



Review article

Prioritizing strategies for wheat biofortification: Inspiration from underutilized species

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ABSTRACT

The relationship between malnutrition and climate change is still poorly understood but a comprehensive knowledge of their interactions is needed to address the global public health agenda. Limited studies have been conducted to propose robust and economic-friendly strategies to augment the food basket with underutilized species and biofortify the staples for nutritional security. Sea-buckthorn is a known “superfood” rich in vitamin C and iron content. It is found naturally in northern hemispherical temperate Eurasia and can be utilized as a model species for genetic biofortification in cash crops like wheat. This review focuses on the impacts of climate change on inorganic (iron, zinc) and organic (vitamin C) micronutrient malnutrition employing wheat as highly domesticated crop and processed food commodity. As iron and zinc are particularly stored in the outer aleurone and endosperm layers, they are prone to processing losses. Moreover, only 5% Fe and 25% Zn are bioavailable once consumed calling to enhance the bioavailability of these micronutrients. Vitamin C converts non-available iron (Fe^{3+}) to available form (Fe^{2+}) and helps in the synthesis of ferritin while protecting it from degradation at the same time. Similarly, reduced phytic acid content also enhances its bioavailability. This relation urges scientists to look for a common mechanism and genes underlying biosynthesis of vitamin C and uptake of Fe/Zn to biofortify these micronutrients concurrently. The study proposes to scale up the biofortification breeding strategies by focusing on all dimensions i.e., increasing micronutrient content and boosters (vitamin C) and simultaneously reducing anti-nutritional compounds (phytic acid). Mutually, this review identified that genes from the Aldo-keto reductase family are involved both in Fe/Zn uptake and vitamin C biosynthesis and can potentially be targeted for genetic biofortification in crop plants.

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1. Introduction

Food security is defined in four dimensions: availability, accessibility, utilization, and stability. Although climate change threatens food security in all dimensions, the most vulnerable is food availability related to its production system [1]. All four dimensions are governed by climate regimes that have been changed in the last couple of decades. It is anticipated that by 2050, the hunger rate will increase by 20% and most of the child deaths occurring due to consequences of climate change will be driven by malnutrition [2]. The impacts of climate change have pushed more than 33 countries into the “high-risk” class of malnutrition. Since 1990s, climate shocks have doubled especially for countries that are already vulnerable to climate change like Pakistan [3]. Such countries are more likely to face intensified food insecurity and malnutrition if they fail to act for climate change mitigation and adaptation [2].

Climate change is a long-term process and potential threat to global food security and nutrition [4]. Indeed, crop productivity and nutrient availability are strongly tied to CO₂ concentration through photosynthesis and CO₂ fixation rate [5]. Researchers have used free-air CO₂ enrichment (FACE) technology to assess plant growth and nutrients by manipulating CO₂ concentrations. Several FACE experiments found decreased nutrient and biological molecule contents (especially iron, zinc, and proteins) associated with elevated CO₂ concentration in important food crops like rice [4], wheat [6,7], maize [4], sorghum [8], barley [9], soybean [8], and peas [4]. Basically, nutrients are up-taken by the roots through sugar-sensing mechanisms mediated by photosynthesis [10]. How the CO₂ increase affects root function for nutrient uptake is not entirely known, but it very likely affects the acquisition of soil nutrients [11,12]. A study reports that CO₂ may reduce nutrient allocation due to dilution effects caused by enhanced carbohydrate production [13]. As plant nutrients are translocated via either diffusion or mass flow, two theories are proposed for the mechanism underlying this dilution effect [13]. The reduced stomatal conductance and transpiration under elevated CO₂ ultimately either reduce mass flow of nutrients [12] or alter the allocation of nutrients and proteins in the sink due to negative nutrient-protein association [14]. For instance, the content of 17 nutrients decreased due to one or the other mechanism caused by the elevated CO₂ in wheat [13]. Thus, cascading effects of nutrients depletion in staple crops will likely have serious implications on human health.

However, not only negative but also positive impacts of climate change are projected and already visible. Among those are for instance increased food production via a CO₂ fertilization effect (>550 ppm), decreased low-temperature damage (an increase by 1.8 °C temperature), and yield increase due to a prolonged growth period by 20% in 2021, especially for C3 crops like wheat [15] and rice [16]. A simulated study in northern Germany reported yield enhancement for ten crops including wheat due to CO₂ fertilization [17]. On the other hand, climate change could adversely affect wheat yields in areas where Sub-optimum growth conditions already prevail and where the frequency of rainfall and high temperature events is intensified as recently witnessed in many South-East Asian countries like India and Pakistan [18]. Being a C3 plant, elevated levels of CO₂ increase photosynthesis and decrease transpiration rate, while additional rise in temperature levels counterweights these phenomena [19], leading to decline in grain protein content [15,20], nutrient content (for instance iron by 4–10% [21]), and grain yield (−7.5% per 1 °C warming [22]). Moreover, increased CO₂ demands more nutrients to balance the plant's physiological response, thus the CO₂ fertilization effect will be neutralised due to plant nutrient deficits [23,24].

Modern technologies, popularly known to have emerged and promoted under the Green Revolution (GR) agenda, may also be cause of nutrients dilution [25]. The introduction of input-responsive, short-stature wheat and rice cultivars owing to the GR has left us with few choices of crops. As a result, the diverse food sources have become expensive and hidden hunger has risen due to consumptions of more available and affordable grain crops [14]. Moreover, the high yielding crop varieties developed through conventional breeding programs were raised under high input systems focusing on yield but marginalising nutrient contents [26]. Overall, although calorie intake increased with GR-driven varieties, dietary diversity and nutrient intake (such as Fe, Zn, Vit A and C) decreased as the monoculture (single species, variety or hybrid) cropping system was preferred [27]. For example, the introduction of Rht-dwarfing genes revolutionised grain yields, but reduced Fe and Zn contents by 3.2 ppm and 3.9 ppm, respectively, along with reduction in many other minerals [28–30] such as magnesium (−94 ppm) and manganese (−6 ppm) [29]. The percentage of modern varieties carrying these Rht genes exceeds 90% [31]. Moreover, the conventional crop production techniques cause nutrient dilution due to high water content as compared to the organic fertilization techniques, which may enhance ascorbic acid (AA), nutrient levels, and protein content [32].

Malnutrition involves both insufficiency and excess of nutrients being termed as overnutrition and undernutrition. In case of micronutrient malnutrition, also known as hidden hunger, more than two billion people are affected globally [33]. It is caused by the deficiency of essential vitamins (e.g., vitamins A, B and C) and minerals e.g., iron (Fe) and zinc (Zn). Diseases like diarrhoea, malaria, measles, and pneumonia, commonly spread in developing countries, are also caused by micronutrient deficiency. The consequences of hidden hunger are long-lasting and sometimes known to pass on through generations [34].

However, important nutrients like Fe and Zn, even though sufficiently present, may not be available [35]. The bioavailability of micronutrients largely depends on the antinutritional compounds mainly phytic acid (PA), polyphenols, and the content of enhancer molecules like vitamin C. Phytic acid, the primary P-storage molecule (50–85% of P stored in plants) frequently present in generative organs, reduces the bioavailability of the bivalent and trivalent minerals by 5–15% through a chelation mechanism [36]. Safe levels of PA reduction during biofortification breeding in cereals are debatable. On the other hand, vitamin C (L-ascorbic acid) is an excellent enhancer for iron absorption [37]. It reduces the ferric to ferrous iron that is bound in the form of absorbable chelate and does not react with inhibitors like phytate [38]. It also inhibits synthesis of carcinogenic nitrosamines [39]. Additionally, AA is considered an essential co-factor only for a few vertebrates like humans, bats, and birds that have lost the ability to synthesize AA due to mutations in the last enzymatic pathway, i.e. L-gulonolactone oxidase [40]. Moreover, the pattern of food (based on vegetables and fruits) reduced the selection pressure to keep the pathway functional [41]. Hence, the aforementioned taxa depend on the plant-based

vitamin C for their metabolism. Although the vitamin C supplements are abundantly available, it has been revealed that food-based vitamin C is more readily available [42]. Thus, it should be prioritized for biofortification breeding programs.

The options for improved nutrition are however limited (Fig. 1). A promising approach, might be to employ the neglected and underutilized species (NUS) that are climate resilient, nutrient dense, environmentally and locally viable [43] to gain insights for enhanced nutrition in staple crops. For instance, NUS have been potentially utilized to biofortify staples for micronutrients in Africa: maize with amaranth, moringa with cowpea, cassava with yam bean, and pigeon pea with bread fruit [44]. NUS have also been utilized as frontline food to combat health problems with nutrigenomic studies – an approach to understand nutritional science through compartmentalization of genes using applied genomics [45]. Similarly, sea-buckthorn is an excellent source of vitamin C (200–1500 mg 100 g⁻¹: 15 times higher than orange fruit [46], anthocyanin (0.5–25 mg l⁻¹) and 24 minerals including calcium, iron, magnesium, phosphorus, potassium [47], and zinc [48]. Thus, such plants provide a great potential for common underlying mechanisms to upscale biofortification breeding efforts and thus enhance nutrient contents.

In this review, several approaches are discussed that can be used solely or in combination to enhance micro-nutrient contents and their bioavailability in crops. For this, wheat (*Triticum aestivum* L.), a staple crop with varying quantities of nutrients [30] but low caryopsis-vitamin C contents [49], will be discussed as model species among cereals in comparison to the nutrient-and vitamin C-rich [50] but underutilized species sea-buckthorn (*Hippophae rhamnoides* L.) [51]. Similarities of biochemical pathways and underlying genes responsible for the translocation and absorption of Fe and Zn and the biosynthesis of AA will be mined to bridge the gap among them. The vitamins- and minerals-rich NUS may be an asset to conduct such studies, while this information may then be translated to upscale the biofortification breeding of staple cereals, available for a larger human population. This paper thus hypothesizes that (i) the bioavailability of micronutrients can be enhanced by increasing ascorbic acid and reduced phytic acid content (ii), some common genes are involved in the biosynthesis of AA and micronutrient uptake, and (iii) the simultaneous biofortification of these nutrients can be a targeted approach for the biofortification of staple crops like wheat.

2. Methods

A systematic review on prioritizing strategies for iron, zinc, and ascorbic acid biofortification in wheat was conducted by utilizing NUS as an inspiration for nutrigenomics following the PRISMA methodology [52]. As the review covers three broad topics, literature was searched individually (Fig. S1). Comprehensive figures were generated using information from different research and review studies presented in the manuscript. Information on genes and mechanisms underlying iron and zinc translocation and vitamin C biosynthesis were compiled from different sources. Data were compared in tabular form and a common gene family was identified in all three nutrient/co-factor pathways. A detailed keywords list can be found in the [Supplementary File 1](#).

2.1. Identification of relevant records

The review articles and peer-reviewed research articles from 1982 to July 2023 published in the English language were browsed and identified from different electronic databases including PubMed, HEC Digital Library, Science Direct, Web of Science, JSTOR, Scopus, BioMed Central, and CAB Direct. The identified articles were screened, duplicates removed, and transferred to EndNote v. X7 (Thomson Reuters, New York City, US).

Further, key and seed articles were identified for specific search in [inciteful.com](#) and [connectedpapers.com](#). The included studies met following eligibility criteria: (i) case studies, opinion papers, and letters to editor were removed; (ii) studies in the domain of medical science were excluded except of studies concerning recommended dietary allowance and disease burden statistics; (iii) studies narrowly targeting a geographical region without global information were excluded; (iv) only peer-reviewed articles in English language with specific outcome or problem-orientation rather than narrative literature were selected. At least one of the outcomes of the study was related to malnutrition, iron and zinc uptakes as well as AA biosynthesis, and/or tapping potential of underutilized species to combat malnutrition; (v) reports were included in case of defining specific terms like “food security” and “malnutrition” or statistical data (FAOstat, for instance).

2.2. Criteria of selection

After applying the five eligibility criteria, three reviewers (one from agronomic and climate change background, second from breeding and genetics background and third from biodiversity conservation and utilization background) screened the articles at two stages. First, the articles were selected on title and abstract basis, then a full-text scanning was done to identify most related articles. Opposite opinions among reviewers during the screening process were reached to a consensus by another reviewer (with general agricultural background) and a third-party reviewer was consulted at the end.

2.3. Data extraction from included articles

Following the articles selection, the data for specific topics of predefined outline of review were recorded in a spreadsheet. Data were validated by two authors and another author cross-checked for quality appraisal.

3. Global prevalence of iron, zinc and vitamin C deficiency: causes and effects

The dietary habits of present-day people are less diverse compared to 30 years ago resulting in a deficiency of vitamin C [92], Fe, Zn, selenium, and iodine [93]. Leafy vegetables and fruits like guava, orange, and papaya are rich sources of carotenoids and vitamins while cereals and legumes are important for iron and zinc intake [94]. Thus, any factor limiting the availability of these crops can lead to greater vulnerability of micronutrient malnutrition. These vulnerabilities might occur when global food prices increase, limiting ultimately their availability and accessibility [95], but also when elevations of CO₂ levels reduce the zinc content by 9% and iron content by 5–7% in wheat grains compared to the normal CO₂ levels [4].

Wheat is one of the major staple food crops being consumed by 30% of the world population and accounts for 20% of the global overall calories [96]. It constitutes a large part of daily intake exceeding 70% in rural areas [14]. Selective breeding has decreased nutrients content due to dilution effects [97]. Most of the Fe and Zn are stored in the aleurone layer of the grain that is lost during milling [98]. The Fe stored in protein storage vacuoles [99] of these tissues is bound to phytate, making it bio-unavailable [100]. The lower level of micro-nutrients in wheat grain leads to malnutrition in people whose diet mainly depends largely on wheat. Among these trace elements, Fe and Zn are important for metabolic processes: impair body growth, brain functionality, and immune system when deficient. About 60% and more than 25% of the world population is Fe and Zn deficient, respectively [101]. Thus, it ranks 5th and 6th among the global disease burden [102]. More than 47% of children suffer from Fe deficiency alone. About one in three adult women are anaemic due to Fe deficiency [103]. Similarly, Zn deficiency also affects millions of people. Out of 768 million undernourished people, around half (418 million) reside in Asia, while one-third (282 million) live in Africa [104]. Similarly, the vitamin C deficiency was found more prevalent (43%) in low to middle income countries (LMIC), although not uncommon in high income countries [105]. The reason behind high prevalence of Fe, Zn and vitamin C deficiency is lowered food diversity, including the reduction to a few staple crops, e.g. wheat and rice [106]. Moreover, 15% of the people with functional iron deficiency were found to have vitamin C deficiency as well [107], calling for a strong interrelation. Global vitamin C deficiency prevalence and status have not been reported, despite of its pleiotropic roles in both infectious and non-infectious diseases. Its prevalence is assessed by the incidence of diseases caused by its deficiency like hypovitaminosis C, which has an incidence of more than 70% in LMIC including India and Pakistan. According to these estimates, more than 50% of the people in these countries suffer from vitamin C deficiency. Climate factors like drought and low temperatures were found to be associated with the increased levels of scurvy caused by vitamin C deficiency [108].

Fruits and vegetables are the only source of vitamin C as it is not accessible to or utilized by all people at the same rate. It is an important antioxidant and plays multiple roles in optimal health and prevention of diseases [109]. In LMIC, staple foods are mostly grains of millets, rice, and wheat with low proportion of vitamins which tend to decrease vitamin C intake [110]. However, locally abundant fruits like sea-buckthorn that are rich in vitamin C can be made available to combat its deficiency.

4. Sea buckthorn potential as a source of minerals and vitamin C: inspirations from “superfoods”

Biodiversity helps to regulate the nutrient and water cycle and mitigate climate change impacts on native as well as crop plants. Provision of a diverse food supply chain is important for food security. The expansion of croplands for enhanced production to ensure food security affects biodiverse regions, demanding the need for context-specific assessment of food security strategies [111]. Investment for nutritional security is a highly-paid back job with several benefits for developing countries, as it contributes to achieve two of the sustainable development goals (SDG), including zero hunger – SDG 2 (sustainable agriculture, improved nutrition and food security), and healthy lives – SDG 3 (wellbeing at all stages for everyone) [112]. Food patterns with diverse food groups ensure a balanced diet and nutritional food security. However, out of 300,000 known vascular plant species only 20,000 to 30,000 are edible, yet their nutritional value remains uncharacterized, neglected and underutilized, but can be a key to eradicate malnutrition [113]. This knowledge gap paralyses our ability to cherish plant diversity for our food basket and support our existing strategies to address micronutrient deficiencies [90]. Alone more than 6400 plant species are considered important for vitamin B [113]. Many NUS are often characterized by advantageous levels of energy, promising assemblies of micronutrients, amino acids, proteins, and fibre [43]. Moreover, these species are adapted to marginal lands and can therefore be commercialized without competition to many staple crops [114]. While these edible wild plants diversify local food systems, their availability to the international community is limited [115]. For example, 15 out of 91 countries reported daily use of wild species in their national diets [116]. Fortunately, many countries also maintain their nutrient-rich biodiversity hotspots for several reasons [117]. The FAO has called for the regional initiative on future smart food (FSF) to achieve zero hunger (SDG2). However, for a NUS to qualify as FSF, it must be 1) climate resilient, 2) economic friendly, 3) nutrient dense, and 4) native or locally adapted [43,87]. Large scale focus on NUS for the dietary diversity to prevent anaemia is being targeted. For example, the Indian government encourages the consumption of vitamin C-rich fruits which are abundantly available but underutilized by the majority of the nutrient-deficient population [43,118]. Similarly, Nepal has recognized buckwheat (*Fagopyrum esculentum* MOENCH), grass pea (*Lathyrus sativus* L.), jackfruit (*Artocarpus heterophyllus* (PARKINSON) FOSBERG), Chiuri (*Diploknema butyracea* (ROXB.) H.J.LAM), and taro (*Colocasia esculenta* (L.) SCHOTT) as important source of rutin, proteins, minerals, and vitamins, respectively (s. also Table 1).

Among those NUS is sea-buckthorn, a widespread plant species of the northern hemisphere, considered a “superfood” owing to the rich nutritional set of its fruits. It is of great national and international marketing potential in Europe and China. The fruits contain vitamin C (200–1500 mg 100 g⁻¹: 15 times higher than orange fruit [46]), oil (mainly sterols, 1.5–3.5% in fruit pulp and 9.9–19.5% in seed [119]), vitamin D, E, K and P [120], anthocyanin (0.5–25 mg l⁻¹), and 24 minerals including calcium (70–98 ppm), Fe (40–150 ppm), magnesium (150–240 ppm), phosphorus (110–133 ppm), potassium (140–360 ppm) [47], Zn (32 ppm) [48], and 190 bioactive compounds [121]. A study has also revealed its potential for fortification of vitamin D in food products [120]. However, not much has

Table 1
Potential neglected and underutilized species to be targeted for dietary diversity and future smart foods.

Common name	Scientific Name	Edible portion	Family	Country of utilization	Novel trait/utilization	References
Vegetables						
Chaya/Mayan Spinach	<i>Cnidoscolus aconitifolius</i> (Mill.) I. M. Johnst.	Leaves	Euphorbiaceae	Guatemala	Nutrient rich leaves year-round	[85]
Kale	<i>Brassica oleracea</i> v. <i>acephala</i> L.	Leaves	Brassicaceae	ubiquitous	Superfood providing $\geq 10\%$ of 17 essential nutrients	[86]
Roquette/arugula	<i>Eruca vesicaria</i> (L.) Cav.	Leaves	Brassicaceae	ubiquitous	Rich in vitamin C, phenols, carotenoids	[87]
Sea asparagus	<i>Salicornia</i> spp. L.	Stems, leaves	Amaranthaceae	USA, Mexico, Canada, Europe	Halophyte, rich in minerals, vitamins, fatty acids	[87]
Pulses/Legume						
Tepary bean/ <i>escumite</i>	<i>Phaseolus acutifolius</i> A. Gray	Seed	Fabaceae	Guatemala	1.5 times out yielding common bean in hot environments with same nutrition	[85]
Lentils	<i>Lens culinaris</i> Medik.	Seed	Fabaceae	Sri Lanka	Rich source of Fe, Zn, and selenium	[88]
Cereals						
Millet	<i>Panicum</i> spp. L.	Grain	Poaceae	Bangladesh, India	“High-energy” cereals, higher protein, oil, and vitamin A contents than maize	[89]
Tartary Buckwheat	<i>Fagopyrum tataricum</i> (L.) Gaertn.	Leaves and grain	Polygonaceae	Nepal	Good source of rutin and dietary fibre	[90]
Buckwheat	<i>Fagopyrum esculentum</i> (L.) Gaertn.	Grain	Polygonaceae	Bhutan	Rich source of Fe and Zn	[43]
Quinoa	<i>Chenopodium quinoa</i> Willd.	Grain	Amaranthaceae	Nepal, Bhutan	23 times more folate, five times dietary fibre, four times Fe, and two times more protein than rice	[91]
Roots and Tubers						
Taro	<i>Colocasia esculenta</i> (L.) Schott	Corms, petioles, and leaves	Araceae	Cambodia, Nepal, Vietnam	Rich in vitamin A	[43]
Multi-coloured carrots	<i>Daucus carota</i> L.	Tuber	Apiaceae	Central Asia, Italy/Apulia	High levels of minerals, polyphenols, and carotenoids especially B-carotene	[87]
Trees						
Drumstick	<i>Moringa oleifera</i> Lam.	Young seeds, pods, and leaves	Moringaceae	Bhutan, Myanmar, Vietnam	Nutritious vegetable rich in vitamin A	[43]
Jackfruit	<i>Artocarpus heterophyllus</i> Lam.	Ripe and unripe fruit, mature seed	Moraceae		Fruit is rich in carbohydrates, protein, calcium, potassium, and vitamins A, B and C	[43]
Butter Tree	<i>Madhuca longifolia</i> (J. Koenig ex L.) J.F. Macbr.	Seed oil	Sapotaceae		35-40 extractable oil; twice than soybean and <i>Brassica</i>	[43]
Sea-Buckthorn	<i>Hippophae rhamnoides</i> L.	Fruit, seed oil	Elaeagnaceae	China, Europe India, Pakistan	Rich in vitamin C; 15 times higher than orange fruit	[46]

been reported on prioritizing the strategies for genetic biofortification of sea-buckthorn by understanding genetic pathways for these minerals and vitamins. It is adaptable to a wide range of harsh biophysical conditions, including hot summers, and cold winters. In recent years, the inhabitants of northern Pakistan have recognized the importance of sea buckthorn as a medicinal and food plant, and have expanded the area under cultivation. It is cultivated on 5700 ha, however, only 285 ha (around 5%) is harvested and utilized due to untraditional cuisine, thorny nature, alternate bearing, and its dioecious nature [51]. Due to their cultivation in limited regions of the world, the potential of these NUS can only be partially utilized as they cannot be immediately and widely consumed by the global community. It is therefore important to improve the nutritional value of staple foods such as wheat or rice, which are consumed in many parts of the world, to combat malnutrition.

5. Setting up targets: translating the information for wheat biofortification

Biofortification is an approach to enrich the agricultural produce with micronutrients and vitamins by modulating and targeting the movement pathway of nutrients (uptake, translocation, storage, and bioavailability). Before setting targets to achieve nutritional food security, it is imperative to know the estimated average requirements of the human body for the essential micro-nutrients. There are three main steps for setting up the priorities for biofortification (Fig. 1).

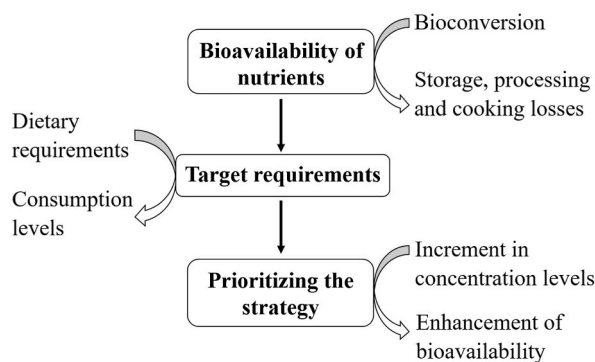


Fig. 1. Important steps to consider while setting up priorities to address micronutrient malnutrition. The side arrows represent factors that need to be considered during each successive step.

5.1. Nutrient content and their bioavailability

5.1.1. Grain micronutrient contents

The content of Fe and Zn in modern wheat cultivars ranges from 24 to 51 mg kg⁻¹ and 20–35 mg kg⁻¹, respectively [122] compared to 15–109 mg kg⁻¹ Fe, and 14–190 mg kg⁻¹ Zn in *Triticum turgidum* (wild emmer) [123]. Similarly, Zn content varies from 8 to 85 mg kg⁻¹ compared to wild emmer having 14–190 mg kg⁻¹ dry weight [123]. The wild resources have been exploited to fortify Fe and Zn in wheat, for example, *NAM-B1* gene increased Fe and Zn content by 18 and 12%, respectively, which was originally identified in emmer wheat [124,125]. Due to less availability of Fe as compared to Zn (5% vs. 25%) in wheat, higher Fe content is needed to achieve target levels [14].

5.1.2. Micronutrient bioavailability

Bioavailability is defined as the ratio of micronutrients absorbed by the body to the total intake/undigested micronutrient content. It is determined as a product of interaction between phosphorus storage as phytate in grain. Wheat flour contains higher levels of PA (6–10 mg g⁻¹ [126]) presenting the need to reduce it in wheat flour. The PA:Fe and PA:Zn molar ratios are used to estimate the potential bioavailability of these micronutrients [127]. The lower molar ratios represent higher bioavailability and *vice versa*. Vitamins, especially vitamin C, help in Fe absorption in plants and animals. It enhances Fe availability by converting non-heme iron (Fe³⁺) into the available form (Fe²⁺ [41]) and synthesizes ferritin while protecting it from degradation [128]. However, AA as a ligand to increase the Fe content in original and fortified foods is only effective if it is already present in the food.

5.1.3. Target requirements

According to the WHO, the recommended dietary allowance of Fe and Zn is 10 and 15 mg d⁻¹ for men and 10 and 12 mg d⁻¹ for women (25–50 age group), respectively. According to HarvestPlus – an IFPRI associated institute for biofortification research and implementation – 59 μg g⁻¹ of Fe and 38 μg g⁻¹ of Zn are estimated to be required for an adult human diet [129]. To significantly improve iron absorption, the molar ratio of PA:Fe should be < 1 [130], while PA:Zn should be < 5, 5–15 and > 15 corresponding to high, moderate and low bioavailability associated with 50%, 30% and 15% Zn availability [131]. The recommended dietary allowance (RDA) for vitamin C is 90 mg day⁻¹ for males and 80 mg day⁻¹ for females [132]. Although no standardized targets for AA have been set for wheat grain, studies report that 6–8 mg kg⁻¹ [49,133] to 92 mg kg⁻¹ AA [134] is present in wheat flour. To achieve the declared RDA, cereal grains must have an additional 40–60 mg kg⁻¹ of Fe and Zn and around 50–100 mg kg⁻¹ of AA [122]. The recommended molar ratio of AA to Fe in food was 2:1 [135]. However, increasing it to 4:1 ratio may be impractical for the cereal-based food due to instability of AA during cooking and processing and unwanted sensory changes [37]. The recommended suitable sources of AA as either vitamin fortificant or Fe enhancer are dry-blended foods like cereal based precooked foods for children, powdered milk or complementary foods [37].

5.1.4. Prioritizing the strategies

The most suitable strategy to eliminate and prevent micronutrient malnutrition is to ensure a nutritionally balanced diet. Dietary diversification, post-harvest biofortification, and mineral supplementation are some common and effective strategies. Food fortification is the most practical approach to overcome micronutrient deficiency without changing the feeding pattern of a large population. Many food products like butter, sugar, and margarine have a history of being fortified with vitamin A, B, and iodine. Vitamin B fortified cereals are being introduced in some countries; even milk and oil fortification is mandatory in 14 and 27 countries, respectively [136]. Around 83 countries are implementing legislation to fortify their staple food crops [136]. Biofortification of different nutrients and essential minerals with edible oil is also a good and cheap source to nourish nutrient deficient people and eradicate hidden hunger in the world [136]. However, food fortification is reserved to countries with well-developed and properly monitored food processing systems and pharmaceuticals. Moreover, it is costly being dependent on year after year investment for fortification. Therefore, such fortification intervention is not effective for societies that mainly depend on on-farm produce as food source [137,138]. Biofortification

to improve the nutritional quality of the staple crops instead, is a cost-effective, sustainable, and environmentally-safe choice to address the issue of malnutrition globally [14]. Rather than using a single and costly method, the combination of cost-effective approaches might be more operative to cap micronutrient deficiency (Fig. 2). The agronomic approaches focus on the optimization of the fertilizers that are directly taken up like Zn but are ineffective for nutrients/co-factors that are synthesized by the plant like vitamins. Thus, it provides temporary increment in nutrients through fertilizers [139]. Moreover, fertilizers applied either foliar or through soil, may cause air and water pollution. On the other hand, the latter approach targets the better nutrient accumulation ability, increased bioavailability and reduced anti-nutritional compounds making it long-term and sustainable. This strategy also focusses on the underlying mechanisms and genes, making it a sustainable approach. Since wheat is a staple of around 2.5 billion people; increasing Zn and Fe content in wheat grain will have important health implications to combat these micronutrient deficiencies globally.

There are five major ways to facilitate and address genetic biofortification. i) Increased acquisition of Fe and Zn from soil; ii) enhanced translocation/loading from source to sink i.e., from leaves/stem to grain, in case of cereals; iii) specialized storage of Fe in the endosperm (to avoid losses during processing); iv) decreased anti-nutrient compounds (like phytic acid, phenols); and v) increased bioavailability by enhancers like vitamin C and other vitamins [140]. These approaches either solo or in combination have been utilized for targeted genetic biofortification and will be discussed targeting wheat in upcoming sections.

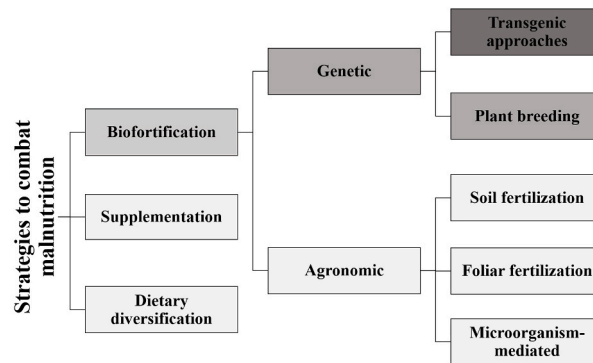


Fig. 2. Prioritizing the strategy for effective and long-term fortification of micronutrients. The darker colour indicates the strategy of choice for genetic biofortification.

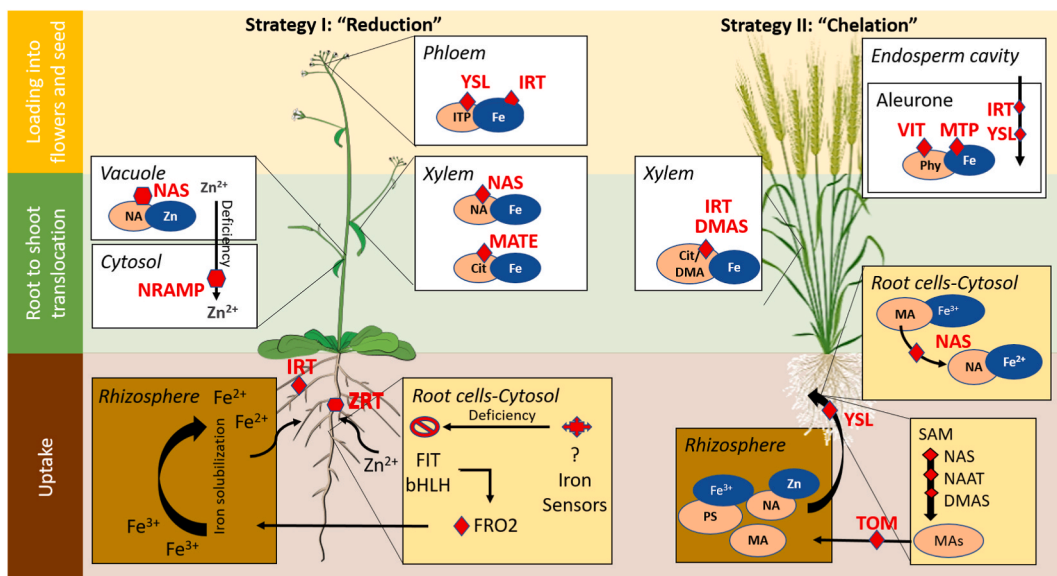


Fig. 3. Comparative mechanisms of iron acquisition and translocation in plants under strategy I and strategy II. The red colour indicates the key genes involved in successive pathways. Basic helix-loop-helix (bHLH) transcription factor (inducible under Fe deficiency); FIT: T3238FER/ FER-like Fe deficiency-induced transcription factor; FRO2: Ferric reductase oxidase; ZRT: Zn regulated transporter; IRT: Fe regulated transporter; NAS: Nicotinamide synthase; MATE: Multidrug and toxin efflux; NRAMP: Natural resistance-associated macrophage protein; YSL: Yellow stripe like proteins; TOM: Transporter of mugineic acid family Phyto-siderophores; SAM: S-adenosyl methionine; NAAT: NA aminotransferase; DMAS: Deoxymugineic acid synthase; VIT: Vacuolar Fe transporter; Cit: Citrate; MTP: Metal tolerance proteins. Source: *Arabidopsis* plant taken from Illustrations, Plant (2017): Shoot illustrations. figshare. Collection. <https://doi.org/10.6084/m9.figshare.c.3701035.v13>; Wheat plant created in bioRender.com.

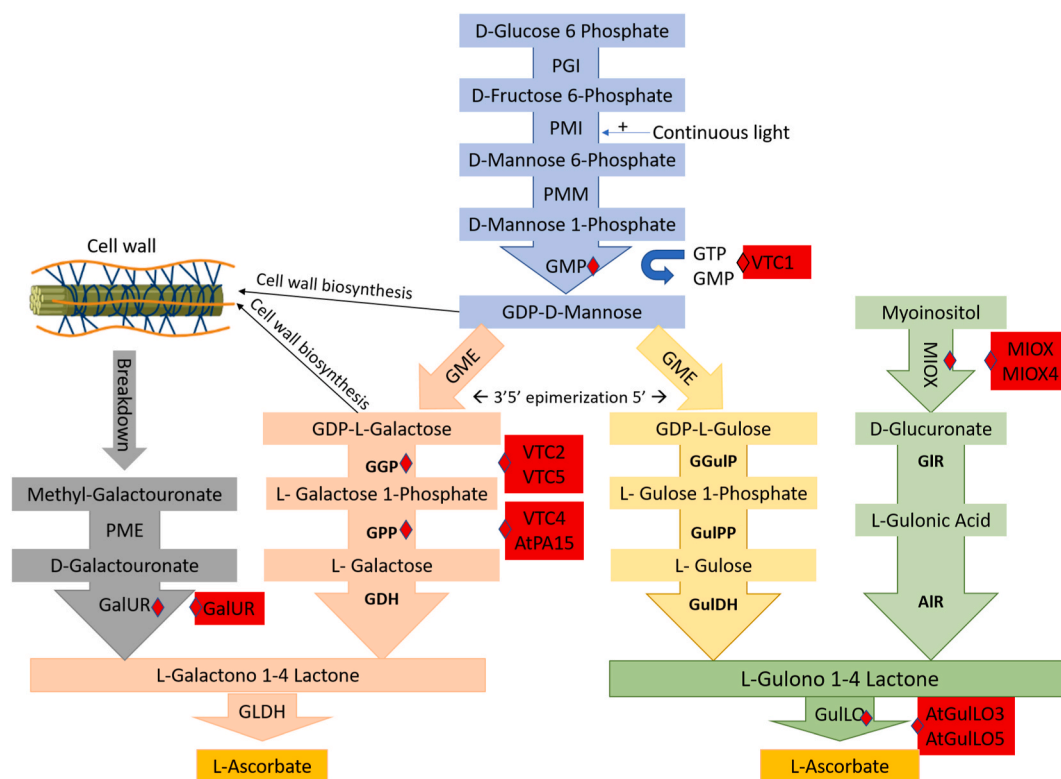


Fig. 4. Pathways for biosynthesis of vitamin C in plants. Green: Myo-inositol pathway, yellow: Gulose pathway, pink: Galactose pathway, grey: Galacturonate pathway (salvage pathway), blue: common pathway of synthesis of D-Mannose, red: key enzymes and genes involved at each successive step. VTC1: codes GMP that converts Guanosine triphosphate into Guanosine diphosphate. PGI: Phosphor-glucose isomerase; PMI: Phosphomannose isomerase; PMM: Phosphomannose mutase; GMP: GDP-D-Mannose pyrophosphorylase; GME: GDP-D-mannose epimerase; GGP: GDP-L-Galactose-phosphorylase; GDH: L-Galactose dehydrogenase; GLDH: L-Galactono-1,4-lactone dehydrogenase; PME: Pectin methyl esterase; GalUR: D-Galacturonate reductase; GGulP: GDP-L-Gulose pyrophosphatase; GulPP: L-Gulose-1-phosphate phosphatase; GulDH: L-Gulose dehydrogenase; MIOX: Myo-inositol oxygenase; GIR: Glucuronate reductase; AIR: Aldono reductase; GulLO: Gluconolactone oxidase. The cell wall structure was created with biorender.com.

5.2. Bridging the gap in translocation of iron, zinc, and vitamin C biosynthesis

5.2.1. Iron and zinc uptake by the plant

To genetically biofortify wheat, it is important to assess the biochemical and genetic background for the transport of Fe and Zn and AA biosynthesis and to identify common pathways and restrictions by one or the other species. Under natural soil conditions, Fe and Zn micronutrients are not readily available. For example, Fe in soil is present in its ferric form that is unavailable by plants. However, plants have developed unique strategies to acquire this unavailable form by either reducing it to an available form or by chelation with other molecules [141].

Two mechanisms are reported for the uptake of Fe and Zn in plants. Strategy I is the direct uptake mechanism utilized by non-gramineous (all incl. sea buckthorn, except grasses), in which Fe and Zn are carried by the IRT and ZRT-like proteins (ZIPs). It involves H^+ extrusion that promotes reduction of ferric chelate on root surface into ferrous (Fe^{2+} is ultimately up-taken via plasma membrane [142]). Therefore, the key gene involved in this strategy is ferric reductase oxidase (*FRO2*) that codes for root ferric chelate reductase [142] and *IRT1* which codes for the ferrous Fe transporter [143].

The other is via chelation in which Phyto-siderophores bind with Fe^{3+} and are later up-taken by yellow stripe like transporters (YSLs) in strategy II plants like grasses, incl. wheat (Fig. 3 [144]). Normally, Mugenic acids (MA) are excreted into the rhizosphere which forms chelates with Fe and are taken up by the roots. DMAS is the key enzyme responsible for synthesis of MA. The ascorbate also plays important role by reducing Fe^{3+} -MA to Fe^{2+} -NA in cytosol. From here to phloem and grain loading, Fe^{2+} binds to citrate and Deoxy-MA again [145]. The key genes involved in Fe uptake have been presented in Table 2 and reviewed by Kobayashi and Nishizawa [146]. The strategy II is known to arise as an adaptation to the alkaline soils whereas acidification (as done in strategy I) is difficult [147].

Table 2
Key genes involved in the acquisition of Fe and Zn from soil, its translocation and biosynthesis of vitamin C in plants.

Gene	Family	Organism	Reported members	Protein domain	Function	Reference
NAS	Nicotinamide synthase	<i>Oryza sativa</i> L.	3 (<i>OsNAS1</i> , <i>OsNAS2</i> , <i>OsNAS3</i>)	PF03059	Enhanced accumulation of Fe and Zn in rice endosperm (14 $\mu\text{g g}^{-1}$ Fe)	[53]
		<i>Hordeum vulgare</i> L.	7 (<i>NAS1-4</i> , <i>5-1</i> , <i>5-2</i> , and <i>NAS6</i>)		Encoding NAS and NAS-like proteins under Fe deficiency; root specific expression	[54]
		<i>Arabidopsis thaliana</i> (L.) Heynh.	4		Loading of Fe into the seed mediated by NA	[55]
FRDL	Multidrug and toxic compound extrusion (MATE) transporters	<i>Oryza sativa</i> L.	2 (<i>OsFRDL1</i> , <i>OsFRDL2</i>)	PF01554	<i>OsFRDL1</i> is involved in root-shoot Fe translocation	[56]
		<i>Arabidopsis thaliana</i> (L.) Heynh.	56 (FRD)		<i>OsFRDL2</i> is involved in secretion of citrate phytosiderophore and aluminium tolerance	[57]
		<i>Oryza sativa</i> L.	18	PF03169	Constitutive Fe^{3+} chelate-reductase activity and over accumulates a variety of metals including manganese Translocation of Fe and Mn via the phloem pathway	[58] [59]
YSL	Yellow stripe like protein sub-family of oligopeptide transporter protein	<i>Arabidopsis thaliana</i> (L.) Heynh.	8		Translocation of Fe and copper, remobilization of Fe from senescing leaves to sink; pollen development	[60,61]
		<i>Oryza sativa</i> L.	1 (<i>OsDMAS1</i>)	PF00248	Synthesizing 2'-Deoxymugineic acid for acquisition of insoluble Fe	[62]
DMAS1	Aldo-keto reductase	<i>Triticum aestivum</i>	3 (<i>TaDMAS1-A</i> , <i>B</i> & <i>D</i>)		Synthesizing 2'-Deoxymugineic acid for acquisition of insoluble Fe	[63]
		<i>Oryza sativa</i> L.	2 (<i>OsFRO1</i> , <i>OsFRO2</i>)	PF08030	Encodes Fe-chelate reductase to reduce Fe^{3+} into Fe^{2+}	[64]
FRO	Ferric-chelate reductase/oxidase protein	<i>Arabidopsis thaliana</i> (L.) Heynh.	8 (<i>AtFRO1-8</i>)		Fe metabolism in a variety of locations	[65]
		<i>Oryza sativa</i> L.	2 (<i>OsIRT1</i> , <i>OsIRT2</i>)	PF02535	Involved in Strategy I Fe transport	[66]
IRT	Iron like transporter sub-family of ZIP	<i>Arabidopsis thaliana</i> (L.) Heynh.	15 (<i>AtIRT1</i> and 2 are involved in Fe/Zn transport)		Encodes the high-affinity Fe transporter	[67]
		<i>Oryza sativa</i> L.	6 (<i>OsNAAT1-6</i>)	PF00155	Catalyses NA to the 3'-keto form	[68]
NAAT	Nicotianamine aminotransferase	<i>Oryza sativa</i> L.	2 (<i>OsVIT1</i> and 2)	PF01988	Fe translocation between flag leaves and grain	[69]
VIT	Vacuolar iron transporter	<i>Oryza sativa</i> L.	1 (<i>OsNAC5</i>)	PF01849	Remobilization of Fe from senescing leaves to grains	[70]
NAC	NAC TF	<i>Triticum aestivum</i> L.	1 (<i>NAM-B1</i>)		Remobilization of Fe from senescing leaves to grains	[70]
NRAMP	Natural resistance-associated macrophage protein	<i>Oryza sativa</i> L.	7 (<i>NRAMP1-7</i>)	PF01566	Transports several metal ions like Fe^{2+} , Zn^{2+} , Mn^{2+} , Cd^{2+}	[71]
		<i>Arabidopsis thaliana</i> (L.) Heynh.	6		Modulates Cadmium and Fe toxicity	[72]
MTP	Cation diffusion facilitator (metal tolerance protein)	<i>Oryza sativa</i> L.	10 (Only <i>MTP6</i> and <i>MTP7</i> belong to Fe/Zn CDF class)	PF01545	Transports Fe and Zn into vacuoles	[73]
		<i>Arabidopsis thaliana</i> (L.) Heynh.	12		Three classes of this gene family are involved in Fe, Zn and Mn (Fe, Zn and Mn_CDFs)	[74]
FER	Ferritin	<i>Oryza sativa</i> L.	2 (<i>OsFER1</i> and 2)	PF07690	Defends plants against oxidative stress caused by Fe-deficiency and enhanced accumulation of Fe in ferritin form	[55]
		<i>Arabidopsis thaliana</i> (L.) Heynh.	4		Tolerance to Fe toxicity	[75]

(continued on next page)

Table 2 (continued)

Gene	Family	Organism	Reported members	Protein domain	Function	Reference
Zinc						
HMA	Heavy metal ATPase	<i>Oryza sativa</i> L.	9 (Only <i>OsHMA1</i> -3 belong to Zn/Co/Cd/Pb P1B-ATPase group)	PF00403	Zn absorption and transport	[76]
		<i>Arabidopsis thaliana</i> (L.) Heynh.	8		Zn homeostasis and Cadmium detoxification	[77]
ZIFL	Zn-induced facilitator-like (ZIFL) family	<i>Oryza sativa</i> L.	13 (<i>OsZIFL1-13</i>)	PF07690 (MSF-1)	Homeostasis of Zn induced by Zn availability	[76]
		<i>Arabidopsis thaliana</i> (L.) Heynh.	3		Zn homeostasis	[78]
HRZ	Hemerythrin motif-containing Really Interesting New Gene (RING)- and Zn-finger protein 1	<i>Oryza sativa</i> L.	2 (<i>HRZ1</i> and 2)	PF00098	Negatively regulates Fe deficiency responses; intracellular Fe sensors	[79]
Vitamin C						
GalUR	Aldo-keto reductase	<i>Fragaria × ananassa</i> Duchesne	1	PF00248	Encodes an NADPH-dependent D- Galacturonate reductase	[80]
PAP15	Metallophosphatase family	<i>Arabidopsis thaliana</i> (L.) Heynh.	29	PF00149	Hydrolyses Myoinositol hexakisphosphate to Myoinositol and free phosphate.	[81]
VTC	Glycosyltransferase superfamily A	<i>Arabidopsis thaliana</i> (L.) Heynh.	5 (<i>VTC1-5</i>)	PF00483	Encodes two isoforms of GDP-L- galactose phosphorylase, one of the last enzymes of the ascorbate biosynthetic pathway	[82]
APX	Peroxidase superfamily	<i>Oryza sativa</i> L.	8	PF00141 (Heme Peroxidase)	Plays role in vitamin C recycling mechanism	[83]
DHAR	Glutathione-dependent dehydroascorbate reductase	<i>Arabidopsis thaliana</i> (L.) Heynh.	3	PF13417	Encodes DHA reductase during vitamin C regeneration cycle	[84]

5.2.2. Biosynthesis of vitamin C in plants

The biosynthesis of vitamin C has been a matter of dispute for many years. A defined mechanism was formulated only twenty-three years earlier [148]. Unlike animals, the Smirnoff-Wheeler pathway, also known as D-Mannose/L-Galactose pathway named after the molecule used for vitamin C synthesis, is the major route for biosynthesis of vitamin C in plants [149]. Besides this, three other pathways have been proposed namely Myoinositol, Galacturonate (Salvage pathway) and Gulose pathways (Fig. 4 [80,84,150,151]). As the *GalUR* gene involved in the Galacturonate is of our interest, only the Salvage pathway will be discussed in detail.

The Galacturonate pathway is called salvage pathway as it utilizes the sugars released during the breakdown of cell wall [80]. The key enzyme involved in this pathway is D-Galacturonate reductase (GalUR). The process starts with the release of Methyl galacturonate due to breakdown of pectin. It is then converted into D-Galacturonate and successively into L-Galactonate by GalUR. Finally, 1-Galactono-1,4-lactone is converted into L-Ascorbate by GLDH [149]. The *GalUR* gene was first identified in strawberry [80] and belongs to Aldo-keto reductase superfamily. Overexpression of this gene enhanced vitamin C in tomato [152]. As it encodes for the synthesis of last dedicated molecules for vitamin C synthesis by Salvage pathway, it is important to target this gene for vitamin C biofortification.

5.3. Aldo-keto reductase: a targeted gene family for simultaneous biofortification of iron, zinc, and vitamin C

The Aldo-keto reductase (AKR) gene was first identified in strawberry as a gene responsible for coding GalUR [153]. The genes of this family are not only involved in ascorbic acid (AA) biosynthesis, but also play a vital role in biotic and abiotic stresses, as found for tomato [154]. A study conducted to explore the phylogenetic relationships of this gene across plant species identified 18 paralogues in citrus [155] and 17 in kiwi genomes [156]. Although diverse expression patterns were observed for different genes, GalUR12 was identified as a key contributor for the AA accumulation upregulated in fruits like citrus [157].

The AKR is a superfamily of diverse enzymes involved in catalysis of carbonyl compounds to respective alcohols or responsible for the reverse oxidation in several processes. These genes are widely present in plants, algae, and animals [141]. In rice, the putative substrate binding site was identified as A(50)-HY(52-53)-W(85)-HW(116-17)-V(119)-KA(127-28)-G(305)-Y(307)-S(309). The conserved domain NADPH is also an important feature of this family. The AKR has 15 sub-families and genes are classified into four major groups based on the diverse functionality *viz* osmolyte biosynthesis, membrane transport, secondary metabolism, and detoxification of aldehyde. These functional groups are further categorized into respective tasks like Fe-acquisition from soil and plant-microbe interactions [141].

5.3.1. Role of AKRs in iron acquisition

The mechanism of Fe acquisition from soil falls under category I: detoxification of reactive aldehydes but interestingly the genes involved in this group do not detoxify aldehydes rather they catalyse the biosynthesis of phyto-siderophores or metal chelators like DMAS (Fig. 5). However, it is categorized under this group due to indirect involvement in alleviating metal stress [141].

The Gramineae family plants utilize MAs for the acquisition of Fe from soil, thereby helping plants to adapt in Fe deficient soils. The enzymes involved in catalysis of 3' keto intermediate to DMAs are NADPH dependent and were named as DMAS (Fig. 1). The genes responsible to code DMAS have been isolated in rice (*AKR4C14*) by Narawongsanont et al. [158], and for wheat (*AKR4B8*), barley (*AKR4B7*), and maize (*AKR4B5*) by Bashir and Nishizawa [62]. These genes showed huge similarity (>82%) and classified as a new sub-family (*AKR4*) in the AKR superfamily [159]. All these transcripts were up-regulated under Fe-deficiency conditions in root tissues indicating their active involvement in Fe-acquisition in respective crop plants.

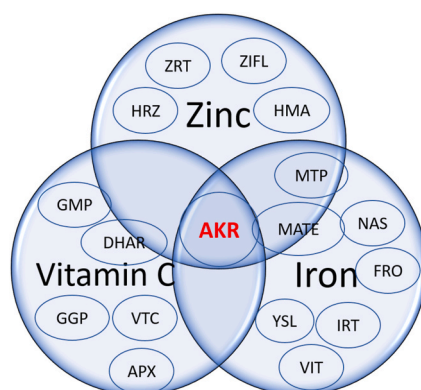


Fig. 5. Different gene families involved in the uptake/biosynthesis of respective mineral and vitamin. Red: potential candidate family/genes for the genetic biofortification. ZIFL: Zn-induced facilitator-like family, HMA: Heavy metal ATPase, ZRT: Zn regulatory transporter protein family, HRZ: Hemerythrin motif-containing Really Interesting New Gene (RING)- and Zn-finger protein 1 family, MTP: Metal tolerance protein, MATE: Multidrug and toxic compound extrusion (MATE) transporters, NAS: Nicotinamide synthase gene family, YSL: yellow stripe like transporter family, IRT: Iron regulatory transporter protein family, VIT: Vacuolar iron transporter family, FRO: Ferric reductase oxidase, DHAR: Glutathione-dependent dehydro-ascorbate reductase, GMP: GDP-D-Mannose pyro-phosphorylase, VTC genes: vitamin C genes belonging to Glycosyltransferase superfamily A, GGP: GDP-L-Galactose-phosphorylase, APX: Ascorbate peroxidase.

5.3.2. Role in vitamin C biosynthesis

Biosynthesis of vitamin C falls under the functional group III of AKR gene family responsible for biosynthesis of commercial secondary metabolites [141]. A single member of plant AKRs (coded as *AKR4B4*) encode GalUR, an important enzyme in the pectin degradation pathway or Galacturonate pathway (Fig. 5). The gene was reported in strawberry as a key gene involved in vitamin C biosynthesis. The overexpression of this gene also showed enhanced (2 to 3-fold) vitamin C content in *Arabidopsis thaliana* (L.) HEYNH [80].

6. Conclusions

Around 2 billion people suffer from micronutrient (minerals and vitamins) malnutrition around the world. Changing climate and intensive breeding efforts have further diluted nutrients content in staple food crops. Countries where cereals are a major part of the daily food intake, especially developing countries, are more prone to deficiency of Fe and Zn. Vitamin C is another important nutrient for human health but being a vitamin, it can only be obtained from plant sources naturally. It is not only essential to combat diseases but also responsible to enhance Fe absorption. Food diversification by utilizing NUS may solve problem partially but they cannot be made available to the global community due to their limited spatial distribution and cultivation. This study thus suggested that seabuckthorn – a vitamin C and mineral rich plant – could be utilized to study the genetic pathways underlying vitamin C biosynthesis, and Fe and Zn uptake. This information can be translated to scale-up the biofortification breeding efforts in wheat. Moreover, the review also identified a novel gene family (Aldo-keto reductase) involved both in the uptake of Fe and Zn (in strategy II plants like wheat) and synthesis of precursor for vitamin C. Thus, the biofortification of cereals indicates a more sustainable approach to enhance micronutrient content and at the same time stimulate their bioavailability. However, there is a need to evaluate the phenotypic and genotypic response of these genes for micronutrients and yield traits under natural conditions to develop biofortified wheat cultivars. Last but not least, biofortification of staple foods alone will certainly not be the solution to global micronutrient deficiencies, especially for the group of thermolabile compounds such as vitamins that are largely destroyed during the long and temperature-intensive processing of carbohydrate-rich foods. Vitamin-preserving techniques and processes must therefore be considered and adopted to local food traditions when introducing biofortified foods.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Data availability statement

Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

List of abbreviations

AA	ascorbic acid
AKR	Aldo keto reductase
DMAS	Deoxymugineic acid synthase
Fe	iron
FSF	future smart food
GR	green revolution
GalUR	D- Galacturonate reductase
LMIC	low to middle income countries
MA	mugineic acid
NUS	neglected and underutilized species

PA	phytic acid
RDA	recommended dietary allowance
Zn	zinc.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2023.e20208>.

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