Open Access
Full Length Article

REVIEW ARTICLE

Advancements in Food Biofortification: Enhancing Nutrient Density to Combat Global Malnutrition

Hafiza Zara Saeed[1], Musrat Shaheen[2], Burhan Khalid[3]*, Aftab Umar[4], Hina Anwar[2], Talha Riaz[5], Iqra Irfan[1], Muhammad Atiq Ashraf[6], Saira Batool[6]
[1]Department of Botany, Government College University Faisalabad, 38000, Pakistan.
[2]Department of Chemistry, Government College University Faisalabad, 38000, Pakistan.
[3]College of Plant Science and Technology, Huazhong Agricultural University, Wuhan-430070, China.
[4]Department of Botany, University of Agriculture Faisalabad, 38000, Pakistan.
[5]College of Food Science and Technology, Huazhong Agricultural University, Wuhan-430070, China.
[6]College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan-430070, China.

ABSTRACT

Background: Micronutrient deficiencies impact over two billion people globally, particularly in low- and middle-income countries, leading to severe health issues. Traditional interventions such as supplementation and dietary diversification face limitations, necessitating sustainable solutions like biofortification to enhance the nutritional content of staple crops.

Objective: This review aims to synthesize advancements in biofortification techniques, including agronomic, conventional breeding, and genetic engineering approaches, to address micronutrient deficiencies while ensuring crop yield stability and consumer acceptability.

Methods: A systematic analysis of peer-reviewed literature from 2000 to 2024 focused on biofortification strategies for key crops such as vitamin A cassava, iron pearl millet, and zinc wheat. Data were evaluated for nutritional efficacy, scalability, and socioeconomic impacts.

Result: Biofortified crops demonstrate significant efficacy: provitamin A maize improved serum retinol levels by 30% in deficient children, while iron-biofortified pearl millet increased hemoglobin concentrations by 15%. Agronomic techniques (e.g., foliar fertilization) and CRISPR-Cas9 gene editing emerged as scalable solutions, though challenges like GMO regulations and farmer adoption persist.

Conclusion: Bio fortification offers a cost-effective, sustainable strategy to combat malnutrition. Future success depends on interdisciplinary collaboration, policy harmonization, and community engagement to optimize crop nutrient profiles and ensure widespread adoption.

Keywords	*Address of Correspondence	Article info.
Bio-Fortification, Micronutrients, Health Benefits, Conventional Approaches, Agronomic Techniques.	*burhankhalid1859@gmail.com*	*Received: October 04, 2024* *Accepted: December 28, 2024*

***Cite this article:** Saeed HZ, Shaheen M, Khalid B, Umar A, Anwar H, Riaz T, Irfan I, Ashraf MA, Batool S. Advancements in Food Biofortification: Enhancing Nutrient Density to Combat Global Malnutrition. RADS J Biol Res Appl Sci. 2024; 15(2):99-113.*

INTRODUCTION

The WHO is strengthening efforts to combat acute malnutrition in children under five by issuing new guidelines on preventing and treating wasting and nutritional edema[1]. In 2015, the globe committed to attaining the Sustainable Development Goals (SDGs), with the ambitious goal of eradicating hunger in all forms by 2030[2]. On September 24, 2024, the WHO collaborated with a coalition of Nutrition for Growth (N4G) host states, international institutions, and global nutrition networks at the 'Together for Nutrition', a high-level event held alongside the 79th United Nations General Assembly[3].

Malnutrition takes several forms, including undernutrition, stunting, micronutrient deficits, and wasting. This can be attributed to unhealthy diets that are high in calories, fat, sugar, and salt. Malnutrition can lead to stroke, cardiovascular disease, type 2 diabetes, and some types of cancer[4,5]. Undernutrition accounts for about half of all fatalities among children under the age of five worldwide. The majority of these deaths occur in low- and middle-income nations, mainly in Africa and Asia[6]. The global burden of malnutrition has extensive developmental, economic, social, and medical consequences, affecting individuals, families, communities, and entire countries[7].

To address these problems and make meaningful progress toward reaching the Sustainable Development Goals, notably SDG2 on zero hunger, would require cost-effective initiatives, realistic resource mobilization methods, clear measurements, and accountability from all stakeholders[8]. Poverty increases the likelihood of malnutrition and its associated risks. People with limited resources are more susceptible to different types of malnutrition. Furthermore, malnutrition hinders economic progress, reduces productivity, increases healthcare expenses, and leads to ill health[9,10]. Micronutrient deficiencies affect almost two billion people globally, accounting for one-third of the population. Malnutrition is a condition in which the body does not obtain sufficient nutrients to function correctly. This might be due to a deficiency in vitamins and minerals[11]. Malnutrition can be induced by a variety of reasons, including food deprivation, anorexia nervosa, fasting, vomiting, and drug-nutrient interactions. Pregnant women and children under the age of five are especially vulnerable to nutritional deficiencies such as Fe, I, and Zn[12,13]. Climate change is anticipated to increase the number of people experiencing vitamin deficiencies. Climate change makes crops less resilient to drought and other stresses, lowering the nutritional content of the food they produce[14].

Many organizations, each with distinct goals and working at varying scales, have promoted agricultural intensification in developing nations in recent decades. To fulfill the food demands of a growing population, the majority of agricultural researchers and food security specialists believe that intensification is required on a worldwide scale[15-17]. But the production of healthy non-staple foods, such as vegetables, legumes, and animal products, has not increased as quickly[18,19]. Low-income people are finding it more and more difficult to afford a healthy diet due to the steady and notable increases in the cost of non-staple food items[20].

One-third of the world's population, or around 2 billion people, are deficient in some micronutrients. Iron, zinc, and vitamin A deficiencies in particular cause serious health problems, such as reduced immune systems, poor cognitive development, and higher death rates, especially in children and pregnant women[12,13]. Micronutrient deficits will be significantly decreased in the long run by promoting dietary variety and expanding the production of nutrient-dense foods[21]. By increasing people's daily intake of important micronutrients throughout their lives, eating biofortified crops can help prevent micronutrient deficiencies[22]. Since micronutrient deficiencies cannot be resolved by a single treatment, biofortification complements current programs such as commercial food fortification and supplementation[23]. Agronomic techniques, transgenic technology, and cultivation are all used in the biofortification process to enhance crops. Regular use of these crops improves human nutrition and well-being[24-26].

Biofortification Advantages

Biofortification has two major benefits. First, it is cost-effective and well-suited for rural communities. Second, it delivers long-term health advantages[27], plant breeding, and excellent yield[28]. To optimize investment returns, biofortified crops can be enhanced nutritionally and adopted in new settings and geographies[26]. When the micronutrient trait becomes a fundamental breeding goal in national and international crop breeding initiatives, the ongoing expenses for monitoring and maintenance by agricultural research organizations are negligible[29]. These crops are good for rural people who have inadequate access to a balanced diet and vitamin supplements. The micronutrient targets for biofortification are intended to address the specific food needs of children and women while considering current consumption patterns[30]. Biofortification offers farmers a solution by merging micronutrient traits with agronomic and consumer preferences[31].

Evidence of Nutritional Bioavailability and Efficacy

By improving bioavailability and uptake of vital micronutrients, biofortified crops can greatly improve human nutrition. For example, it has been demonstrated that biofortified orange sweet potatoes (OSP) raise vitamin A levels, which lowers the frequency and severity of diarrhea in kids under five[32]. Similarly, youngsters with vitamin A insufficiency have shown improved visual function when given provitamin A maize [33,34]. Additionally, iron biofortification yields encouraging outcomes. Adolescents with iron deficiency who consumed biofortified pearl millet flatbread for four months saw a significant rise in blood ferritin and total body iron levels[35]. These studies demonstrate how certain micronutrient deficits may be addressed by biofortified crops. Researchers carry out controlled studies to assess the influence of various genotypes on micronutrient bioavailability to guarantee the efficacy of biofortified crops. Validating the long-term advantages of biofortified crops on micronutrient status and health outcomes requires randomized controlled trials[36].

Agriculture-Based Nutrition

Designing agriculture-based nutrition-sensitive projects, such as biofortifying staple crops, is a long-term, cost-effective way to improve crop nutritional content and, in turn, the health of vulnerable communities. In 2024, Grabowski *et al.* Energy-rich crops and staple carbohydrates like wheat, maize, rice, sweet potatoes, pearl millet, lentils, beans, and cassava are staples in many low- and middle-income countries (LMICs) with high prevalence of micronutrient deficiencies. Although these crops are cheap, they lack the micronutrients needed by humans, particularly when the agricultural industry turns them more and more into highly processed foods[37].

Vitamin A Crops

Vitamin A bioavailability has shown that provitamin A can be effectively converted to retinol, vitamin A preferred for the body. Efficacy has demonstrated that increasing provitamin A consumption by vitamin A-biofortified crops improves circulation beta-carotene and has a moderate effect on vitamin A status, as measured by serum retinol[38]. Biofortified crops with vitamin A improve circulation and beta-carotene content and have a moderate effect on vitamin A status as measured by serum retinol levels. Studies have demonstrated the efficacy of increasing provitamin A consumption. Orange sweet potato (OSP) significantly increases vitamin A in the body[39–41]. Child health can be improved by consuming biofortified orange sweet potato (OSP), as demonstrated by studies showing a reduction in the rate and duration of diarrhea in children under the age of five who ingest biofortified OSP [[32]]. Children with vitamin A deficiency show significant improvement in visual function when consuming maize biofortified with provitamin A[33,34].

Zinc in Crops

Zinc is a fundamental nutrient for plants that promotes growth and production. Wheat tends to be deficient in zinc, making it less likely to respond to Zn treatment. Zinc can be applied through soil incorporation, broadcasting, or foliar application; however, incorporating it with initial fertilizers is the most cost-effective option. Zinc-biofortified seeds can increase grain output and help plants tolerate environmental stress[42,43]. Numerous qualitative trait loci for wheat's grain iron and zinc concentration have been identified (Table **1**). These can be used in superior breeding lines to increase micronutrient levels. The concentration of zinc increases in plants by injecting genetic markers through biofortification[44].

Table 1. Nutrient Concentration (mg/kg Dry Weight) in Different Crops.

Crops	Zinc	Iron	Calcium	Magnesium	Potassium	Phosphorus	References
Maize	10-35	15-40	80-200	80-250	2000-4000	300-600	(57)
Soybean (Grain)	25-50	50-100	200-600	200-400	1500-3000	400-800	(58)
Wheat (Grain)	20-50	30-60	100-300	100-300	2000-4000	300-500	(57)
Rice (Grain)	15-40	20-50	50-150	50-150	1500-3000	250-450	(57)
Potato (Tuber)	10-25	20-40	200-500	100-300	2000-4000	300-600	(59)
Spinach (Leaf)	40-70	100-300	800-2000	600-1500	5000-8000	400-1000	(60)

Iron in Crops

Iron is a necessary nutrient for living creatures, as it plays an essential role in both animal and plant metabolic processes. Iron is necessary for all human growth and development as well as for many physiological processes, including the formation of oxygen transport proteins like hemoglobin and myoglobin, the development of the immune system, and the movement of oxygen from the lungs to tissues[45,46]. Iron pearl millet and biofortified iron beans have been shown to improve the nutritional status of target populations, according to iron nutrition studies[47]. After four months of twice-daily consumption of biofortified pearl millet flatbread, blood ferritin, and total body iron levels significantly improved in iron-deficient teenage boys and girls[35]. Iron is required for many basic activities in crop plants, including photosynthesis, respiration, and antioxidant defense. It is also important in many metabolic pathways, including hormone and secondary metabolism, making it a critical micronutrient[48]. Biofortification, initially increasing Fe levels in plants, is a potential technique for improving crop nutritional value and reducing Fe insufficiency in humans[49].

Cassava Crop

Vitamin A is not found in commonly farmed cassava. Biofortified vitamin A cassava varieties can contain up to 120 micrograms (ug) of provitamin A carotenoids per 100 grams (g) of cassava; the extra carotenoids accumulate in the plant's edible sections during the usual development process. Cassava contains chemicals such as beta-carotene, which can benefit eye health and prevent poor vision or blindness. Cassava contains vitamins A and C, both of which are beneficial for the digestive system. Vitamin A deficiency increases the risk of infections, including diarrhea and measles. In the case of vitamin A-rich yellow cassava, farmer marketing was at the foundation of this project. First, landowners received free bundles of stems, along with agricultural instruction and details about nutrition[50]. Farmers who received free stems were required to distribute an equivalent number of free stems to two other farmers in the following season, which significantly reduced delivery costs[51].

Self-Pollinated Crops

It is possible to cultivate self-pollinated crops year after year since they yield seeds that are exactly like those of their parent plants[52]. The ability to self-produce seeds frequently restricts private sector investment in seed production for self-pollinated crops, even though farmers must replenish their seeds regularly to preserve desired agronomic features[53]. Self-pollinated seeds are instead multiplied and dispersed by the public sector in many nations, and farmer-to-farmer distribution is also typical. Zinc rice in Bangladesh, zinc wheat in India and Pakistan, and iron beans in Rwanda and the Democratic Republic of the Congo are examples of self-pollinated biofortified crops[54].

Hybrid Crops

Hybrid crops, which need seed renewal to maintain constant production and agronomic traits, have the most potential for commercialization by private organizations[55]. While engaging the private sector for distribution may result in long-term sustainability, the rate of private sector absorption is determined by their evaluation of demand. Thus, biofortification advocates must focus their efforts on creating targeted demand for both farmers and consumers[56].

Biofortification Techniques

Biofortification is enhancing agricultural products with micronutrients to promote good health for humans. Notable biofortification methods are included here.

Agronomic Techniques

Agricultural biofortification with micronutrients through foliage spraying promotes the uptake of more nutrients in the reproductive system, resulting in more nutritious food for consumers[61]. Nutrients are supplied in liquid form to the apical regions of plants, they are absorbed by the pores of the stomata and epidermis and enter the dietary chain. Crops can also be biofortified by flooding them with minerals. Minerals like calcium (Ca), selenium (Se), and zinc (Zn) are given to crops during irrigation, which facilitates their absorption and raises their concentration in the plant's edible section [[62]]. In order to biofortify crops, mineral fertilizers-usually NPK (nitrogen, phosphorus, potassium) fertilizers-are given to the soil. Before planting or during multiple seed combination fertilization drilling, they are applied to the garden surface. Consequently, nutrients are taken up by roots and move up the food chain[63]. Enhanced microbial uptake of nutrients is another method of crop biofortification. Various species of microbes, including the bacteria rhizobium and mycorrhizal fungus, assist plants in acquiring nutrients through mutualistic relationships[64].

Agronomic Biofortification

Agronomic biofortification is a technique for increasing the nutritional content of crops by introducing micronutrient fertilizers to the Earth's soil or leaves of plants. It has the potential to significantly boost the content of nutrients in crops' consumable components as well as productivity[63]. The above is considered the conventional approach of supplementing cultivated crops with essential nutrients[65]. Vitamins and minerals can be found in various amounts in different plant parts and are usually absorbed from the soil. Adding micronutrients as fertilizer can increase the levels of these nutrients in the soil and address deficiencies in plants and humans. Several factors influence (Figure **1**) the efficacy of agronomic biofortification, as nutrients are lost during the transition cycles from ground soil to plant and then transferred into human food[66]. Zinc is essential for immune system function, mineralization of bones, growth of tissues, sperm production, and fertility rates, as well as for the replication of cells, DNA, and protein synthesis. Furthermore, zinc has powerful antibacterial and antimicrobial properties in the human body and plays a role in strengthening the immune system against numerous viruses, such as the coronavirus. Agronomic biofortification's ability to alleviate iron (Fe) deficiencies in humans depends on many factors. The amount of easily accessible iron for plant intake, the location and mobility of iron into edible food consumed, the accessibility of iron in food that is prepared for people to eat, and the current state of the human body, influence its capacity for absorption and utilization of iron, are all factors that rely on the presence of iron at different levels[67]. Species of wild plants have nutrient components that can also be utilized as food sources for humans. Fertigation with low electrical conductivity and moderate pH resulted in excellent production and nutrient amounts such as manganese (Mn), iron (Fe), zinc (Zn), and copper in species of wild dune spinach[68,69].

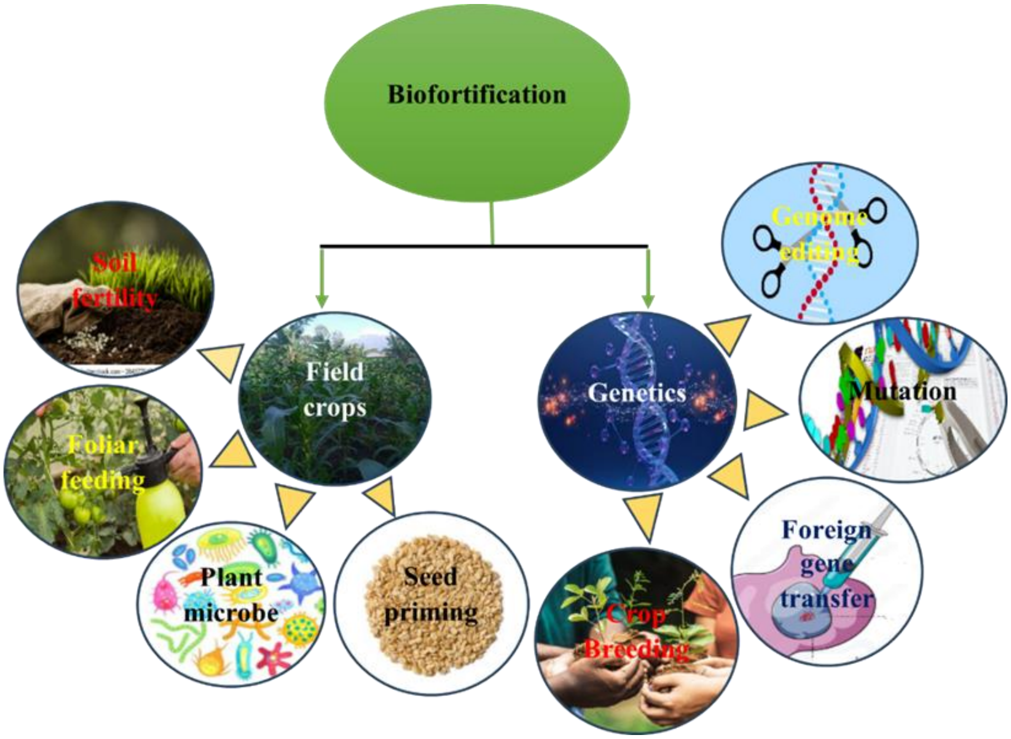

Figure. 1. Strategies and classification of biofortification. The figure outlines key approaches such as seed priming, foreign gene transfer, and mutation breeding, alongside critical elements like plant-microbe interactions, field crops, and genetic modifications to enhance nutrient content in crops.

Conventional Breeding

Crossing two parent plants with different phenotypes and choosing desirable features in the following segregating generations is known as crop biofortification[70]. Eliminating genes that produce antinutrients is one of the new breeding techniques. Crop nutrient deficits can result from anti-nutrients like tannins, lectins, α-amylase inhibitors, phytic acid, saponins, lathyrogens, and protease inhibitors that reduce the bioavailability of essential micronutrients[71]. By inhibiting the genes that produce antinutrients, RNA interference (RNAi) can lessen the buildup of harmful compounds. When overexpressed, the genes in plants that store micronutrients also improve micronutrient biofortification[72]. Certain genes that are overexpressed accumulate more micronutrients, which causes more deposition in the plant sections that can be eaten[73]. Through biofortification, genes are transferred from one plant to another, improving nutritional quality and lowering hunger[74]. Several genes involved in this biosynthesis have been introduced across species for biofortification in the areas of provitamin A, iron homeostasis, and flavonoid production[75]. By promoting a balanced diet, these genes aid in the management of a number of illnesses linked to malnutrition. For instance, Golden Rice has been used to treat night blindness and other conditions associated with a lack of pro-vitamin A[76].

However, there are a number of issues with this technique. Its widespread use may be constrained by the fact that it is frequently challenging, costly, and time-consuming[77]. Perception among the general population is one of the biggest obstacles. Concerns regarding the long-term health and environmental impacts of genetically modified organisms (GMOs) and biofortified crops have made many consumers wary of them. Misinformation and a lack of knowledge on the advantages and scientific foundation of biofortification frequently serve as the fuel for this doubt. The adoption of biofortified crops may also be made more difficult by regulatory and legislative obstacles. To address these issues and foster acceptance and trust, scientists, legislators, and business executives must engage in extensive public education efforts and communicate openly. To overcome these obstacles and guarantee the effective adoption of biofortified crops, public participation and engagement in the creation and execution of biofortification initiatives are essential.

Genetic Biofortification

Identifying beneficial genotypes or genes and creating cultivars that are micronutrient-dense and reduced in antinutrients require genetics and biochemical characterization. Advanced wheat breeding lines with genes associated with high zinc and iron concentrations in grains have been identified[78,79]. Selected 14 wheat lines high in iron (Fe), zinc (Zn), and yield for further study doubled haploid lines from a population. Baiyeri *et al.*[80] crossed a wild-type with subtropically modified low-phytate mutations (lpa1-1) to identify many potential maize hybrids. Additionally, novel micronutrient-biofortified crops can be created using gene modification technologies like CRISPR/Cas9 with minimal linkage drag and no safety or health risks[81].

Golden Rice is a transgenic rice (*Oryza sativa*) that produces beta-carotene, a precursor to vitamin A. The rice's characteristic color comes from beta-carotene, a substance that gives vegetables and other plants their golden hue[82]. Although this crop was intended to tackle vitamin A deficiency, especially in children in low-income nations where rice is an essential source of nutrition, it has attracted significant debate[83]. Vitamin A is one nutrient that is essential to human health and can only be obtained from diet. It is present in milk, eggs, and liver fats, but fish fat, especially fish liver oils has the highest concentration of it. While vitamin A is not naturally found in plants, the body can produce it from beta-carotene or other pigments found in many fruits and vegetables[84].

Genetically Modified Organisms (GMOs)

An organism whose genome has been modified in a lab for better expression of desired physiological features or the production of desired biological products is known as a genetically modified organism (GMO)[85]. Crop farming, pet breeding, and traditional livestock production, which involve breeding selected members of a species to generate offspring with desirable characteristics, are widespread methods. Hybrid plants can dramatically enhance crop productivity per unit area and reduce the demand for pesticides containing chemical ingredients[86]. In many areas, the need for broad-ranging insecticides has reduced since plants such as corn, potatoes, and cotton were genetically modified with a gene from the bacteria *Bacillus thuringiensis*, which generates an organic pesticide known as genetically modified Bt toxin[87]. In many areas, the bacteria *Bacillus thuringiensis*, which generates an organic pesticide known as Bt toxin, has been genetically modified and transferred into corn, potatoes, and cotton[74]. This modification has reduced the need for broad-ranging insecticides in plants. Bt cotton planting increases up to 36% of the income of farmers while reducing their use of pesticides by 50-80%. There is ongoing research underway to create "edible vaccines" from genetically modified plants. An antigenic protein produced in a plant's edible portions (like fruit) and transferred into the circulatory system, when taken, is known as a consumable vaccine[88]. Innovative DNA vaccines may help prevent illnesses such as cancer, TB, and HIV/AIDS that have shown resistance to conventional immunization methods[89].

Biotechnological Innovations

Cell Culture Technologies

Most other techniques are based on cell cultivation technology. They involve microbial cell culture (mycoprotein), animal cell culture (cultured meat), and plant cell culture, all of which have potential applications in food technology[90]. Cell culture technology involves promoting cell growth outside of an organism's body using specialized techniques. It offers various benefits such as non-seasonality, independence from geography, homogeneity, and controlled output[91]. The isolation of cells from plants or animals, which are then cultivated in a laboratory atmosphere in controlled units[92] (Figure **2**). This helps to reduce the chance of exposure, cell culture must be conducted in an aseptic, sterile environment.

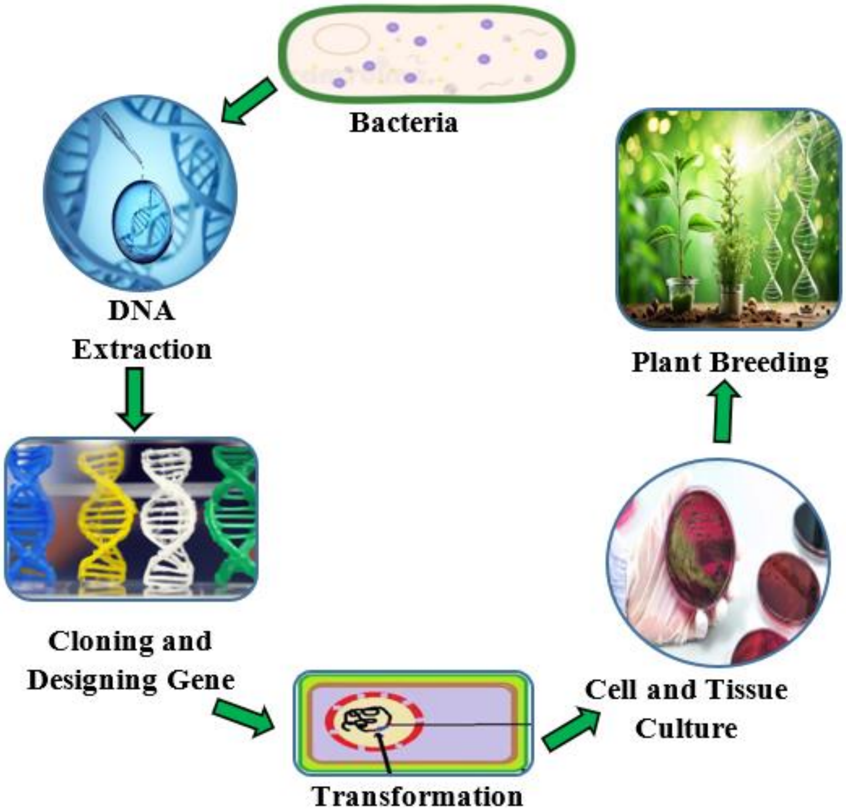

Figure. 2. Key techniques in modern biotechnology. The figure highlights fundamental processes such as DNA extraction, bacterial transformation, plant breeding, gene cloning, and design, as well as cell and tissue culture, which are pivotal in genetic engineering and biotechnological applications.

Animal Cell Culture

The production of food is a significant advancement using animal cell culture technology. Cells from cattle, fish, or poultry, are grown under controlled conditions, and specific cell and tissue types are differentiated, and then collected and processed into new food technology. Customer demand for meat is predicted to rise by 72%, putting a strain on available resources[93]. For example, 8 kilograms of grain are required to produce 1.5 kilograms of livestock, in addition to the duration and energy required for breeding cows until they reach the required age and weight[94]. The use of meat from cultured sources instead of traditional beef could help reduce the amount of energy, water, and land used in livestock production[95]. It would minimize deforestation caused by the development of animal pastures and reduce greenhouse gas emissions from animal production[96].

Microbial Cell Culture

Specifically, the production of microbiological proteins, such as mycoproteins, has the potential to either entirely or partially complement proteins from animal sources such as meats[97]. The utilization of waste from agriculture and industries to produce mycoprotein has several advantages, particularly in terms of environmental impact[98]. Nutritious proteins provide enough crucial nutrients such as amino acids, dietary fiber, carotenoids, vitamins, and minerals. Additionally, nutritious proteins can be produced at a low overall cost, regardless of environmental conditions such as drought or flood and nature constraints[99]. Protein is a vital ingredient in our bodies. It is required for the development, restoration and preserve general good health. Protein is used to form bone, muscle, cartilage, and skin[100]. Meat is one of the most widely consumed protein sources globally, and the Need for meat is bound to rise by 76% by 2050 compared to 2005[14]. A sustainable and nutritious supply of food is required to feed the world's expanding population. Cellular agriculture, which utilizes different fruit and vegetable plant cell cultures to produce agricultural products, could enhance current production methods that rely on farmed animals or crops[18].

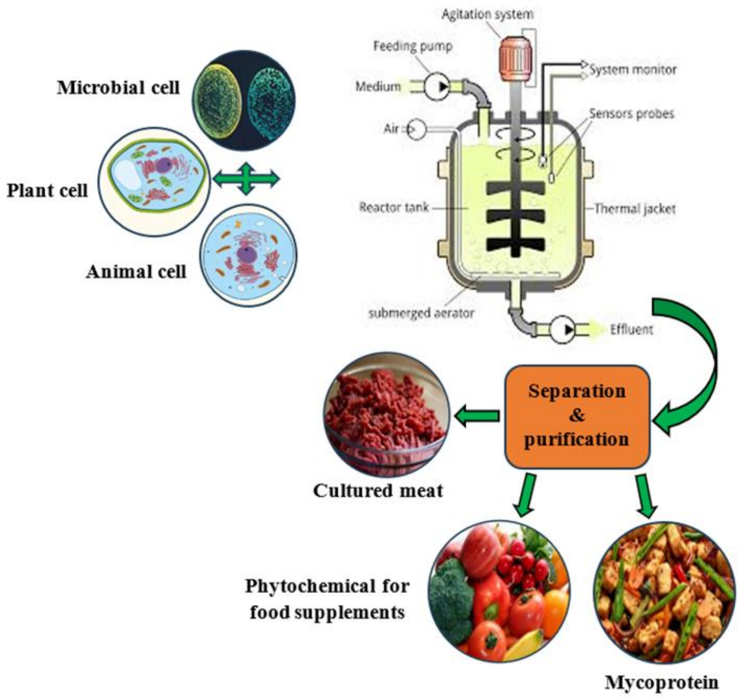

Figure. 3. Schematic representation of microbial, plant, and animal cell culture systems for biotechnological applications. The diagram illustrates key components such as agitation systems, reactor tanks, and monitoring probes used in cell culture processes. It also highlights downstream applications, including cultured meat production, phytochemical extraction for supplements, and mycoprotein synthesis.

Nutritional Safety

Cultured meat is expected to have a nutritional value comparable to meat produced from consumed livestock, with the added option of modifying its composition to suit specific dietary preferences or specialized diets via dietary supplementation, genetic engineering, and collaborative cultures[32,37,101]. Culturing techniques also offer the potential to produce antioxidants, vitamins, and oxidatively predictable substances[18]. Other components to consider in meat from animals include amino acids such as carnosine, anserine, creatine taurine, and hydroxyproline[102]. Taurine has been shown to benefit cardiac health and function. Hydroxyproline supports intestinal health. Carnosine and anserine are powerful antioxidants, while arginine has been shown to improve lean mass, physical strength, and cognitive function in older individuals[9]. Modifying the lipid profile, primarily by enhancing the levels of essential fatty acids such as linolenic acid through a combination of cultivation and adipocyte cell methods, remains a complication because the accumulation of essential fatty acids in ruminants is entirely diet-dependent. Genetic manipulation at the cellular level may enhance linolenic acid content. For example, transgenic swine that have inserted two genes from spinach, coding for the enzyme 12 linoleic acid desaturase, showed increased linolenic acid levels[103]. Regarding environmental and safety concerns, several aspects of current cell-based meat technology should be considered, including bovine serum, antibiotic or antibacterial agents, hormones and developmental process in the cultivation media, gene modification, and sterile packaging[32].

Agricultural Biotechnology and Food Security

Agricultural biotechnology is a field of science that employs modern techniques to enhance agricultural productivity and sustainability[13]. This technology is used to boost crop nutritional content, develop novel plant varieties that are resistant to pests and diseases, and improve agricultural practices. As the world's population continues to increase, agricultural biotechnology is becoming increasingly important in meeting the rising demand for food, while also addressing environmental issues and supporting sustainable agricultural methods. The availability of food can be enhanced by promoting environmentally conscious farming, emphasizing variation in crop cultivation, and contributing to reducing the effects of global warming[63,68]. The use of bioengineering techniques (Figure **3**), such as planting trees as wind barriers or fire barriers and growing a diverse range of plant species, can also help protect crops Worldwide nutritional stability includes supporting safety precautions for food and minimizing wasteful consumption and damages, which account for around one-third of total food production[18]. The loss after harvest is predominantly an issue in industrialized countries, as most consumers are unwilling to purchase cosmetically flawed fruit[80]. Furthermore, implementing governmental initiatives that target the fundamental causes of economic hardship and make food both inexpensive and available for poor populations will help minimize hunger. In the USA, the cost of assistance with food aid payments increases by two times if utilized to purchase products at market prices and farming stalls. These kinds of initiatives offer additional advantages of preserving agricultural producers' livelihoods while simultaneously helping meet consumer's nutritional needs[15,104].

Challenges and Future Direction

Food biofortification innovation has the potential to significantly reduce world hunger by increasing the nutritional value of staple crops. To attain wide-ranging and durable benefits, this field must overcome obstacles that call for targeted research, stricter laws, and creative approaches. It's challenging to biofortify crops without compromising their yield and growth. The health and productivity of the plant must not be harmed by the addition of micronutrients like iron or zinc. Furthermore, it may be difficult to create biofortified crops that are uniformly effective due to differences in nutrient absorption in fortified cultivars and genetic variability among staple crops. The acceptability of biofortified foods may be restricted by cultural preferences, and many consumers are not familiar with them. Biofortified crops need to taste and look like their non-fortified counterparts in order to be accepted by consumers. Public education campaigns regarding the health benefits of biofortified foods are necessary to debunk fallacies. GMO and fortified crop regulations vary by region and generally lag behind technological development. For instance, the adoption of biofortified crops has been considerably hampered in the European Union by strict laws on GMOs. On the other hand, nations with more lenient laws, such as the US and Brazil, enable quicker adoption. More research may be done on genetically engineered biofortified crops. Thus, aligning policies and establishing global standards may aid in their expansion.

It can be costly to create, distribute, and grow biofortified crops. Lack of funding and infrastructure may make it difficult to carry out biofortification initiatives in many developing countries. Inadequate resources make it difficult for smallholder farmers, who are essential to food security, to acquire and preserve biofortified seeds. Sustainability requires that biofortified crops be able to withstand environmental stresses including pests, salt, and drought. To ensure that they don't negatively impact ecosystems or soil health, more research is required. Farmers' and consumers' adoption of biofortified crops is significantly influenced by socioeconomic conditions. Smallholder farmers' adoption of these crops is hampered by a lack of funding and availability to biofortified seeds. Additionally, the success of biofortified crops depends on consumer awareness and market demand. Targeted advertising and community involvement can raise demand and acceptance in areas where malnutrition is common. For example, by interacting with farmers and local people, the Harvest Plus program has effectively promoted biofortified crops in many poor nations.

CRISPR-Cas9 technology for gene editing may enable greater accuracy in biofortification with fewer unexpected effects. These approaches have the potential to improve nutrient profiles while maintaining crop production and other desired traits. Combining conventional breeding with gene editing technology could help in the development of robust and nutrient-dense cultivars, potentially extending biofortification to new crops. Addressing various micronutrient shortages (e.g., Fe, Zn, and vitamin A) in a single crop may enhance health benefits. Maize, rice, and wheat are already being biofortified with many micronutrients. Developing crops to meet regional nutritional deficits depending on population demands might increase the impact of biofortification. Digital devices can monitor soil and crop nutrient levels, improving biofortification processes. Precision agriculture technologies, like soil nutrient monitors, may optimize fertilizer consumption and boost yields. Additionally, mobile apps and digital platforms may educate farmers and customers on the benefits of biofortified crops and offer insights into crop management. Governments, non-governmental organizations, academic institutes, and the commercial sector may all work together to scale up biofortification efforts. Nutritional outcomes have been shown to improve as a result of programs like Harvest Plus, which promotes biofortified crops worldwide. The market release of biofortified crops could be accelerated and approval procedures shortened by developing a unified regulatory framework for biofortified commodities. It will be crucial to combine drought-tolerant crops with biofortification in climate-sensitive regions. Research on crop types that can withstand severe weather conditions and yet provide vital nutrients is becoming more and more significant. "Neglected" crops like sorghum and millets, which are naturally resilient and nutrient-dense and offer a dual benefit in terms of nutrition and climate adaptation, are the focus of several biofortification initiatives. Involving communities in the creation and promotion of biofortified crops guarantees that the cultivars reflect regional preferences and tastes. Including local leaders can assist boost adoption and acceptance rates, especially in rural areas. Cultural shifts like adding biofortified crops to traditional dishes could make these foods more enticing and promote frequent intake.

CONCLUSION

Food biofortification has significant potential for reducing global hunger by boosting the nutritional density of basic crops. Overcoming consumer acceptability, regulatory barriers, economic constraints, and environmental concerns necessitates a multidimensional approach that combines innovative technology, legislative reform, and community-based initiatives. Biofortification projects, by continuing to develop and collaborate, may play a critical role in delivering sustainable, nutrient-rich food supplies to needy communities across the world.

ACKNOWLEDGMENT

We want to express our gratitude to the Government College University, Faisalabad, Pakistan for providing the necessary resources and support throughout the preparation of this review paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Farah AM, Gebremedhin S, Rago B, Kebede A, Barsenga K, Chiriga M, *et al*. Effective coverage of management of wasting in ethiopia. MedRxiv. 2024; 2024.
2. Mutunga M, Chiwile F, dos R. de Araujo Moniz N, Bahwere P. Improving case-detection of severe wasting among under-five-year-old children in Timor Leste: A secondary analysis of data from the 2020 national cross-sectional food and nutrition survey. PLoS One. 2024; 19: e0308208.
3. Kozuki N, Seni M, Sirat A, Abdullahi O, Adalbert MFE, Biotteau M, *et al*. Adapting acute malnutrition treatment protocols in emergency contexts: a qualitative study of national decision-making. Confl Health. 2020; 14:1–12.
4. Jackson A, Ashworth A, Annan RA. The International Malnutrition Task Force: a model for the future?. Trends Food Sci Technol. 2022; 130:11-19.
5. Cazes C, Phelan K, Hubert V, Boubacar H, Bozama LI, Sakubu GT, *et al*. Simplifying and optimizing the management of uncomplicated acute malnutrition in children aged 6–59 months in the Democratic Republic of the Congo (OptiMA-DRC): a non-inferiority, randomised controlled trial. Lancet Glob Health. 2022; 10:e510-e520.
6. Bailey J, Opondo C, Lelijveld N, Marron B, Onyo P, Musyoki EN, *et al*. A simplified, combined protocol versus standard treatment for acute malnutrition in children 6-59 months (ComPAS trial): A cluster-randomized controlled non-inferiority trial in Kenya and South Sudan. PLoS Med. 2020; 17: e1003192.
7. Dalglish SL, Seni Badou M, Sirat A, Abdullahi O, Adalbert MFE, Biotteau M, *et al*. Combined protocol for severe and moderate acute malnutrition in emergencies: Stakeholder perspectives in four countries. Matern Child Nutr. 2020; 16:e12920.
8. Atukunda P, Eide WB, Kardel KR, Iversen PO, Westerberg AC. Unlocking the potential for achievement of the UN Sustainable Development Goal 2'Zero Hunger' in Africa: targets, strategies, synergies and challenges. Food Nutr Res. 2021; 65:10-29219.
9. Chen X, Shuai C, Wu Y. Global food stability and its socio-economic determinants towards sustainable development goal 2 (Zero Hunger). Sustain Devel. 2023; 31:1768–1780.
10. Arora NK, Mishra I. Current scenario and future directions for sustainable development goal 2: A roadmap to zero hunger. Environ Sustain. 2022; 5:129-133.
11. Darnton-Hill I, Mkparu UC. Micronutrients in pregnancy in low-and middle-income countries. Nut. 2015; 7:1744-1768.
12. Keats EC, Oh C, Chau T, Khalifa DS, Imdad A, Bhutta ZA. Effects of vitamin and mineral supplementation during pregnancy on maternal, birth, child health and development outcomes in low-and middle-income countries: A systematic review. Campbell Syst Rev. 2021; 17:e1127.
13. Salam RA, Das JK, Bhutta ZA. Multiple micronutrient supplementation during pregnancy and lactation in low-to-middle-income developing country settings: impact on pregnancy outcomes. Ann Nutr Metab. 2014; 65:4–12.
14. Khan R, Brishti FH, Arulrajah B, Goh YM, Abd Rahim MH, Karim R, *et al*. Mycoprotein as a meat substitute: production, functional properties, and current challenges-a review. Int J Food Sci Technol. 2024; 59:522-544.
15. Uccello E, Kauffmann D, Calo M, Streissel M. Nutrition-sensitive agriculture and food systems in practice. Unspecified J. 2017.
16. Hunter MC, Smith RG, Schipanski ME, Atwood LW, Mortensen DA. Agriculture in 2050: recalibrating targets for sustainable intensification. Biosci. 2017; 67:386–391.
17. Tilman D, Balzer C, Hill J, Befort BL. Global food demand and the sustainable intensification of agriculture. Proc Nat Acad Sci. 2011; 108:20260-20264.
18. Alioma R, Zeller M, Ling YK. Analysis of long-term prices of micronutrient-dense and starchy staple foods in developing countries. Agr Food Econ. 2022; 10:24.
19. Erokhin V, Diao L, Gao T, Andrei JV, Ivolga A, Zong Y. The supply of calories, proteins, and fats in low-income countries: a four-decade retrospective study. Int J Environ Res Public Health. 2021; 18:7356.
20. Gomez-Zavaglia A, Mejuto JC, Simal-Gandara J. Mitigation of emerging implications of climate change on food production systems. Food Res Intl. 2020; 134:109256.
21. Timsina J. Can organic sources of nutrients increase crop yields to meet global food demand? Agron. 2018; 8:214.
22. Avnee, Sood S, Chaudhary DR, Jhorar P, Rana RS. Biofortification: an approach to eradicate micronutrient deficiency. Front Nutr. 2023; 10:1233070.
23. Dhaliwal SS, Sharma V, Shukla AK, Verma V, Kaur M, Shivay YS, *et al*. Biofortification-A frontier novel

approach to enrich micronutrients in field crops to encounter the nutritional security. Mol. 2022; 27:1340.

24. Rai D, Chaudhary C, Khatak A, Banyal S. A Sustainable approach to combat micronutrient deficiencies and ensure global food security through biofortification. Eur. J. Nutr. Food Saf. 2024; 16:15–30.
25. Saltzman A, Birol E, Oparinde A, Andersson MS, Asare-Marfo D, Diressie MT, *et al*. Availability, production, and consumption of crops biofortified by plant breeding: current evidence and future potential. Ann N Y Acad Sci. 2017; 1390:104-114.
26. Bouis HE. Biofortification: an agricultural tool to address mineral and vitamin deficiencies. In: Food Fortification in a Globalized World. Elsevier, 2018: pp. 69–81.
27. Monika G, Kim SRM, Kumar PS, Gayathri KV, Rangasamy G, Saravanan A. Biofortification: A long-term solution to improve global health-a review. Chemosphere. 2023; 314:137713.
28. Srivastav P, Vutukuru M, Ravindran G, Awad MM. Biofortification-present scenario, possibilities and challenges: a scient metric approach. Sustain. 2022; 14:1632.
29. Mishra GP, Dikshit HK, Priti, Kukreja B, Aski M, Yadava DK, *et al*. Historical overview of biofortification in crop plants and its implications. In: Biofortification of Staple Crops. Springer, 2022: pp. 31-61.
30. Kiran A, Wakeel A, Mahmood K, Mubaraka R, Hafsa, Haefele SM. Biofortification of staple crops to alleviate human malnutrition: contributions and potential in developing countries. Agron. 2022; 12:452.
31. Bachewe F, Genye T, Girma M, Samuel A, Warner J, van Zyl C. Biofortification in Ethiopia: opportunities and challenges. Food Nutr Bull. 2023; 44:151-161.
32. Lee H. The status of Uganda's food-based Vitamin A deficiency mitigation strategies. Afr J Food, Agr Nutr Devel. 2022; 22:19929-19944.
33. Mudadirwa D, Ndhlela T, Olakojo SA, Gonhi T. The potential of provitamin A maize (Zea mays L.) biofortification in sub-Saharan Africa: Current status, opportunities, challenges and perspectives: A review. CABI Reviews. 2023.
34. Talsma EF, Borgonjen-van den Berg KJ, Melse-Boonstra A, Mayer EV, Verhoef H, Demir AY, *et al*. The potential contribution of yellow cassava to dietary nutrient adequacy of primary-school children in Eastern Kenya; the use of linear programming. Public Health Nutr. 2018; 21:365-376.
35. Natekar P, Deshmukh C, Limaye D, Ramanathan V, Pawar A. A micro review of a nutritional public health challenge: iron deficiency anemia in India. Clin Epidemiol Glob Health. 2022; 14:100992.
36. Shaheen C, Ahmad IA, Aslam R, Naz S, Mushtaq S, Ahmed S, *et al*. A review of therapeutic and medicinal uses of Fenugreek (Trigonella foenum-graceum L.). J Res Appl Sci Biotechnol. 2024; 3:39–50.
37. Huey SL, Krisher JT, Bhargava A, Friesen VM, Konieczynski EM, Mbuya MNN, *et al*. Review of the impact pathways of biofortified foods and food products. Nutrients. 2022; 14:1200.
38. Giuliano G. Provitamin A biofortification of crop plants: a gold rush with many miners. Curr Opin Biotechnol. 2017; 44:169–180.
39. Onwuchekwa O, Ene CO, Ebenyi LN, Frederick OO, Okpuru IG, Nwankwo SE. Comparative assessment for nutritional and antinutritional qualities revealed better performance of traditional white-fleshed sweet potatoes than orange-fleshed sweet potatoes. Acta Agric Slov. 2024; 120:1-17.
40. Gilligan DO, Kumar N, McNiven S, Meenakshi JV, Quisumbing A. Bargaining power, decision making, and biofortification: The role of gender in adoption of orange sweet potato in Uganda. Food Policy. 2020; 95:101909.
41. Muchiri MN, McCartney AL, Methven L. Sensory profile and consumer preference of novel probiotic yoghurt enriched with orange sweet potato (Ipomoea batatas). Afr J Food, Agr, Nutr Devel. 2020; 20:16471-16489.
42. Gupta PK, Balyan HS, Sharma S, Kumar R. Biofortification and bioavailability of Zn, Fe and Se in wheat: present status and future prospects. Theo Appl Genet. 2021; 134:1-35.
43. Akram MA, Depar N, Irfan M. Agronomic zinc biofortification of wheat to improve accumulation, bioavailability, productivity and use efficiency. Eur J Soil Sci. 2020; 9:75-84.
44. Knez M, Pantovic A, Tako E, Boy E. FADS1 and FADS2 as biomarkers of Zn status–a systematic review and meta-analysis. Crit Rev Food Sci Nutr. 2024; 64:3187-3205.
45. Schmidt W, Thomine S, Buckhout TJ. Iron nutrition and interactions in plants. Front Plant Sci. 2020; 10:1670.
46. Kumar A, Sheik AG, Bux F, Kumari S. Machine learning and artificial intelligence application in automotive water quality monitoring, analysis, and management. Comput Automation Water Sec. 2025; 89-109.
47. Awuchi CG, Igwe VS, Amagwula IO, Echeta CK. Health benefits of micronutrients (vitamins and minerals) and their associated deficiency diseases: A systematic review. Intl J Food Sci. 2020; 3:1-32.
48. Sarraf M, Janeeshma E, Arif N, Farooqi MUQ, Kumar V, Ansari NA, *et al*. Understanding the role of beneficial elements in developing plant stress resilience: Signalling and crosstalk with phytohormones and microbes. Plant Stress. 2023; 10:100224.

49. Kaur H, Kaur H, Kaur H, Srivastava S. The beneficial roles of trace and ultratrace elements in plants. Plant Growth Regul. 2023; 100:219-236.
50. Bouis H, Low J, Zeigler R. Delivering biofortified crops in developing countries. In: Sustaining Global Food Security: The Nexus of Sci Policy. 2019; 82-96.
51. Igbaifua WE, Adeniyi OR, Omolehin RA. Comparative analysis of technical, allocative and economic efficiencies of vitamin a cassava farmer with other improved cassava farmers in Oyo state, Nigeria. J Res Agr Ani Sci. 2021; 8:60-73.
52. Saleem A, Anwar S, Nawaz T, Fahad S, Saud S, Rahman TU, *et al*. Securing a sustainable future: the climate change threat to agriculture, food security, and sustainable development goals. J Umm Al-Qura Uni Appl Sci. 2024; 1–17.
53. Spielman DJ, Gatto M, Wossen T, McEwan M, Abdoulaye T, Maredia MK, *et al*. Regulatory options to improve seed systems for vegetatively propagated crops in developing countries. Intl Food Policy Res Inst. 2021.
54. Joshi AK, Braun HJ. Seed systems to support rapid adoption of improved varieties in wheat. In: Wheat improvement: food security in a changing climate. Springer Intl Pub Cham. 2022; 237-256.
55. Ter Steeg EMS, Struik PC, Visser RGF, Lindhout P. Crucial factors for the feasibility of commercial hybrid breeding in food crops. Nat Plants. 2022; 8:463–473.
56. Saxena KB, Sharma D, Vales MI. Development and commercialization of CMS pigeonpea hybrids. Plant Breed Rev. 2018; 41:103-167.
57. Pissinati A, Moreira A, Santoro PH. Biomass yield and nutrients concentration in shoot dry weight of winter cover crops for no-tillage system. Commun Soil Sci Plant Anal. 2016; 47:2292–2305.
58. Bagale S. Nutrient management for soybean crops. Intl J Agron. 2021; 2021:3304634.
59. Torabian S, Farhangi-Abriz S, Qin R, Noulas C, Sathuvalli V, Charlton B, *et al*. Potassium: A vital macronutrient in potato production-A review. Agron. 2021; 11:543.
60. Levine CP, Mattson NS. Potassium-deficient nutrient solution affects the yield, morphology, and tissue mineral elements for hydroponic baby leaf spinach (Spinacia oleracea L.). Horticulturae. 2021; 7:213.
61. Ofori KF, Antoniello S, English MM, Aryee ANA. Improving nutrition through biofortification–A systematic review. Front Nutr. 2022; 9:1043655.
62. Sandhu R, Chaudhary N, Shams R, Singh K, Pandey VK. A critical review on integrating biofortification in crops for sustainable agricultural development and nutritional security. J Agric Food Res. 2023; 14:100830.
63. Bhardwaj AK, Chejara S, Malik K, Kumar R, Kumar A, Yadav RK. Agronomic biofortification of food crops: An emerging opportunity for global food and nutritional security. Front Plant Sci. 2022; 13:1055278.
64. Wahab A, Muhammad M, Munir A, Abdi G, Zaman W, Ayaz A, *et al*. Role of arbuscular mycorrhizal fungi in regulating growth, enhancing productivity, and potentially influencing ecosystems under abiotic and biotic stresses. Plants. 2023; 12:3102.
65. Jaiswal DK, Krishna R, Chouhan GK, Pereira AP de Araujo, Ade AB, Prakash S, *et al*. Bio-fortification of minerals in crops: current scenario and future prospects for sustainable agriculture and human health. Plant Growth Regul. 2022; 98:5–22.
66. Singh SK, Barman M, Sil A, Prasad JP, Kundu S, Bahuguna RN. Nutrient biofortification in wheat: opportunities and challenges. Cereal Res Commun. 2023; 51:15–28.
67. Sharma D, Ghimire P, Bhattarai S, Adhikari U, Khanal S, Poudel PB. Biofortification of wheat: Genetic and agronomic approaches and strategies to combat iron and zinc deficiency. Intl J Environ Agr Biotechnol. 2020; 5.
68. Shahane AA, Shivay YS. Agronomic biofortification of crops: Current research status and future needs. Ind J Fertilizers. 2022; 18:164-179.
69. Stangoulis JCR, Knez M. Biofortification of major crop plants with iron and zinc-achievements and future directions. Plant Soil. 2022; 474:57-76.
70. Shahzad R, Jamil S, Ahmad S, Nisar A, Khan S, Amina Z, *et al*. Biofortification of cereals and pulses using new breeding techniques: current and future perspectives. Front Nutr. 2021; 8:721728.
71. Gaikwad KB, Rani S, Kumar M, Gupta V, Babu PH, Bainsla NK, *et al*. Enhancing the nutritional quality of major food crops through conventional and genomics-assisted breeding. Front Nutr. 2020; 7:533453.
72. Duraiswamy A, Sneha A NM, Jebakani KS, Selvaraj S, Pramitha JL, Selvaraj R, *et al*. Genetic manipulation of anti-nutritional factors in major crops for a sustainable diet in future. Front Plant Sci. 2023; 13:1070398.
73. Mandal S, Gupta SK, Ghorai M, Patil MT, Biswas P, Kumar M, *et al*. Retracted Article: Plant nutrient dynamics: a growing appreciation for the roles of micronutrients. Plant Growth Regul. 2023; 100:435-452.
74. Khaleeq K, Ashna S, Ehsan Q, Rathore SS, Ahmadi A, Samim M, *et al*. Optimization of crop establishment methods and phosphorus fertilizer levels on growth and economic efficiency of groundnut under semiarid region of afghanistan. J Res Appl Sci Biotechnol. 2024; 3:46-51.

75. Shoeb E, Venkataraman S, Badar U, Hefferon K. Genome editing for crop biofortification. In: Applications Genome Eng Plants. 2024; 91-121.
76. Medina-Lozano I, Díaz A. Applications of genomic tools in plant breeding: crop biofortification. Int J Mol Sci. 2022; 23:3086.
77. Amna, Qamar S, Tantray AY, Bashir SS, Zaid A, Wani SH. Golden rice: genetic engineering, promises, present status and future prospects. In: Rice Research for Quality Improvement: Genomics and Genetic Engineering: Volume 2: Nut Biofort Herbicide Biotic Stress Resist Rice. 2020; 581-604.
78. Janghel DK, Sharma V. Genomics-assisted breeding approaches in lentil (Lens culinaris medik). In: Technologies in Plant Biotechnology and Breeding of Field Crops. Springer, 2022; 201-237.
79. Wang H, Jia J, Cai Z, Duan M, Jiang Z, Xia Q, *et al*. Identification of quantitative trait loci (QTLs) and candidate genes of seed Iron and zinc content in soybean [Glycine max (L.) Merr. BMC Genom. 2022; 23:146.
80. Baiyeri SO, Samuel-Baiyeri CCA, Jandong EA, Otitoju GTO. Evaluation of micronutrients, bioavailability micronutrients and antinutrients in african yam bean (Sphenostylis stenocarpa) Seeds. J Home Econ Res. 2023; 30:85-96.
81. Sathee L, Antoo S, Balamurugan B, Vadakkethil AA, Prabhakar GD, Arpitha S, *et al*. 9 CRISPR-Based Genome Editing. In: CRISPR and Plant Funct Genom. 2024; 144.
82. Welsch R, Li L. Golden Rice-lessons learned for inspiring future metabolic engineering strategies and synthetic biology solutions. Methods Enzymol. 2022; 671:1–29.
83. Kumari K. Adoption of golden rice: a boon for the children suffering from vitamin A deficiency. J Adv Sci Res. 2024; 15:6-10.
84. Kobayashi K, Wang X, Wang W. Genetically modified rice is associated with hunger, health, and climate resilience. Foods. 2023; 12:2776.
85. Yadav V, Pal D, Poonia AK. A study on genetically engineered foods: need, benefits, risk, and current knowledge. Cell Biochem Biophys. 2024; 82:1931–1946.
86. Gao C. Genome engineering for crop improvement and future agriculture. Cell. 2021; 184:1621-1635.
87. Belousova ME, Malovichko YV, Shikov AE, Nizhnikov AA, Antonets KS. Dissecting the environmental consequences of Bacillus thuringiensis application for natural ecosystems. Toxins (Basel). 2021; 13:355.
88. Sorokan A, Gabdrakhmanova V, Kuramshina Z, Khairullin R, Maksimov I. Plant-associated Bacillus thuringiensis and Bacillus cereus: inside agents for biocontrol and genetic recombination in phytomicrobiome. Plants. 2023; 12:4037.
89. Scott GY, Worku D. HIV vaccination: Navigating the path to a transformative breakthrough-A review of current evidence. Health Sci Rep. 2024; 7:e70089.
90. Hadi J, Brightwell G. Safety of alternative proteins: Technological, environmental and regulatory aspects of cultured meat, plant-based meat, insect protein and single-cell protein. Foods. 2021; 10:1226.
91. Barzee TJ, El Mashad HM, Cao L, Chio A, Pan Z, Zhang R. Cell-cultivated food production and processing: A review. Food Bioeng. 2022; 1:4-25.
92. Benny A, Pandi K, Upadhyay R. Techniques, challenges and future prospects for cell-based meat. Food Sci Biotechnol. 2022; 31:1225–1242.
93. Kirsch M, Morales-Dalmau J, Lavrentieva A. Cultivated meat manufacturing: Technology, trends, and challenges. Eng Life Sci. 2023; 23:e2300227.
94. Tubb C, Seba T. Rethinking food and agriculture 2020-2030: the second domestication of plants and animals, the disruption of the cow, and the collapse of industrial livestock farming. Indus Biotechnol. 2021; 17:57-72.
95. Chodkowska KA, Wódz K, Wojciechowski J. Sustainable future protein foods: the challenges and the future of cultivated meat. Foods. 2022; 11:4008.
96. da Silva BD, Conte-Junior CA. Perspectives on cultured meat in countries with economies dependent on animal production: a review of potential challenges and opportunities. Trends Food Sci Technol. 2024; 104551.
97. Molfetta M, Morais EG, Barreira L, Bruno GL, Porcelli F, Dugat-Bony E, *et al*. Protein sources alternative to meat: state of the art and involvement of fermentation. Foods. 2022; 11:2065.
98. Kanwal R, Haq MHU, Waseem A, Riaz T, Rehman ZU, Fazal A, *et al*. Fungitoxic properties of essential oils to treat tinea. In: complementary and alternative medicine: essential oils. Unique Scientific Publishers, Faisalabad, Pakistan, 2024; 81-89.
99. Huling R. Applications of fungi for alternative protein. In: Fungi and fungal products in human welfare and biotechnology. Springer, 2023; 365-395.
100. Coelho MOC, Monteyne AJ, Dunlop V M, Harris HC, Morrison DJ, Stephens FB, *et al*. Mycoprotein as a possible alternative source of dietary protein to support muscle and metabolic health. Nutr Rev. 2020; 78:486-497.
101. Snapp S, Sapkota TB, Chamberlin J, Cox CM, Gameda S, Jat ML, *et al*. Spatially differentiated nitrogen supply is key in a global food–fertilizer price crisis. Nat Sustain. 2023; 6:1268-1278.
102. Virk PS, Andersson MS, Arcos J, Govindaraj M, Pfeiffer WH. Transition from targeted breeding to mainstreaming of biofortification traits in crop

improvement programs. Front Plant Sci. 2021; 12:703990.

103. SUN B, Hong Z, Lü Y, Fei LU, WANG X. The effects of nitrogen fertilizer application on methane and nitrous oxide emission/uptake in Chinese croplands. J Integr Agric. 2016; 15:440-450.

104. Díaz-Gómez J, Twyman RM, Zhu C, Farré G, Serrano JCE, *et al*. Biofortification of crops with nutrients: factors affecting utilization and storage. Curr Opin Biotechnol. 2017; 44:115-123.