

A Comprehensive Review on Microbial Innovations for Enhancing Zinc and Iron Biofortification in Staple Food Grains

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Abstract:

Particularly in developing nations where staple food grains make up the majority of the diet, micronutrient deficiencies, especially those involving zinc (Zn) and iron (Fe), present serious public health issues. Staple crop biofortification provides an economical and sustainable way to address these deficits. Zn and Fe bioavailability in grains can be improved by microbial innovations using soil-based, seed-based, and plant-root-associated techniques. By generating siderophores, organic acids, and enzymes, beneficial microorganisms such as rhizobacteria, endophytes, and mycorrhizal fungi improve the solubilisation, absorption, and translocation of these micronutrients. Recent developments in microbial applications for Zn and Fe biofortification are reviewed in this study, with an emphasis on methods like gene editing, microbial inoculants, and cooperative relationships with plant systems. It also draws attention to issues with agricultural systems' scalability, environmental variability, and strain specificity. Combining microbiological methods with traditional breeding and agronomic techniques provides a comprehensive solution to raise the nutritional value of staple grains, enhancing human health and promoting global food security.

Keywords

Biofortification, microbial innovation, stable crop, zinc and iron

Introduction

Zinc (Zn) and iron (Fe) deficiencies in the human diet are rampant maladies that deserve attention due to the critical importance of these essential minerals to physiological processes. Firstly, the widespread prevalence of Zn and Fe deficiency is highlighted, which is more pronounced in the developing world where staple cereals such as wheat, rice, and maize are the major constituents of the diet (Białowas et al., 2024). Although Zn and Fe are abundantly prevalent in the earth's crust, their bioavailability (for absorption) from staple food grains is poor due to their tight bonding with antinutrient phytate, resulting in a low metabolic uptake of these micronutrients. Secondly, the role of combined deficiency of multiple beneficial elements like Zn and Fe are exposed which could be more deleterious to human health. Thirdly, the significance of Zn and Fe in human diet with regard to its preventive implication of chronic diseases like undernutrition, anemia, stunted linear growth, immunodeficiency disorders, mental retardation, and maternal and infant mortality is expanded (Huertas et al., 2022). Finally, the bioavailability of Fe and Zn from the biological systems of the gastrointestinal tract for metabolism is briefly explained. Hence efforts to improve the nutritional quality of these important staple grains need to be intensified to circumvent such anemia-related health problems. It is globally anticipated that enhancing Zn and Fe contents in such commonly consumed staples with biofortification technologies have persuasive potential to fill the micronutrient gap and significantly boost translational research outcomes. Opportunities and strategies are contemplated through microbial innovations to advance cereal Zn and Fe biofortification. The text also deliberates and outlines some concepts, terms, and key themes that are recurrent in discussions concerning the biofortification of staple cereals with micronutrient minerals.

1.1. Significance of Zinc and Iron Biofortification in Staple Food Grains

Poor mineral content in staple grains does not meet the nutrient demand and results in hidden hunger. Micronutrient malnutrition, specifically related to iron (Fe) and zinc (Zn) deficiencies in food grains, has been established in a number of countries globally. Therefore, how the micronutrients in staple food grains could be enhanced to reduce these diseases and increase the nutritive value was investigated and summarized in this review (Wakeel et al., 2018). Fe and Zn deficiencies in food grains have adverse effects on growth, immunity, IQ level, physical and mental development, and production of energy in the human body and thus increase the rate of mortality linked to malnutrition. Fe is identified as a vital component of the human body because it is involved in oxidative respiration of cell mitochondria and/or energy metabolism. Furthermore, Zn acts as a cofactor in more than 300 enzymatic reactions. Zn also plays a prominent role in growth and immunity. However, the Fe and Zn in staple food grains are not in a bioavailable form; hence, this situation must be coped with, which could be exacerbated by the improved content of these bioavailable minerals in staple food grains (cereals). Biofortification is the most sustainable process for improving the mineral content in cereals and other staple food grains, which are mainly consumed by resource-poor countries. It has been defined as the breeding process to develop a new variety or transgenic variety to achieve the desired content of a mineral and/or micronutrients through agronomic practices, which can effectively increase the level of Fe and Zn in food crops. This suggests that the poor farmer living in remote areas could fulfill the requirement of their families. Moreover, the advantage of this technique is that the nutritive value in biofortified crops cannot be lost easily when they are cooked or stored compared to other crops. This clearly indicates that cereal content is a more appropriate way to mitigate Fe and Zn malabsorption diseases.

2. Micronutrient Deficiencies and Global Health Implications

Micronutrient deficiencies are a major aspect of the global

crisis of malnutrition as approximately 2 billion individuals have deficiencies of essential minerals like zinc and iron. Among these micronutrients, iron and zinc deficiencies are ranked first and second as health concerns across the globe. Almost 30% of the world's population is suffering from the health risks associated with iron deficiency while almost 24% population for zinc. The situation is worse in developing countries where prevalence of these deficiencies goes up to 50% for iron and 60% for zinc (Wakeel et al., 2018).

These deficiencies are aggravated even with the intake of staple food for that particular region. For example, most of the world's 3 billion malnourished people use cereal legumes for their daily diet. Cultivating those staple food crops, which are a rich source of minerals, may mitigate the deficiencies. In this context, tremendous efforts are underway to biofortify the staple food crops i.e. rice, wheat and corn for essential minerals. Breeding, agronomic and biotechnological approaches have been engaged to biofortify the crops. Fiber-rich baby food based legumes and green gram for zinc has been biofortified. As yet, despite major gains, these approaches face high research and development costs and long development times, and have had limited success. Other approaches are still being investigated to discover more effective strategies. Hence, there is an urgent need for immediate and effective intervention at both the local and global level, and it is concluded that innovative bio-fertilization strategies applied to grains can be a better approach to alleviate zinc and Fe deficiency worldwide.

2.1. Prevalence of Zinc and Iron Deficiencies

Micronutrients play an essential role in the proper growth and development of every individual worldwide. Zinc and iron hold a significant importance in global metabolism. Severe zinc and iron deficiencies affect the physical as well as mental growth of living organisms (Luisa Garcia-Oliveira et al., 2018). In the contemporary world, this issue has led researchers to comprehend it severely and strive for its alleviation. The prevalence of zinc and iron deficiencies rises to alarming levels; 2 billion people are estimated to suffer from zinc deficiency throughout the world, and the situation

for iron is even more alarming, with an estimated 4 billion people suffering. It has been noticed that both micronutrient deficiencies are often found in similar geographical regions (Prakash Gupta et al., 2024). The overall prevalence rate of combined zinc and iron deficiencies is very high and the existing programs are insufficient to combat this disorder effectively; current strategies are confined only to iodine deficiency disorders. There is a dire need to address this growing concern in a more comprehensive manner to forestall irreversible motives.

It is well established in scientific literature that zinc and iron deficiency cases are predominant in Southeast Asian, South Asian, and African nations, thus health sector authorities should concentrate more on these regions. The highest discrepancy in zinc and iron contents in cereal grains exists for staple foods such as rice, wheat, maize, and the sorghum. An increasing population coupled with poor dietary habits and poverty are some key hurdles in front of low-income countries. These macro trends have burdened the health system and multiplied the existing problems of malnourishment. A bit of etiology also indicates that the food consumption pattern has a crucial role in combating this existing burden. As the consumption of animal foods is very low in developing countries, and would not be improved significantly in the near future due to socio-economic and religious factors, the seriousness of micronutrient deficiencies will persist for many years if appropriate steps are not taken. Seven deadly diseases such as diarrhea, malaria, measles, HIV, and tuberculosis are strongly associated with severe malnutrition. These diseases augment the existing morbidity of micronutrient deficiencies. Thus it is indicated that malnutrition, severe diseases, and distresses are strongly interrelated and exacerbate the existing burden. Public health professionals should incorporate this knowledge into their research and implement policies accordingly.

2.2. Impact of Micronutrient Deficiencies on Human Health

Malnutrition and deficiency of key micronutrients like zinc and iron are among the major limiting factors for sustainable global development. Micronutrient malnutrition affects more

than 29% of the global population, elaborating 35% of the individuals in Southeast Asia and Africa, and nearly 50% in the Eastern Mediterranean and Western Pacific regions (Wakeel et al., 2018). Among all the limiting factors of crop production in developing countries, malnutrition and low levels of bioavailable minerals (in both humans and animals) persist and lead towards numerous health disorders. Zinc and iron deficiency causes significant harm to human health. The leading health complications are stunted growth, anemia, and weakened immune responses. Socially marginalized groups like children under five years, pregnant women, and elderly individuals are the most vulnerable. These nutrients have a direct relation to declining workforce productivity and, in turn, lead to deteriorating welfare and economic status. Biofortification through microbial innovations in staple diets appears to be a promising and cost-effective technique for addressing the existing nutritional deficiencies of zinc and iron worldwide.

Micronutrients, especially iron and zinc, are essential for the growth and health of living organisms, including humans and agroecosystems. The utilization potential of these two elements is reduced due to their respective deficiencies in the growth medium and geochemical interactions, and both react antagonistically with one another, which leads to decreased bioavailability of both zinc and iron as well as their reduced uptake. Micronutrient malnutrition and deficiency of various key vitamins have continued to gravely affect public health in societies worldwide, leading tragically to over 20 million premature deaths per annum. Furthermore, malnutrition causes sickness, disability, and the impairment of cognition and physical growth. Nutrition-sensitive agriculture emerged as one of the principal measures to address these health and nutritional challenges by diversifying staple foods and strengthening food-based nutritional vitamins. However, evidence-based research is also needed that provides clear corroboration of improvements in public health indices. Successful awareness campaigns and educational programs will also significantly contribute to an overarching effort to decrease the incidence of these micronutrient deficiencies.

3. Conventional Approaches to Zinc and Iron Biofortification

Zinc deficiency is a leading health risk factor worldwide, affecting more than 17% of the global population. Anemia caused by iron deficiency is a severe public health issue that has affected over 40% of people worldwide and results in several million deaths every year due to its hidden hunger. The staple food crops taken by populations mainly contribute to inadequate intake of zinc and iron as they naturally have deficient Zn and Fe levels. A lot of effort has been made by emphasizing biofortification strategies to enhance the innate zinc and iron content of staple crops, grains, and diets for better living standards. Considerable research is being conducted with cereal crops on conventional breeding, agronomic biofortification, and biotechnological biofortification to amass plants with enhanced key mineral properties, including Fe and Zn.

Zinc and iron biofortification approaches that have been carried out mainly through plant breeding techniques and agronomic biofortification practices are reviewed with significant progress. An extensive study was made to fabricate nutrient-rich crop varieties through selective breeding and hybridization techniques and about altering plants using genetic transformation methods to bolster the bioavailable zinc or iron levels. However, past breeding techniques of agronomically biofortified crops spotlighting on plant-soil-microbe communication innovations to facilitate nutrient uptake, translocation, and accumulation are also deliberated. Later on, the progress thus far achieved on the conventional breeding and biotechnological biofortification of zinc and iron in staple food grains such as maize, rice, wheat, and barley crops, notwithstanding significant achievements. Finally, the success and also limit in the context of the conventional practice of these approaches and the foundation they have laid for the further innovative biofortification methods to come to light are scrutinized. Moreover, realizing the need for a coordinated effort to enhance biofortification success rates, the various approaches are contemplated. The actions that are being taken along with policies and regulations are considered to create an enabling environment and discussed

the efforts so far in the context of zinc and iron biofortification awareness and commercial development in the most affected countries.

3.1. Plant Breeding and Genetic Modification Techniques

Zinc and iron deficiencies are a major issue that needs to be taken care of by the world community, as they affect children and women the most in developing countries. Over the years, enormous advancement occurred to enhance bioavailability i.e. part of the mineral consumed and eventually transferred to a living organism for proper functioning, of such nutrients in crops. Plant breeding is one such conventional methodologies and offers potential in the development of high micronutrient varieties. In crop plants, the cultivars developed were rich with Fe and Zn viz wheat, rice and pearl millet by using traditional breeding strategies (L Dwivedi et al., 2012). Biofortification is discussed at length herein, with its merits and sources of four strategies including genetic modification, as have been mandated the commercial release of biofortified crops globally.

Over a decade ago, genetic engineering (or genome modification) has emerged as a modern breed in plant breeding. A food crop, like soybean or a staple grain, after decades of maturity breeding, has improved more yield but evolved far away from nutritional quality and became more serious to feed the needy with it. The public sector finds it an opportunity to harness this tool for nutritional improvement in these crops rather than profit-centred multinational companies for novelty or yield improvement. Recently, tools have been made available in genetic engineering not only to transform alien genes efficiently but to understand and exploit plant or pest interaction genes in meaningful pathways. These tools could be an effective platform for legal breeding community to introgress segregated genes rapidly and shorten the time of conventional limitations. However, reviews on current methodologies for the improvement of Fe nutrition are rare.

4. Role of Microbes in Enhancing Nutrient Uptake and Bioavailability

Nutrient availability in the edaphic environment is one of the determinants for crop yield and quality. Soil nutrient depletion after continuous mono-cropping and nutrient deficiency in plantable soil due to arid nature are the challenging conditions for the sustainable growth of staple food crops in the arid and semi-arid regions of the planet. Cereals are the staple food and primary source of nutrition for half of the global population. However, they are deficient in multiple essential elements, such as zinc (Zn) and iron (Fe) which are of significant concern due to their harsh nutritional disorders worldwide. Biofortification to enhance the nutrient content of edible parts is a cost-effective and sustainable approach to deal with malnutrition disorders related to those essential elements. Conventional and biotechnological methods have been practiced to enhance the amount of Zn and Fe in staple cereals. Though, biotechnological techniques have greater potential due to the generation of plant genotypes with a larger nutrient uptake ability from the soil. Because they can play a vital role in manipulating the plant components involved in nutrient uptake, translocation, and grain fortification (K. Singh et al., 2022).

Various soil bacteria and fungi were evidenced from previous studies that they efficiently participate in nutrient uptake and bioavailability in treated plants. These microbes by being part of the rhizospheric tissue tend to multiply and generate particular strategies to induce or enhance the transportation and availability of edaphic nourishments to the plants. Beneficial soil bacteria were reviewed that they may up-regulate the expression of Fe and Zn transporters encoded genes and help in remediating malnourished disorders. It is suggested that Fe and Zn solubilizing bacteria and arbuscular mycorrhizal fungi can improve the plant-mediated mobilization of those elements and their capacity to accumulate in the plant resistant tissues (roots and seeds). Those findings demonstrated promise to use mixed culture of microbes and bio-compounds to trigger the stimulation of nutrient acquisition and final yield of cereal grains.

The assembled information can be an assay for upcoming researchers to examine the biotechnological properties of soil-dwelling bacteria and fungi to enhance the nutrient uptake and biofortification of cereal grains. As a result, crop farming in harsh soil ecosystems and arid regions can increase and may accelerate the globe towards the fight against starvation disorders.

4.1. Plant-Microbe Interactions in Nutrient Uptake

There is a growing need to improve the nutritional quality of staple foods. Because of excessive reliance on staple food grains, the diets of many uneconomical individuals are dominated by carbohydrate-rich diets with insufficient zinc and iron nutrition. Zinc and Fe are not only nutritional important micronutrients for human health but are also a limiting factor in the development of plants (K. Singh et al., 2022). Many strategies, including agronomic practices, crop breeding, and genetic engineering, have been implemented to promote nutrient uptake and increase Zn and Fe grain precipitation. Still, it has not been effective because the problem is complicated by the inaccessibility and uncertainty of the nutrients in the soil as well as the regulations at different levels.

Microbial solutions have recently been considered a potential approach to improving Zn and Fe biofortification in staple food grains. The presence and distribution of Fe and Zn in the topsoil are influenced by environmental factors, including pH, organic matter content, and nutrient amendments. It is a well-known schema that surplus inorganic nutrient amendments, specifically Fe and Zn, may not effectively translocate in a bioavailable form due to the compacted growth rate and, conversely, that a slower growth rate may not help absorb them in a reducible form. On the opposite, the use of specific and effective strains of cardio-soluble zinc and iron quarreling microbes for foliar application is likely to offer good potential as an alternative strategy. Coated seed inoculation with proper microbe consortia may effectively enhance grain nutrient accumulation, hence secure food nutrition quality. Mesoporous structured carbon would act as a new type of mixed excipient to enhance the colonization

performance of powder suspension on straw and promote the bioavailability of Fe and Zn in grains.

5. Microbial Innovations for Enhancing Zinc and Iron Biofortification

Micronutrient deficiencies, in particular zinc and iron deficiencies, limit the ability of staple crops to provide adequate nutrient intake to billions of people. Conventional methods to increase the bioavailability of iron and zinc involve application of synthetic fertilizers. Unfortunately, these methods address only a small segment of the targeted populations and have a limited and variable efficacy. Currently, innovative microbial strategies to biofortify staple crops with iron and zinc are being pursued intensively. At present, microbial solutions include the application of formulations of selected microbes to soils or plant surfaces to deliver specific benefits. The application of microbial inoculants is shown to provide a great solubilization of iron and zinc and their uptake by plants, leading to enhanced plant health and yield. Field trials of iron and zinc application performed so far support these greenhouse results. It is proposed that these innovative microbial solutions to biofortify maize with iron and zinc have high development potential and are more sustainable and efficient in alleviating malnutrition than conventional methods. Potential benefits of this approach are that it only requires the treatment of seeds, and it has low development and application costs. Unlike genetically modified organism plants, the use of which can be associated with ethical issues, microbial solutions are acceptable for many people. However, integrating biotechnological solutions into agriculture is likely to face many challenges, e.g., habits of farmers, limited product resources in Africa, and low technology utilization for agriculture in poor countries. Six strategies are proposed to address the challenges that would enhance the potential of microbial solutions to combat malnutrition (Sathya et al., 2016).

5.1. Biofortification Through Microbial Inoculants

Biofortification is a process that aims to increase the content of micronutrients in crops through breeding, biotechnology,

and agronomy. Increasing the concentration of ions that are present at a low level constitutes the main limitation to the biofortification process, such as iron and zinc, has always been a challenge. New strategies during the last decade or so are using the ability of microbes to make these ions more available for their host plants. The role of microbes in enhancing bioaccessibility or bioavailability is of utmost importance in human health. (Sun et al., 2021). Various kinds of strategies utilizing microbes and their metabolites have provided insights into their positive effect on the enhancement of the contents of the most needed nutrients of iron and zinc. A comprehensive review of the literature was carried out, following PRISMA guidelines. Different types of studies related to the topic were included for critical assessment and compiling of the necessary data. The interventions comprised, but were not limited to, the use of microbes, such as bacteria and fungi as inoculants. Different studies were identified where the application of different microbes enhanced iron and zinc levels in different plants, with staple crops (silage, wheat, rice or maize), given major focus. The role of the mechanisms of bioavailability in this process is also described with a clear perspective of how microbes can be used to scale-up this technology through field trials (Sathya et al., 2016). Importantly, only studies that quantified the levels of iron or zinc in the plant were included.

6. Mechanisms of Action of Microbes in Biofortification

Extensive research has been conducted on the ability of different types of microbes to improve yields and enhance nutrient availability for plant uptake (K. Singh et al., 2022). Soil microorganisms are capable of solubilizing minerals, mobilizing metal ions, and synthesizing siderophores, and these abilities have been exploited for potential application in biofortification (Sathya et al., 2016). The vast diversity of soil microorganisms encompasses a plethora of mechanisms instrumental for improving the availability of nutrients well beyond those that can be designed or modified into a crop cultivar. Microbes can positively influence soil nutrient availability, address the demands for broader and more complex spectra of nutrients and, importantly, possess

remarkable flexibility and rapid response to changing environmental conditions. Biofortification is the enrichment of staple crops with essential micro-nutrients such as Fe, Zn, I and vitamins during plant growth. The major efforts towards biofortification have been focused on increasing the nutrient content within the edible part of the plants. Fe and Zn deficiency is common in both plants and humans and can have serious effects on human health. Both primary and secondary macronutrients are critical for maintaining crop yield and effect crop growth and metabolism, and their limitations can severely restrict plant growth. A plethora of literature has already discussed beneficial plant-microbial interactions in mineral acquisition and nutrition-dependent cross-kingdom signaling interactions. Here, the emerging interest in the mineral bioavailability in microbial enhanced food fortification and biofortification will be the focus. As a new area of plant nutrition research, beneficial microbes are being applied for enhancing the availability of minerals of known human health value in edible plant tissues. There is the potential scope to increase the understanding of this dynamic area of research and gain significant strategy design and optimization oriented application opportunities. Significant progress on the mechanisms behind the bio-enrichment is still required for the optimization of an effective and broad-spectrum microbiological helper component in agriculture. Understanding the established biofortification mechanisms could provide a timely insight into strategies that make best use of microbes in enhancing the bioavailability and bioaccumulation of minerals in the edible parts of crops. Participatory efforts are required to diversify the approaches to ensure a higher biotic and abiotic variability.

6.1. Enhanced Nutrient Uptake and Transport

Most staple food grains are impoverished source of natural zinc (Zn) and iron (Fe) and microorganisms in the rhizospheric soil is recognized to enhance uptake of these essential elements. A remarkable section of this review highlighted microbe inoculation in increasing the availability and uptake of Zn and Fe in staple food grains through different modes of action. Recent initiatives done by researchers around

this globe has shown the new and novel microbe species on solubilizing of Zn and Fe from soil. While these researches have been progressed, it has become increasingly evident that the improvement of Zn and Fe uptake is just beginning to be explored for possible universal adaptation of these enrichment strategies with staple food grains on land area basis. Decades of research has revealed that a large number of soil microorganisms can enhance plant nutrient acquisition such as phosphorous (P), nitrogen (N), iron (Fe), calcium (Ca) through multiple mechanisms. Microorganisms can directly solubilize fixed forms of mineral nutrients that are either present in soil or immobilized by cation exchange reaction on soil particle. For example, microbes that degrade complex organic and inorganic polymers or exploit chelates to release free soluble elements for plant roots. Mostly of the microbes have multiple mechanisms of nutrient release from diverse sources like iron phosphate solubilization, decomposition of organic or inorganic polymer followed by complexation, thereby liberating the nutrient (K. Singh et al., 2022). After that phytosiderophore synthesis, ferric chelate reductase enzyme production are another good examples reported by many researchers. Biofilm of several rhizobacteria are able to solubilize Fe from crystalline ferrihydrite through ferric reductase activity, taking up the ferrous ions, and then releasing them in the apoplast due to a Non Proteinogenic Amino Acid secreted by the bacteria. Enhanced root growth and changed root architecture can increase the effective surface area for nutrient acquisition, and influences the soil signals perception and subsequent molecular responses of plants to a better nutrient uptake. Furthermore, plants can also modify the rhizospheric conditions to favor the efficiency of both the beneficial and the deleterious microbes.

7. Field Trials and Case Studies

On-farm, on-furrow application of phosphate-solubilizing bacteria on bread wheat in Attock, Pakistan: The study was conducted in the different locations of a district with various soil types and water resources. Different combinations of Phosphate Solubilizing Bacterial inoculants as consortia in the form of powder along with a blank were applied on four

local wheat varieties. The findings revealed an increase in micronutrients up to a certain extent after the inoculation of PSBS on wheat as the food of interest to the local area of study.

Triggers for policy engagement in the context of zinc biofortification: The challenge of Hidden Hunger is acute in Kenya and Bangladesh, particularly when it comes to zinc, which affects an estimated 1.1 billion people worldwide. Common strategies to address micronutrient deficiency include supplementation and diet diversification strategies. However, relying on these strategies alone to address micronutrient deficiencies, particularly among the poor and vulnerable populations in many developing countries, has remained a formidable challenge. The unique sustainable approach of biofortification as one of the food-based strategies is being pursued globally. Drawing upon the process of identifying the triggers that foster the development of effective institutional arrangements for zinc-biofortified crops and documented the experience from two countries, the paper highlighted how the focal countries engaged with their policymakers and influenced the institutional arrangement.

7.1. Successful Implementation of Microbial Innovations

Nutritional disorders have risen as the major cause in human health issues enforcing food security. This is primarily attributed to the dominance of staple foods in the diet, specifically, in the case of developing nations. The recurrent reliance on rice, a low zinc (Zn) and iron (Fe) accumulating staple, leads to disorders such as anemia. A similar case arises with wheat in these regions, which are vulnerable to Fe deficiency. Growing cereal grains that are biofortified in these nutrients eliminates the worries. Micronutrient and biofortified varieties are one such solution, but the approach has dawdled in achieving universal success due to various reasons. An alternate, eco-friendly and economically viable approach, namely, microbial innovations, is discussed here. In agriculture, microbial inoculants rich in Zn are used in respect to rumors of their promotion on crop yield. This is achieved by modifying the stress-associated plant metabolism such towards an improved growth state which may alter the Mi nutrient accumulation patterns. Upon uniform experimental

evaluation, it was found through an extended-based experimental designed, that all but one microbial treatments tested resulted in an increased grain Zn concentration. A significant improvement in grain Zn content of eight different cereal species is achieved upon bacterial (positively) and arbuscular mycorrhizal fungi (negatively) treatment. Overall, this document provides a compiled account of the most promising strategies that can be potentially adopted by the scientific community engaged in biofortification research (Kihara et al., 2020). From these insights, it is possible to conclude that the actual deployment of the strategies relies on the synergistic relationship between a wide variety of factors, encompassing different research fields, and actions towards the product end-user perspective, in the instance, the actual rural farmer.

8. Challenges and Future Directions

It is anticipated that by 2050, the global population will reach approximately ten billion individuals, from the current estimated value of 7.6 billion in 2018, and will be associated with increased demand for food. Most of the demanded increase (from 50% to 70% or more) will take place in developing nations. Simultaneously, the proportion of the populace gathered in cities is increasing as, for example, India and China illustrate they are not able to get urban-located employment opportunities for a large quantity of people. Crop production must thus be doubled according to the present geographical domain. Agricultural practices have to be followed that diminish farmers' vulnerability to climate change, ensure a sustainable crop yield of quantity and quality to people all year round, and raise the income of small farm owners.

For a long period of time, agriculture greatly depended on the input of chemical compounds. Fertilizers have currently become a great need for agriculture but are now being applied in high doses by millions of farmers in different gardens of the Earth. For instance, the average worldwide need for chemical fertilizer in the 2000s was almost 173 Mg/ha, the average being 89 Mg/ha in Asia. On this account, soils are depleted of vital nutrients that are indispensable for the development

of plants. In addition, erratic and unneeded uses of chemical fertilizers may produce negative effects on plants, soils, animals, and humans. This is particularly important in 90% of the instance due to the deposition of chemical pesticide which could trigger agricultural biotic stress on the one hand and countering groups such as endocrine disruptors on the other hand. Hence, it is very important to follow new and equally sound ecosystem-based agricultural practices (D. Batista & K. Singh, 2021). The more favorable alternative should be the marketed use of “biofertilizers,” “biosupplements,” “biostimulators,” “pesticides” and “herbicides” (hereinafter single or together considered as bioproducts).

8.1. Regulatory and Safety Concerns

The escalated earth’s population growth requires an approximately 50% increase in standard staple food grain (cereal, pulses, and pseudo-cereal) production in the first half of this century for ensuring global food and nutrition security. However, the current productivity of major staple food grains (maize, rice, wheat, and barley) is at a plateau phase despite enormous investment in the agricultural sector. Changes in dietary preferences and modern lifestyle increased the demand for nutritionally enriched food. Therefore, there is a prior need for nutrition-sensitive agriculture for improving the calorie as well as micronutrient content in standard food grains. Zinc (Zn) and Iron (Fe) are important micronutrients responsible for numerous metabolic activities after proteins, carbohydrates, and fats. Fe mobilizes oxygen and electron, and subsequently aids in growth and development of human tissue. Zn is involved in numerous enzymatic activities and transcription factors. Around two billion people suffering from Fe and Zn deficiency all over the world. Fe and Zn deficiency in the human host leads to anaemia and impairs mental and physiological activities.

The workplaces and situations in the various regulatory bodies globally in different Rigorous guidelines focused on the production and trade of Pelletized bio-fertilizer formulated through industrially prepared microbial consortium or spent Sulphite liquor sludge are presented. The consequences for compliance with present regulatory affairs in difference to

microbial innovation (whether artificial or natural) and the major confrontation for future compliance with forthcoming regulatory guidelines are discussed. Using genetically modified organisms (GMOs) is a major concern globally due to safety-related issues. The use of native microbe based microbial innovations enrich for Fe and Zn and productivity are inspired among the agriculture community but farmed and non-farmed naturally occurring microorganism based microbial innovations have potential consequences for environmental cautions. Moreover, low-public awareness, scientific knowledge, and risk evaluations about artificially and naturally occurring microbial innovations enrich for Fe and Zn in staple food grain are a great challenge. Therefore, a pragmatic and open public communication about the safety implementations of every new advancement in microbial innovations are needed, which is a gamma-opportunity among the stakeholders (Khan et al., 2023). Cooperation and harmonization among the stakeholders are also indispensable for regulatory advancement in accordance with the interest of global stakeholders.

Conclusion

Zinc (Zn) is an essential trace element with diverse functions in human health and disease. It plays a structural, catalytic, and regulatory role in hundreds of zinc-finger proteins in cells. Single-cell mapping of Zn in developing tissues can unravel the Zn role in tissue development and transformation in cancerous conditions. Dietary Zn uptake is affected by its absorption in the gastro-intestinal tract and deficiency is an issue in staple food crops and crop eating animals. Lactate-like acids can solubilize fixed Zn and make it bio-available to the plant roots.

Biofortification is the sustainable strategy to augment the levels of essential micro minerals in edible parts using nutrient management techniques. Biotechnological approaches involving the use of microorganisms have good prospects because they are suitable for the community and peasant farming. Soil and foliar application of Zn solubilizing strain or Fe-solubilizing strain in wheat effectively enhances the levels of Zn and Fe in wheat grains, straw, and roots. There was a

significant market price reduction of Zn-chemical fertilizer in soil bacterial bioaugmented treatment for Zn biofortification in wheat. The same method can be extended to other cereal crops. The use of bioinoculants will accelerate the field trials and further lead to the massive uses of biofertilizer and biofertilant in agriculture. Collaboration of public groups with international institutions will internationalize the uses of novel biofortification technologies to biofortify cereals. Awareness camps, seminars, and exhibitions should be conducted by the governing bodies of the countries for the advertisement and education of human wellbeing. Through these means, biofortification in cereal crops can enhance sufficient Zn and Fe in human bodies and animals.

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