maize (0.6 mg). This is because the net absorbed zinc is a function of both fractional zinc absorption and the total

Table 3 Studies assessing the impact of consuming zinc biofortified cereals and millets

	Study	Country	Type	Biofortification	Population	N	Additional daily zinc intake
Wheat	Gupta et al. (2022a)	Pakistan	Effectiveness	Conventional breeding (Zincol-2016) + agronomic	Adolescent girls aged 10–16 years	517	21%
	Lowe et al. (2021)	Pakistan	Efficacy	Conventionally bread (Zincol-2016) + agronomic	NPNL women of reproductive age (16–49 years)	50	30–60%[a]
	Sazawal et al. (2018)	India	Unclear	Conventionally bread (PBW 550) + agronomic	Children aged 4–6 years	6050	50%
	Sazawal et al. (2018)	India	Unclear	Conventionally bread (PBW 550) + agronomic	NPNL woman of child-bearing age (15–49 years)	6050	39%
	Rosado et al. (2009)	Mexico	Efficacy (absorption)	Conventionally bread (combined from six landraces and included: DGO95.1.17; DGO95.3.2; CHIH95.2.1; CHIH95.2.47; CHIH95.3.47; JAL95.4.10 and LGP2 and LGP12)	NPNL adult women aged 18–42 years	27	68–72%[a]
	Signorell et al. (2019)	Switzerland	Efficacy (absorption)	Agronomic biofortification (foliar)	NPNL women aged between 18 and 45 years	55	52–54%[a]
	Signorell et al. (2023)	India	Efficacy	Agronomic biofortification (foliar spray)	School aged children (4–12 years)	273	169%
Pearl millet	Mehta et al. (2022)	India	Efficacy	Iron- and zinc-biofortified pearl millet cultivar (*Dhanashakti*, ICTP-8203Fe)	Children aged 12–18 months	223	220%

Rice	Jongstra et al. (2022)	Bangladesh	Unclear	Conventionally bread high zinc strain (BRRI42) + agronomic (zinc foliar spraying)	Children aged 12–36-months	523	85%
Maize	Chomba et al. (2015)	Zambian	Efficacy (absorption)	Conventionally bread maize variety	Children aged 1–5 years	60	117%

[a]Depending on the extraction rate
NPNL non-pregnant non-lactating

zinc content of the food. The authors concluded that supplying biofortified maize can meet zinc requirements and provide an effective dietary substitute for regular maize for young children.

In a double-blind intervention trial, 1 to 3-year-old rural Bangladeshi children (n = 530), most of whom exhibited zinc-deficiency and stunted growth, were recruited and randomly assigned to receive either control rice (non-biofortified) or the biofortified variety for 9 months. While there was no significant difference between the amounts of rice consumed by the two groups (control: 232.7 ± 49.8 g/d; biofortified: 239.1 ± 43.4 g/d), the average daily zinc intake from the study rice was 1.20 ± 0.34 mg for the control group and 2.22 ± 0.47 mg for the biofortified group. However, this additional 1 mg per day of zinc did not translate into improvements in plasma zinc status, growth, or zinc-related morbidity among the participants (Jongstra et al. 2022).

Small-scale trials, including absorption studies primarily conducted on non-pregnant non-lactating (NPNL) women, suggest that the consumption of zinc-biofortified wheat results in an additional daily intake of approximately 2.5 mg to 6 mg, depending on flour extraction rates. Higher intake is observed with greater extraction rates (Lowe et al. 2021; Rosado et al. 2009; Signorell et al. 2019). Considering that roughly 75% of dietary zinc in predominantly wheat consuming populations is derived from wheat (as these studies calculated additional intake from bread rather than full meals), substituting the zinc biofortified variety for standard varieties can potentially fulfil 57–115% of the required daily intake (12.7 mg) for adult NPNL women (EFSA Panel on Dietetic Products, Nutrition and Allergies 2014).

It is noteworthy to observe that the study by Signorell et al. (2019) which explored the impact of the two agronomic approaches to biofortification on human zinc absorption found no discernible disparity in zinc absorption (both fractional and total absorbed zinc) in relation to food derived from wheat biofortified via foliar application or hydroponic root enrichment. Concurrently, absorption from the biofortified foods, irrespective of the agronomic biofortification technique employed, exhibited higher net zinc absorption in comparison to the control. Similarly, Rosado et al. (2009) also reported that net absorption from meals (2.1 ± 0.7 for 95% extraction and 2.0 ± 0.4 for 85% extraction) consisting of biofortified tortillas was 0.5 mg higher than from the non-biofortified control (1.6 ± 0.4 for 95% extraction and 1.5.0 ± 0.5 for 85% extraction). These values agreed well those predicted by an equation-based zinc absorption model that predicted 0.6 mg or 0.7 mg additional absorption from fortified meals made using 95% extraction or 80% extraction respectively, compared to the control wheat flour.

A recently published study (Signorell et al. 2023) presents findings from a 20-week double-blind intervention trial involving children aged 4 to 12 years (n = 273). The aim of the trial was to compare the effects of chapati made from agronomically biofortified whole wheat flour (BFW) on PZC when integrated into a mid-day school meal scheme. The study also included fortified control wheat (PHFW) and unfortified control wheat (CW) groups. The results revealed that the mean daily zinc intakes for the study groups BFW, PHFW, and CW groups were

4.4 ± 1.6, 5.9 ± 1.9, and 2.6 ± 0.6 mg of zinc per day, respectively. It is worth noting that these intakes were based on providing just one meal per day, and in the case of "universal biofortification," the zinc intake would likely be more substantial over time.

There was no significant difference in zinc intake between the PHFW and BFW groups, but both were significantly higher than the CW group. Despite the additional daily zinc intake of approximately 1.8 to 3.3 mg when consuming either PHFW or BFW as a single school meal per day, this did not lead to a positive effect on PZC, growth, or morbidity when compared to the control group. The study also included an additional plasma zinc analysis conducted four months after the intervention endpoint to understand the development of PZC post-intervention. In contrast to the PHFW and CW groups, which exhibited lower final PZC values compared to the measurements taken at the end of the intervention, the BFW group did not demonstrate a lower final PZC. This observation is intriguing and warrants further investigation.

Outcomes from two large-scale trials investigating the effectiveness of biofortified wheat have been published thus far. The first conducted in India was a community-based, double-masked randomized controlled trial (RCT) involving 6050 mother-child dyads (Sazawal et al. 2018). A standard commercial variety, PBW 550, was cultivated using standard farming techniques except that the high zinc wheat (HZn) for the intervention group was grown under specific agro-ecological conditions and received additional foliar spraying of 0.5% zinc sulfate fertilizer to enhance zinc uptake by the plants and its deposition, while 'Low zinc wheat' (LZn) control was grown in agro-ecological conditions that limit soil zinc uptake by plants and did not receive any additional zinc fertilization. Zinc content for the agronomically biofortified HZn and control LZn wheat flour was 30 mg/kg and 20 mg/kg, respectively. Participants received either HZn biofortified wheat flour or non-biofortified wheat flour for six months. Mothers enrolled in the study were NPNL women of reproductive age (15–49 years) and children were 4–6 years old. The study reported that compliance with consuming at least half of the recommended intake of flour was approximately 88% days for both women and children, while compliance with consuming the entire recommended amount (350 g for women and 120 g for children) was about 55% days. The zinc biofortified flour was estimated to deliver 3.6 mg/day of zinc to children compared with 2.4 mg/day for the control, providing a differential of 1.2 mgZn/day, and 10.8 mg/day to women compared to 7.8 mg/day for the control, providing a differential of 3 mgZn/day when the complete the recommended intakes was consumed by both the population subgroups.

The second trial was conducted in Pakistan (BiZiFED2) and had a cluster-randomized, double-blind, controlled design to understand the effectiveness of consuming zinc-biofortified wheat flour on the haematological indices of zinc in 517 adolescent girls (aged 10–16 years) in rural Pakistan under real-world scenarios (Lowe et al. 2020; Gupta et al. 2022a). In this study, the biofortified grain, grown by local farmers met the target zinc concentration of >40 mg/kg, averaging 45.3 mg/kg, with some variability (24.3 to 76.3 mg/kg). The provision of flour was made for the entire household, ensuring that family meals using the flour were consumed by the adolescent girls as part of their usual family meals. Similar to the study in India, the intervention lasted approximately 6 months, with the bread from biofortified flour providing 6.9 mgZn/day to the adolescent girls compared with 8.4 mgZn/day from the control flour, providing an average differential intake of 1.5 mgZn/day. The differential was less than expected due to the higher-than-average zinc content of the control flour. An earlier study by the same group, where both the control and biofortified wheat was grown under controlled conditions, reported an intake of 5.5 mgZn/day from biofortified and 2.5 mgZn/day from control flour, giving a differential intake of 3 mgZn/day for NPNL women for a comparable, low extraction (white) flour.

Neither study reported any significant difference in plasma zinc concentration between the intervention and control arms. The study conducted in Pakistan failed to show any intervention effect on linear growth and morbidity for adolescent girls and young children (secondary outcomes), although there was some indication of beneficial effects of the intervention on the incidence of respiratory tract infections towards the end of the study for both the population groups (Gupta et al. 2022b; Gupta et al. 2023). Additionally, this study in Pakistan also found a modest increase in head circumference among children in biofortified group compared to control (unpublished). In India, biofortification showed positive impacts on self-reported morbidity among both the population groups (Sazawal et al. 2018).

Beside the two studies described above, several recent ex-ante studies have examined how biofortified crops impact human well-being using the disability-adjusted life year (DALY) method. The first study focused on the potential health benefits of golden rice in the Philippines (Zimmermann and Qaim 2004). This approach was later expanded to include crops fortified with zinc and iron, and was applied across various countries (Stein et al. 2005). A more recent analysis (Liu et al. 2017) evaluated the effect of agronomic biofortification (via application of six rates of zinc fertilizer to soil) on zinc bioavailability in wheat grain and flour and its impacts on human health using DAILY approach. Zinc bioavailability was estimated using a mathematical model. It showed that the zinc concentration increased in all flour fractions with an increase in rate of zinc fertilization, however the percentages of zinc in standard flour (25%) and bran (75%) relative to total grain zinc were constant. Phytic acid concentrations in grain and flours were unaffected by zinc biofortification. The availability of zinc and its impact on health as measured by saved DALYs, escalated with the zinc application rate. This effect was more pronounced in standard white flour, and highly processed refined flour compared to whole grain and coarse flour. Standard and refined flour from biofortified sources, achieved through agronomic methods, met the target of 3 mg of zinc from 300 g of wheat flour and led to a >20% reduction in DALYs.

Overall, the above studies including ex-ante evaluations and feeding studies provide evidence that traditional breeding and agronomic methods of biofortification led to significantly increased dietary zinc intake compared with controls, without compromising bioavailability.

Forging Ahead with Zinc Biofortification: Navigating Challenges and Impact on Human Health

In general, biofortification has three core requisites for its success (1) Effective breeding that involves combining high nutrient density with substantial yields and profitability; (2) Biofortified crops must gain traction among farmers, with their grain reaching those most vulnerable to micronutrient malnutrition; (3) Ability to demonstrate efficacy by showcasing improved micronutrient status and/or related health outcomes through consuming biofortified varieties within the usual diet (Listman et al. 2019). In the case of zinc biofortification using conventional and agronomic techniques, it is evident that breeding has been effective and can provide several other benefits, such as high yield, improved seed and seedling vigour, reduced root and shoot accumulation of cadmium, as well as offering resistance towards certain pest and pathogens. Thus, the "invisible" nutrient zinc, when integrated into resilient high yielding varieties, acts synergistically to provide 'added market value' for farmers to incentivise adoption. Further to this, concerted efforts from the governments and non-government agencies have facilitated the release and scale up of zinc rich cereals in various regions of the world, in particular South-Asia, where the greatest impact of zinc deficiencies including impaired childhood growth, morbidity and mortality, and adverse maternal health and pregnancy outcomes are witnessed.

Although assessments of intakes through limited human studies and ex-ante evaluations suggest that zinc biofortification of cereals can enhance zinc intake, empirical data supporting its translation into human health benefits remain fragmented, making it challenging to draw definitive conclusions. It is crucial to be able to directly measure changes in the prevalence of deficiency resulting from the consumption of biofortified staples, which necessitates controlled trials to validate the impact at achieved nutrient density levels. Zinc deficiency is associated with impaired growth and immunity (King et al. 2015; Black and Sazawal 2001; Liu et al. 2018). In fact, the percentage of children <5 years of age with height-for-age Z scores (HAZ) below −2 SD of the WHO reference has also been suggested as a proxy indicator for assessing at-risk populations and initiating program planning for zinc interventions (de Benoist et al. 2007). Consequently, several cereal biofortification trials also include height and derived HAZ, as well as zinc-related morbidities such as diarrhoea and respiratory tract infections, as outcome variables alongside PZC.

The effectiveness and efficacy studies done so far have often yielded contradictory results concerning zinc status. The sole study conducted on rice in Bangladesh failed to demonstrate any effect on PZC of consuming zinc-biofortified rice for 9 months which provided approximately 1 mg of additional zinc daily, or on the prevalence of zinc deficiency based on PZC (Jongstra et al. 2022). This intervention had no significant effect on diarrhoea. Surprisingly, an 8% higher overall morbidity rate was reported in the intervention group due to a higher incidence of upper respiratory tract illnesses in this group. Since no reasonable explanation could be identified for this observation, the authors attributed this outcome to coincidence.

The study by Mehta and co-workers (2022) concluded the daily consumption of iron-zinc pearl millet -based complementary foods did not significantly impact iron and zinc status or growth in children living in an urban slum of western India. However, it primarily evaluated the effect on iron status and therefore the sub-group analysis conducted only for iron status, indicated improved haemoglobin concentrations among male children and among individuals who were iron-deficient or iron-depleted at baseline.

All the studies carried out to understand the usefulness of biofortified wheat consumption for improving zinc status consistently fail to exhibit any positive impact on PZC (Gupta et al. 2022a; Sazawal et al. 2018; Signorell et al. 2023) except a short duration 8-week intervention in Pakistan (Lowe et al. 2021). In this study, although a significant increase in plasma zinc concentration after 4 weeks was observed in the intervention arm but not control, this effect was not sustained at 8 weeks which marked the intervention endpoint.

While it could be contended that the additional absorbed zinc from biofortified flour might have limited impact on PZC due to high phytate intake among the participants or modest zinc increments from biofortified meal, it important to acknowledge the well-known constraints of PZC as an indicator (King et al. 2015). PZC is a common measure for evaluating zinc status in populations, however, it is homeostatically controlled and at an individual level, thus responses to modest dietary zinc changes, are subtle (King 2011). This is especially true when the extra zinc is ingested with food, such as from consuming biofortified staples rather as a supplement. Also, challenges in interpreting PZC arise from factors such as concurrent infection, fasting, non-fasting states, and the time of day (McDonald et al. 2020; Arsenault et al. 2011). Considering these limitations of PZC, several novel biomarkers, including products of essential fatty acid metabolism, DNA fragmentation, hair and nail zinc content, are being tested in real-world settings and may enable the effect of modest changes in dietary zinc intake via biofortification to be monitored (Lowe et al. 2020; Signorell et al. 2023; Jongstra et al. 2022; Zyba et al. 2017; Liong et al. 2021). A study nested in the BiZiFED2 effectiveness trial reported a detectable increase zinc counts, adjusted for sulfur (Zn:S count ratio) in individual hair strands, measured using X-ray fluorescence spectrometry, in response to a modest increase in dietary zinc (1.5 mg/day) over 6 months among adolescent Pakistani girls aged 10–16 years (Frederickson et al. 2023). Such methods offer a sensitive, non-invasive method to monitor changes within subjects in response to dietary zinc interventions and should be further tested for robustness in free-living, community settings where confounding co-morbidities may be present.

In terms of functional indicators, studies have generally failed to demonstrate any measurable impact of consuming biofortified foods on anthropometric data. However, there are some findings indicating a positive impact of consuming zinc-biofortified wheat on self-reported morbidities. In 2018, research conducted in India reported that young children who included zinc-biofortified wheat in their diets, commonly consumed through items such as chapatis, puri (flatbreads), or porridge, exhibited a notable 17% decrease in the frequency of days they experienced pneumonia, along with a substantial 39% reduction in the number of days they encountered vomiting, when compared to children who consumed conventional wheat-based products over a period of 6 months (Sazawal et al. 2018).

In the BiZiFED 2 study, a lower incidence of respiratory tract infections (RTIs) was reported in the intervention arm compared to the control arm at the end of the 25-week intervention period, during which the biofortified group of adolescent girls consumed an additional 1.5 mg of dietary zinc daily compared to the control group (Gupta et al. 2022b). Similar intervention effects on incidences of RTI were reported for young children (1-5 years) in this study (Gupta et al. 2023). However, when considering the longitudinal prevalence of RTIs (cumulative days of sickness as a percentage of total observation days) with baseline adjustments, no differences between the groups were observed in either population. The duration of large-scale intervention studies are inevitably limited by cost and resource. The ongoing scale-up of the release of zinc-biofortified cereals provides an opportunity to conduct longer-term (>1 year) observational studies to monitor changes in such functional outcomes over time.

Interaction between phytate and zinc presents another critical impediment in realizing the full potential of the biofortification strategy. Phytic acid, a naturally occurring compound in many plant-based foods, has the ability to form insoluble complexes (phytate) with minerals such as zinc, rendering them less available for absorption by the human body (Lönnerdal 2000; Gibson et al. 2010). Scientists are researching strategies such as low-phytate crops and processing techniques to mitigate this. Evidence suggests that plant breeding techniques hold promise for enhancing zinc bioavailability as well. Previously, maize with low phytate content, developed through plant breeding, showed improved zinc absorption in short-term studies (Adams et al. 2002; Hambidge et al. 2004). However, a longer-term study providing low-phytate maize to Guatemalan schoolchildren was unsuccessful in establishing enhanced zinc absorption compared to control maize (Mazariegos et al. 2006). The reasons behind these unexpected results remain unclear. Validating the long-term effectiveness of low-phytate hybrids is essential, as this approach could significantly improve absorbable zinc intake for populations relying on plant-based diets. Challenges such as reduced yields associated with the low-phytate trait and the need for dedicated long-term breeding projects have hindered further exploration of this strategy.

Studies in wheat and other cereals have shown that transgenic strategies can be used to increase the contents of iron and zinc in white flour by converting the starchy endosperm tissue into a 'sink' for minerals (Harrington et al. 2022). Although such strategies currently have low acceptability, a greater understanding of the mechanisms that control the transport and deposition of iron and zinc in the developing grain should allow similar effects to be achieved by exploiting naturally occurring genetic variation (Balk et al. 2019).

Mechanical treatments and fermentation are two of the most promising processing techniques. Many microorganisms secrete phytase enzymes, which can release minerals from phytate complexes, particularly microorganisms present in sourdough systems (Lopez et al. 2003; Rodriguez-Ramiro et al. 2017). Hence, sourdough wholegrain products may have increased mineral bioavailability. However, this approach may increase mineral bioavailability in foods made from wholegrain and high-extraction flours, but it is not relevant to white flour products, which are preferred in most countries. Micro-milling, a processing technique whereby aleurone cell walls (containing 70% of grain iron and zinc) are ruptured, can increase the availability of minerals from wheat flour. A study to explore whether micro-milling can increase iron and zinc availability from biofortified wholegrain flour as well as from aleurone-enriched white flour is underway (UK Research and Innovation (UKRI) 2016). If successful, such strategies are expected to enhance the mineral absorption potential across various wheat-based products, ranging from refined flour to whole-grain products.

Conclusion

Overall, within the context of cereals, particularly maize and wheat discussed here, the concept of zinc biofortification emerges as a promising strategy for improving nutritional status on a population scale. Zinc biofortification enhances zinc content, yield, and resistance to various pests, which encourages adoption. It undeniably demonstrates its success in increasing zinc intakes in various population sub-groups. However, despite increased zinc intake, its translation into better health is inconclusive, primarily due to a lack of sensitive and reliable biomarkers. Novel biomarkers, such as single hair analysis by X-ray fluorescence spectrometry may offer greater sensitivity and need to be tested alongside widely used PZC. Long-term interventions are warranted to further confirm positive findings related to self-reported morbidities and assess the impact on growth among children.

References

Adams, C. L., Hambidge, M., Raboy, V., Dorsch, J. A., Sian, L., Westcott, J. L., et al. (2002). Zinc absorption from a low-phytic acid maize. *The American Journal of Clinical Nutrition, 76*(3), 556–559. https://doi.org/10.1093/ajcn/76.3.556

Ansari, N., Mehmood, R., & Gazdar, H. (2018). Going against the grain of optimism: Flour fortification in Pakistan. *IDS Bulletin, 49*, 57–71. https://doi.org/10.19088/1968-2018.104

Arsenault, J. E., Wuehler, S. E., de Romaña, D. L., Penny, M. E., Sempértegui, F., & Brown, K. H. (2011). The time of day and the interval since previous meal are associated with plasma zinc concentrations and affect estimated risk of zinc deficiency in young children in Peru and Ecuador. *European Journal of Clinical Nutrition, 65*(2), 184–190. https://doi.org/10.1038/ejcn.2010.234

Bailey, R. L., West, K. P., Jr., & Black, R. E. (2015). The epidemiology of global micronutrient deficiencies. *Annals of Nutrition and Metabolism, 66*(Suppl. 2), 22–33. https://doi.org/10.1159/000371618

Balk, J., Connorton, J. M., Wan, Y., Lovegrove, A., Moore, K. L., Uauy, C., et al. (2019). Improving wheat as a source of iron and zinc for global nutrition. *Nutrition Bulletin, 44*(1), 53–59. https://doi.org/10.1111/nbu.12361

Bhardwaj, A. K., Chejara, S., Malik, K., Kumar, R., Kumar, A., & Yadav, R. K. (2022). Agronomic biofortification of food crops: An emerging opportunity for global food and nutritional security. *Frontiers in Plant Science, 13*, 1055278. https://doi.org/10.3389/fpls.2022.1055278

Bhutta, Z., Soofi, S., Zaidi, S., Habib, A., Hussain, Bhutta, Z., Soofi, S., Zaidi, S., Habib, A., & Hussain, I. (2011). *Pakistan National Nutrition Survey, 2011*. Available at: https://ecommons.aku.edu/pakistan_fhs_mc_women_childhealth_paediatr/262. Accessed on 21.09.2023.

Black, R. E. (2019). Progress in the use of ORS and zinc for the treatment of childhood diarrhea. *Journal of Global Health, 9*(1), 010101. https://doi.org/10.7189/jogh.09.010101

Black, R. E., & Sazawal, S. (2001). Zinc and childhood infectious disease morbidity and mortality. *The British Journal of Nutrition, 85*(Suppl 2), S125–S129. https://doi.org/10.1079/bjn2000304

Bouis, H. E., & Saltzman, A. (2017). Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Global Food Security, 12*, 49–58. https://doi.org/10.1016/j.gfs.2017.01.009

Brown, K. H., Wuehler, S. E., & Peerson, J. M. (2001). The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. *Food and Nutrition Bulletin, 22*(2), 113–125. https://doi.org/10.1177/156482650102200201

Brown, K. H., Rivera, J. A., Bhutta, Z., Gibson, R. S., King, J. C., Lönnerdal, B., et al. (2004). International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food and Nutrition Bulletin, 25*(1 Suppl 2), S99–S203.

Cakmak, I., & Kutman, U. B. (2018). Agronomic biofortification of cereals with zinc: A review. *European Journal of Soil Science, 69*(1), 172–180. https://doi.org/10.1111/ejss.12437

Caulfield, L. E., & Black, R. E. (2004). Zinc deficiency. In *Comparative quantification of health risks: Global and regional burden of disease attributable to selected major risk factors* (Vol. 1, pp. 257–280).

Ceballos-Rasgado, M., Moran, V., Ander, E. L., Ajmal, S., Joy, E., Mahboob, U., et al. (2022). Acceptability of zinc biofortified wheat and flour among farmers in Pakistan: Experiences from the BiZiFED2 project. *Proceedings of the Nutrition Society, 81*(OCE5), E178. https://doi.org/10.1017/S0029665122002117

Chomba, E., Westcott, C. M., Westcott, J. E., Mpabalwani, E. M., Krebs, N. F., Patinkin, Z. W., et al. (2015). Zinc absorption from biofortified maize meets the requirements of young rural Zambian children. *The Journal of Nutrition, 145*(3), 514–519. https://doi.org/10.3945/jn.114.204933

Cui, K., & Shoemaker, S. P. (2018). Public perception of genetically-modified (GM) food: A nationwide Chinese Consumer Study. *npj Science of Food, 2*(1), 10. https://doi.org/10.1038/s41538-018-0018-4

Dapkekar, A., Deshpande, P., Oak, M. D., Paknikar, K. M., & Rajwade, J. M. (2018). Zinc use efficiency is enhanced in wheat through nanofertilization. *Scientific Reports, 8*(1), 6832. https://doi.org/10.1038/s41598-018-25247-5

de Benoist, B., Darnton-Hill, I., Davidsson, L., Fontaine, O., & Hotz, C. (2007). Conclusions of the joint WHO/UNICEF/IAEA/IZiNCG interagency meeting on zinc status indicators. *Food and Nutrition Bulletin, 28*(3 Suppl), S480–S484. https://doi.org/10.1177/15648265070283s306

Dubock, A. (2017). An overview of agriculture, nutrition and fortification, supplementation and biofortification: Golden Rice as an example for enhancing micronutrient intake. *Agriculture & Food Security, 6*(1), 59. https://doi.org/10.1186/s40066-017-0135-3

EFSA Panel on Dietetic Products, Nutrition and Allergies. (2014). Scientific opinion on dietary reference values for zinc. *EFSA Journal, 12*(10), 3844.

e-Pact Consortium. (2019). *Evaluation of the supporting nutrition in Pakistan Food Fortification Programme. Midterm evaluation report*. Available online: https://www.opml.co.uk/files/2021-07/mid-term-evaluation-report-food-fortification-programme.pdf?noredirect=1. Accessed on 21.09.2023.

e-Pact Consortium. (2021). *Evaluation of the supporting nutrition in Pakistan Food Fortification Programme endline evaluation report*. Available at: https://iati.fcdo.gov.uk/iati_documents/90000152.pdf. Accessed on 21.09.2023.

Fischer Walker, C. L., Ezzati, M., & Black, R. E. (2009). Global and regional child mortality and burden of disease attributable to zinc deficiency. *European Journal of Clinical Nutrition, 63*(5), 591–597. https://doi.org/10.1038/ejcn.2008.9

Food and Agriculture Organization of United Nation. (2023). *Food and agriculture database*. https://www.fao.org/faostat/en/#home. Accessed on 27.09.2023.

Frederickson, C. J., Fleming, D. E. B., Asael, D., Zaman, M., Ferguson, R., Kaiser, M. G., et al. (2023). Single hair analysis by X-ray fluorescence spectrometry detects small changes in dietary zinc intake: A nested randomized controlled trial. *Frontiers in Nutrition, 10*, 1139017. https://doi.org/10.3389/fnut.2023.1139017

Gaikwad, K. B., Rani, S., Kumar, M., Gupta, V., Babu, P. H., Bainsla, N. K., et al. (2020). Enhancing the nutritional quality of major food crops through conventional and genomics-assisted breeding. *Frontiers in Nutrition, 7*, 533453. https://doi.org/10.3389/fnut.2020.533453

Gannon, B. M., Thakker, V., Bonam, V. S., Haas, J. D., Bonam, W., Finkelstein, J. L., et al. (2019). A randomized crossover study to evaluate recipe acceptability in breastfeeding mothers and young children in India targeted for a multiple biofortified food crop intervention. *Food and Nutrition Bulletin, 40*(4), 460–470. https://doi.org/10.1177/0379572119855588

Garg, M., Sharma, N., Sharma, S., Kapoor, P., Kumar, A., Chunduri, V., et al. (2018). Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Frontiers in Nutrition, 5*, 12. https://doi.org/10.3389/fnut.2018.00012

Gibson, R. S., & Anderson, V. P. (2009). A review of interventions based on dietary diversification or modification strategies with the potential to enhance intakes of total and absorbable zinc. *Food and Nutrition Bulletin, 30*(1 Suppl), S108–S143. https://doi.org/10.1177/15648265090301s107

Gibson, R. S., & Ferguson, E. L. (1998). Nutrition intervention strategies to combat zinc deficiency in developing countries. *Nutrition Research Reviews, 11*(1), 115–131. https://doi.org/10.1079/NRR19980008

Gibson, R. S., Bailey, K. B., Gibbs, M., & Ferguson, E. L. (2010). A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food and Nutrition Bulletin, 31*(2 Suppl), S134–S146. https://doi.org/10.1177/15648265100312s206

Government of Pakistan and United Nations Children's Fund (UNICEF). (2023). *Pakistan National Nutrition Survey 2018*. Available at: https://www.unicef.org/pakistan/reports/national-nutrition-survey-2018-full-report-3-volumes-key-findings-report. Accessed 21.09.2023.

Gupta, S., Brazier, A. K. M., & Lowe, N. M. (2020). Zinc deficiency in low- and middle-income countries: Prevalence and approaches for mitigation. *Journal of Human Nutrition and Dietetics, 33*(5), 624–643. https://doi.org/10.1111/jhn.12791

Gupta, S., Zaman, M., Fatima, S., Shahzad, B., Brazier, A. K. M., Moran, V. H., et al. (2022a). The impact of consuming zinc-biofortified wheat flour on haematological indices of zinc and iron status in adolescent girls in rural Pakistan: A cluster-randomised, double-blind, controlled effectiveness trial. *Nutrients, 14*(8). https://doi.org/10.3390/nu14081657

Gupta, S., Shahzad, B., Zaman, M., Sinclair, J., Fatima, S., Brazier, A., et al. (2022b). Impact of consuming zinc-biofortified wheat flour on the growth and morbidity status of adolescent girls: A cluster randomised, double blind, controlled trial. *Proceedings of the Nutrition Society, 81*(OCE5), E171. https://doi.org/10.1017/S002966512200204X

Gupta, S., Zaman, M., Fatima, S., Moran, V. H., Sinclair, J. K., & Nicola, M. (2023). Lowe Impact of consuming zinc-biofortified wheat flour on the growth and morbidity status of children aged 1–5 years: A cluster-randomized, double-blind, controlled trial. In *Proceedings of the Nutrition Society*. Accepted for publication.

Hambidge, K. M., Huffer, J. W., Raboy, V., Grunwald, G. K., Westcott, J. L., Sian, L., et al. (2004). Zinc absorption from low-phytate hybrids of maize and their wild-type isohybrids. *The American Journal of Clinical Nutrition, 79*(6), 1053–1059. https://doi.org/10.1093/ajcn/79.6.1053

Harrington, S. A., Connorton, J. M., Nyangoma, N. I. M., McNelly, R., Morgan, Y. M. L., Aslam, M. F., et al. (2022). A two-gene strategy increases iron and zinc concentrations in wheat flour, improving mineral bioaccessibility. *Plant Physiology, 191*(1), 528–541. https://doi.org/10.1093/plphys/kiac499

HarvestPlus. (2022). *The journey of scaling in Pakistan: Zinc-enriched biofortified wheat*. Available at: https://www.harvestplus.org/wp-content/uploads/2022/03/The-Journey-of-Scaling-in-Pakistan.pdf. Accessed on 21.09.2023.

HarvestPlus. (2023). *HarvestPlus Database of Biofortified crop released*. Available at: https://bcr.harvestplus.org/. Acessed on 27.09.2023.

Jongstra, R., Hossain, M. M., Galetti, V., Hall, A. G., Holt, R. R., Cercamondi, C. I., et al. (2022). The effect of zinc-biofortified rice on zinc status of Bangladeshi preschool children: A randomized, double-masked, household-based, controlled trial. *The American Journal of Clinical Nutrition, 115*(3), 724–737. https://doi.org/10.1093/ajcn/nqab379

King, J. C. (2011). Zinc: an essential but elusive nutrient. *The American Journal of Clinical Nutrition, 94*(2), 679s–684s. https://doi.org/10.3945/ajcn.110.005744

King, J. C., Brown, K. H., Gibson, R. S., Krebs, N. F., Lowe, N. M., Siekmann, J. H., et al. (2015). Biomarkers of nutrition for development (BOND)-zinc review. *The Journal of Nutrition, 146*(4), 858s–885s. https://doi.org/10.3945/jn.115.220079

Kumar, A., Mehtre, S., Anuradha, K., Jaganathan, J., Prasanna, H., Gorthy, S., et al. (2018). Delivering bioavailable micronutrients through biofortifying sorghum and seed chain innovations. *Science Forums*. Available at: https://oar.icrisat.org/10924/

Kumar, K., Gambhir, G., Dass, A., Tripathi, A. K., Singh, A., Jha, A. K., et al. (2020). Genetically modified crops: Current status and future prospects. *Planta, 251*(4), 91. https://doi.org/10.1007/s00425-020-03372-8

Liong, E. M., McDonald, C. M., Suh, J., Westcott, J. L., Wong, C. P., Signorell, C., et al. (2021). Zinc-biofortified wheat intake and zinc status biomarkers in men: Randomized controlled trial. *The Journal of Nutrition, 151*(7), 1817–1823. https://doi.org/10.1093/jn/nxab092

Listman, G. M., Guzmán, C., Palacios-Rojas, N., Pfeiffer, W. H., San Vicente, F., & Govindan, V. (2019). Improving nutrition through biofortification: Preharvest and postharvest technologies. *Cereal Foods World, 64*(3), 1–7.

Liu, D., Liu, Y., Zhang, W., Chen, X., & Zou, C. (2017). Agronomic approach of zinc biofortification can increase zinc bioavailability in wheat flour and thereby reduce zinc deficiency in humans. *Nutrients, 9*(5), 465.

Liu, E., Pimpin, L., Shulkin, M., Kranz, S., Duggan, C. P., Mozaffarian, D., et al. (2018). Effect of zinc supplementation on growth outcomes in children under 5 years of age. *Nutrients, 10*(3). https://doi.org/10.3390/nu10030377

Lockyer, S., White, A., & Buttriss, J. (2018). Biofortified crops for tackling micronutrient deficiencies – what impact are these having in developing countries and could they be of relevance within Europe? *Nutrition Bulletin, 43*, 319–357. https://doi.org/10.1111/nbu.12347

Lönnerdal, B. (2000). Dietary factors influencing zinc absorption. *The Journal of Nutrition, 130*(5), 1378S–1383S. https://doi.org/10.1093/jn/130.5.1378S

Lopez, H. W., Duclos, V., Coudray, C., Krespine, V., Feillet-Coudray, C., Messager, A., et al. (2003). Making bread with sourdough improves mineral bioavailability from reconstituted whole wheat flour in rats. *Nutrition, 19*(6), 524–530. https://doi.org/10.1016/s0899-9007(02)01079-1

Lowe, N. M. (2021). The global challenge of hidden hunger: Perspectives from the field. *Proceedings of the Nutrition Society, 80*(3), 283–289. https://doi.org/10.1017/S0029665121000902

Lowe, N. M., Khan, M. J., Broadley, M. R., Zia, M. H., McArdle, H. J., Joy, E. J. M., et al. (2018). Examining the effectiveness of consuming flour made from agronomically biofortified wheat (Zincol-2016/NR-421) for improving Zn status in women in a low-resource setting in Pakistan:

Study protocol for a randomised, double-blind, controlled cross-over trial (BiZiFED). *BMJ Open, 8*(4), e021364. https://doi.org/10.1136/bmjopen-2017-021364

Lowe, N. M., Zaman, M., Moran, V. H., Ohly, H., Sinclair, J., Fatima, S., et al. (2020). Biofortification of wheat with zinc for eliminating deficiency in Pakistan: Study protocol for a cluster-randomised, double-blind, controlled effectiveness study (BIZIFED2). *BMJ Open, 10*(11), e039231. https://doi.org/10.1136/bmjopen-2020-039231

Lowe, N. M., Zaman, M., Khan, M. J., Brazier, A. K. M., Shahzad, B., Ullah, U., et al. (2021). Biofortified wheat increases dietary zinc intake: A randomised controlled efficacy study of Zincol-2016 in rural Pakistan. *Frontiers in Nutrition, 8*, 809783. https://doi.org/10.3389/fnut.2021.809783

Mahboob, U., Ohly, H., Joy, E. J. M., Moran, V., Zaman, M., & Lowe, N. M. (2020). Exploring community perceptions in preparation for a randomised controlled trial of biofortified flour in Pakistan. *Pilot and Feasibility Studies, 6*(1), 117. https://doi.org/10.1186/s40814-020-00664-4

Mahboob, U., Ceballos-Rasgado, M., Moran, V. H., Joy, E. J. M., Ohly, H., Zaman, M., et al. (2022). Community perceptions of zinc biofortified flour during an intervention study in Pakistan. *Nutrients, 14*(4), 817. https://doi.org/10.3390/nu14040817

Maqbool, M. A., & Beshir, A. (2019). Zinc biofortification of maize (Zea mays L.): Status and challenges. *Plant Breeding, 138*(1), 1–28. https://doi.org/10.1111/pbr.12658

Mazariegos, M., Hambidge, K. M., Krebs, N. F., Westcott, J. E., Lei, S., Grunwald, G. K., et al. (2006). Zinc absorption in Guatemalan schoolchildren fed normal or low-phytate maize. *The American Journal of Clinical Nutrition, 83*(1), 59–64. https://doi.org/10.1093/ajcn/83.1.59

McDonald, C. M., Suchdev, P. S., Krebs, N. F., Hess, S. Y., Wessells, K. R., Ismaily, S., et al. (2020). Adjusting plasma or serum zinc concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. *The American Journal of Clinical Nutrition, 111*(4), 927–937. https://doi.org/10.1093/ajcn/nqz304

Mehta, S., Huey, S. L., Ghugre, P. S., Potdar, R. D., Venkatramanan, S., Krisher, J. T., et al. (2022). A randomized trial of iron- and zinc-biofortified pearl millet-based complementary feeding in children aged 12 to 18 months living in urban slums. *Clinical Nutrition, 41*(4), 937–947. https://doi.org/10.1016/j.clnu.2022.02.014

Nasrin, D., Wu, Y., Blackwelder, W. C., Farag, T. H., Saha, D., Sow, S. O., et al. (2013). Health care seeking for childhood diarrhea in developing countries: Evidence from seven sites in Africa and Asia. *The American Journal of Tropical Medicine and Hygiene, 89*(1 Suppl), 3–12. https://doi.org/10.4269/ajtmh.12-0749

Nestel, P., Bouis, H. E., Meenakshi, J. V., & Pfeiffer, W. (2006). Biofortification of staple food crops. *The Journal of Nutrition, 136*(4), 1064–1067. https://doi.org/10.1093/jn/136.4.1064

Ohly, H., Broadley, M. R., Joy, E. J. M., Khan, M. J., McArdle, H., Zaman, M., et al. (2019). The BiZiFED project: Biofortified zinc flour to eliminate deficiency in Pakistan. *Nutrition Bulletin, 44*(1), 60–64. https://doi.org/10.1111/nbu.12362

Praharaj, S., Skalicky, M., Maitra, S., Bhadra, P., Shankar, T., Brestic, M., et al. (2021). Zinc biofortification in food crops could alleviate the zinc malnutrition in human health. *Molecules, 26*(12). https://doi.org/10.3390/molecules26123509

Prasad, A. S. (2013). Discovery of human zinc deficiency: Its impact on human health and disease. *Advances in Nutrition, 4*(2), 176–190. https://doi.org/10.3945/an.112.003210

Prasanna, B. M., Palacios-Rojas, N., Hossain, F., Muthusamy, V., Menkir, A., Dhliwayo, T., et al. (2019). Molecular breeding for nutritionally enriched maize: Status and prospects. *Frontiers in Genetics, 10*, 1392. https://doi.org/10.3389/fgene.2019.01392

Rashid, A., Ram, H., Zou, C.-Q., Rerkasem, B., Duarte, A. P., Simunji, S., et al. (2019). Effect of zinc-biofortified seeds on grain yield of wheat, rice, and common bean grown in six countries. *Journal of Plant Nutrition and Soil Science, 182*(5), 791–804. https://doi.org/10.1002/jpln.201800577

Rizwan, M., Zhu, Y., Qing, P., Zhang, D., Ahmed, U. I., Xu, H., et al. (2021). Factors determining consumer acceptance of biofortified food: Case of zinc-fortified wheat in Pakistan's Punjab Province. *Frontiers in Nutrition, 8*, 647823. https://doi.org/10.3389/fnut.2021.647823

Rodriguez-Ramiro, I., Brearley, C. A., Bruggraber, S. F., Perfecto, A., Shewry, P., & Fairweather-Tait, S. (2017). Assessment of iron bioavailability from different bread making processes using an in vitro intestinal cell model. *Food Chemistry, 228*, 91–98. https://doi.org/10.1016/j.foodchem.2017.01.130

Rosado, J. L., Hambidge, K. M., Miller, L. V., Garcia, O. P., Westcott, J., Gonzalez, K., et al. (2009). The quantity of zinc absorbed from wheat in adult women is enhanced by biofortification. *The Journal of Nutrition, 139*(10), 1920–1925. https://doi.org/10.3945/jn.109.107755

Saha, I., Durand-Morat, A., Nalley, L. L., Alam, M. J., & Nayga, R. (2021). Rice quality and its impacts on food security and sustainability in Bangladesh. *PLoS One, 16*(12), e0261118. https://doi.org/10.1371/journal.pone.0261118

Saltzman, A., Birol, E., Bouis, H. E., Boy, E., De Moura, F. F., Islam, Y., et al. (2013). Biofortification: Progress toward a more nourishing future. *Global Food Security, 2*(1), 9–17. https://doi.org/10.1016/j.gfs.2012.12.003

Sandstead, H. H. (2000). Causes of iron and zinc deficiencies and their effects on brain. *The Journal of Nutrition, 130*(2S Suppl), 347s–349s. https://doi.org/10.1093/jn/130.2.347S

Sazawal, S., Dhingra, U., Dhingra, P., Dutta, A., Deb, S., Kumar, J., et al. (2018). Efficacy of high zinc biofortified wheat in improvement of micronutrient status, and prevention of morbidity among preschool children and women – a double masked, randomized, controlled trial. *Nutrition Journal, 17*(1), 86. https://doi.org/10.1186/s12937-018-0391-5

Signorell, C., Zimmermann, M. B., Cakmak, I., Wegmüller, R., Zeder, C., Hurrell, R., et al. (2019). Zinc absorption from agronomically biofortified wheat is similar to post-harvest fortified wheat and is a substantial source of bioavailable zinc in humans. *The Journal of Nutrition, 149*(5), 840–846. https://doi.org/10.1093/jn/nxy328

Signorell, C., Kurpad, A. V., Pauline, M., Shenvi, S., Mukhopadhyay, A., King, J. C., et al. (2023). The effect of zinc biofortified wheat produced via foliar application on zinc status: A randomized, controlled trial in Indian children. *The Journal of Nutrition, 153*, 3092–3100. https://doi.org/10.1016/j.tjnut.2023.08.013

Sitaresmi, T., Hairmansis, A., Widyastuti, Y., Rachmawati, S. U., Wibowo, B. P., et al. (2023). Advances in the development of rice varieties with better nutritional quality in Indonesia. *Journal of Agriculture and Food Research, 12*, 100602. https://doi.org/10.1016/j.jafr.2023.100602

Stein, A. J., Meenakshi, J. V., Qaim, M., Nestel, P., & Bhutta, Z. (2005). Analyzing the health benefits of biofortified staple crops by means of the disability-adjusted life years approach. In *A handbook focusing on iron, zinc and vitamin A* (Harvest Plus Technical Monograph Series) (pp. 1–32). Food and Agriculture Organization of the United Nations.

Stevens, G. A., Beal, T., Mbuya, M. N. N., Luo, H., & Neufeld, L. M. (2022). Micronutrient deficiencies among preschool-aged children and women of reproductive age worldwide: A pooled analysis of individual-level data from population-representative surveys. *The Lancet Global Health, 10*(11), e1590–e15e9. https://doi.org/10.1016/s2214-109x(22)00367-9

Talsma, E. F., Melse-Boonstra, A., & Brouwer, I. D. (2017). Acceptance and adoption of biofortified crops in low- and middle-income countries: A systematic review. *Nutrition Reviews, 75*(10), 798–829. https://doi.org/10.1093/nutrit/nux037

Tulchinsky, T. H. (2010). Micronutrient deficiency conditions: Global health issues. *Public Health Reviews, 32*(1), 243–255. https://doi.org/10.1007/BF03391600

UK Research and Innovation (UKRI). (2016). *Increasing micronutrient bioavailability from wheat*. https://gtr.ukri.org/projects?ref=BB/N021002/1. Accessed 27.09.2023.

Wani, S. H., Gaikwad, K., Razzaq, A., Samantara, K., Kumar, M., & Govindan, V. (2022). Improving zinc and iron biofortification in wheat through genomics approaches. *Molecular Biology Reports, 49*(8), 8007–8023. https://doi.org/10.1007/s11033-022-07326-z

Wiafe, M. A., Apprey, C., & Annan, R. A. (2023). Dietary diversity and nutritional status of adolescents in rural Ghana. *Nutrition and Metabolic Insights, 16*, 11786388231158487. https://doi.org/10.1177/11786388231158487

Woods, B. J., Gallego-Castillo, S., Talsma, E. F., & Álvarez, D. (2020). The acceptance of zinc biofortified rice in Latin America: A consumer sensory study and grain quality characterization. *PLoS One, 15*(11), e0242202. https://doi.org/10.1371/journal.pone.0242202

World Health Organization (WHO), Johns Hopkins Bloomberg School of Public Health, United States Agency for International Development (USAIDS), United Nations Children's Fund (UNICEF). (2006). *Implementing the new recommendations on the clinical management of diarrhoea: Guidelines for policy makers and programme managers*. World Health Organization. Available at: https://iris.who.int/handle/10665/43456. Accessed online 27.09.2023.

Zimmermann, R., & Qaim, M. (2004). Potential health benefits of Golden Rice: A Philippine case study. *Food Policy, 29*(2), 147–168. https://doi.org/10.1016/j.foodpol.2004.03.001

Zyba, S. J., Shenvi, S. V., Killilea, D. W., Holland, T. C., Kim, E., Moy, A., et al. (2017). A moderate increase in dietary zinc reduces DNA strand breaks in leukocytes and alters plasma proteins without changing plasma zinc concentrations. *The American Journal of Clinical Nutrition, 105*(2), 343–351. https://doi.org/10.3945/ajcn.116.135327