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Agronomic Biofortification of Vegetable Crops–A Systematic Review

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Authors' contributions

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

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ABSTRACT

Mineral deficiency is prevalent in the diets of more than two-thirds of the world's population. This problem can be solved by diversifying the diet, supplementing minerals, enriching foods or increasing the concentration and/or bioavailability of essential mineral elements in products (i.e., biofortification). Vegetables represent the backbone of good nutrition as they are a rich source of dietary fibre, antioxidants, vitamins and minerals, and biofortification is a promising method of increasing the content of these compounds in vegetables. Given the significance of minerals in human diet and metabolism, the possibility of adding minerals to fresh vegetables through the use of specific agronomic methods has been considered. This review thoroughly examines the most recent research on agronomic biofortification of vegetable crops, with an aim of increasing the content of important micronutrients, such as iron, zinc, molybdenum and copper, in the edible parts, with an emphasis on the effects of this strategy. Although agronomic biofortification is considered a practicable technique, the approach is multifaceted due to several interactions occurring at the crop level, as well as the bioavailability of different minerals to plants and consumers.

Keywords: Agronomic biofortification; bioavailability; human diet; minerals; vegetables.

1. INTRODUCTION

Human population is expected to reach 8.54 and 9.74 billion by 2030 and 2050, respectively. In tandem, global crises like climate change [1,2] and pandemics are making agriculture more susceptible, which is further exacerbating the challenges for global food security [3]. A balanced diet is out of reach for many people, especially in the developing and underdeveloped countries, making human malnutrition a serious socio-economic issue. Most of the diets (cereal-based) are rich in carbohydrates; however, the issue of 'hidden hunger' continues to exist due to our inability to satisfy micronutrient necessities [4]. In order to maintain good health, people require specific mineral elements that must be incorporated in their daily diet. Minerals are essential due to the fact that vitamins cannot be absorbed on their own or function without them, and that, they are important in many physicochemical processes. The deficiency of specific mineral elements affects two-thirds of the world's population, in both developed and developing nations [5-7], and malnutrition can negatively affect human health [8]. For instance, a lot of women and children in Central Asia and Europe are at risk for anaemia due to problems of malnutrition associated with diets deficient in essential micronutrients. Furthermore, a study conducted in southern Italy revealed that the population had a low intake of calcium and potassium [9].

There exist a number of strategies to address malnutrition (Fig. 1.), including dietary diversification, food supplementation, food fortification and biofortification, each of which has pros and cons of its own. When it comes to protein, vitamin and mineral intake, dietary diversification and food supplementation are appealing, but in many socio-economic circumstances, they are neither feasible nor affordable [10]. For socio-economic groups with limited access to expensive and commercially-marketed fortified foods, biofortification of edible crops is a promising, sustainable, efficient and cost-effective strategy in alleviating mineral deficiency in humans [11-14]. Biofortification refers to increasing the levels of bioavailable micronutrients using techniques like conventional breeding, biotechnological tools or agronomic procedures. Agronomic biofortification of crops is achieved through the application of mineral fertilisers to increase the concentrations of essential nutrients in the edible parts of plants.

Vegetables are low in calories and fats, cholesterol-free, rich in nutrients and packed with essential vitamins and minerals, and are rightly known as protective food as they protect the human body against many diseases, including cancer. A variety of highly nutritive vegetables are of great importance in combating malnutrition. Considering the importance of minerals in prevention as well curing of various human diseases, the acceptance of vegetables enriched with minerals is increasing. For fresh vegetables, the most effective way to increase the nutrient content before harvest is to use improved genotypes/varieties, or to adopt specific agronomic methods [15].

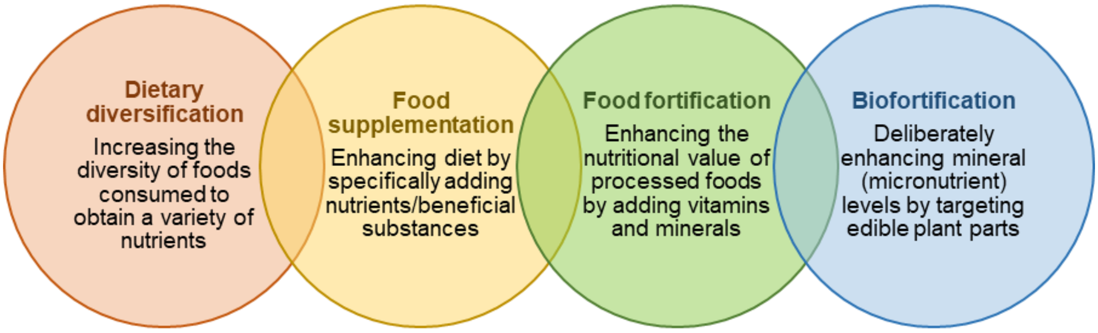

Fig. 1. Different approaches to alleviate malnutrition

The growing interest in enriching fresh vegetables with mineral elements has driven intensive research efforts aimed at developing suitable application methods. This review describes the progress made in agronomic biofortification of vegetables with particular emphasis to trace mineral elements like iron, zinc, molybdenum and copper, that are either absent or inadequately present in human diets. Following a discussion of their significance and role in human nutrition and plant physiology, this review aims to look at the best agronomic practices to enhance the amount of the aforementioned elements in the edible portions of vegetables.

2. VEGETABLES, HUMAN HEALTH AND BIOFORTIFICATION

Plant foods account for a significant portion of the human diet and provide most of the calories, nutrients and bioactive compounds required to maintain good health and prevent diseases. Vegetables constitute one of the main components of a good plant-based diet, which are particularly good sources of dietary fibre, phytochemicals including vitamins and antioxidants, and minerals [16,17]. Minerals are considered essential nutrients since humans cannot synthesise them themselves, and must derive them from food. Mankind has evolved through a diet rich in fruits and vegetables, and not eating enough vegetables is one of the main reasons for many non-communicable diseases. Moreover, vegetables play an important role in the economy, fighting poverty and malnutrition, because they can be grown locally and consumed in a wide variety of shapes, sizes, colours and tastes [18].

Suboptimal micronutrient intake and malnutrition, also known as ‘hidden hunger’, can be particularly serious for people who follow restrictive diets for moral, ethical or medical reasons [6,7,19]. The recommended dietary allowance (RDA) and tolerable upper limits (UL) have been the foundation for establishment of dietary reference intakes (DRI) by health authorities. In general, strategies to address vitamin or mineral deficiencies should focus on meeting the RDA for each component without surpassing the UL [20]. However, the actual contribution of minerals to the human comes from more than just how much of them are present in a particular plant tissue. They must be released from the food matrix as they pass through the gastrointestinal (GI) tract in order to be absorbed into the blood and transported to target tissues. In fact, only a fraction released by the plant tissue is ultimately available for absorption. This fraction is designated as ‘bioavailable’ or ‘bioaccessible’, and increasing the bioavailability of vitamins and minerals in plant tissues is a promising target of agronomic strategies to improve the nutritional quality of vegetables [21]. Vegetable consumption is predicted to increase in the coming years due to the growing concerns about health and sustainability. There will be a greater need for sustainable food supplies to feed the growing population [22]. Tomato, cucurbits (cucumber, pumpkin and squash), bulbs (onion, shallot and garlic), pepper (hot and sweet), brassicas (cabbage and cauliflower), spinach and carrot are the most significant vegetables in the global economy today. Therefore, it makes sense to focus on biofortification of these vegetables.

Biofortification is an effective crop-based approach to address the problem of mineral malnutrition by enriching crops and food products with bioavailable nutrients. Among the various strategies used to obtain biofortified vegetables, there are agronomic and genetic approaches, the latter of which can be carried out by conventional breeding, or by transgenic methods [23]. The goal is to increase the content of minerals, or other health-related compounds like vitamins, antioxidants etc., in the edible portion. Transgenic approach involves biotechnological studies that genetically modify a species to obtain plants with targeted trait, such as higher concentrations of specific nutrients. Although this approach can prove cost-effective in the long run, it is currently the least used method because the research and development phase is still very slow and expensive. In the same spirit, it is possible to cross different genotypes with the aim of introducing desirable characteristics naturally present in plants into new cultivars, but the limitation is finding the desired traits in the available genetic resources [24]. On the contrary, breeding programmes, although effective, can have their effects eliminated due to increased chances of varietal turnover. Therefore, biofortification programmes conducted through the agronomic approach are the best option, as they involve simple methods to accumulate and/or stimulate the production of specific compounds at the plant level.

3. AGRONOMIC BIOFORTIFICATION OF VEGETABLE CROPS

The application of NPK fertilisers to increase crop yields is essential to feed the growing global population and address the problem of hunger in developing countries. However, trace elements such as Zn, Fe and Mo are also crucial for plant growth and human health [25]. These micronutrients are easily accessible to plants and thus, end up as a component of the food chain. However, when plants cannot easily absorb these nutrients, they must be incorporated into the plant system through biofortification programmes [26]. Agronomic biofortification is the simplest method for enriching food crops with necessary trace elements [27,28]. As a result, agronomic biofortification is particularly useful in developing countries as a strategy to increase crop performance and stimulate concentration of trace elements in plant tissues. One of the advantages of this method is also the rapid response of the crop due to the high bioavailability of the supplied trace elements [29].

The application of mineral fertilisers and improving the mobilisation and dissolution of mineral elements in the rhizosphere are the two main agronomic approaches to increase mineral concentration in edible plant organs. Vegetables are usually grown in high-input agricultural systems characterised by a high intensification of production processes, and where food supply largely relies on seed soaking, fertigation, soilless cultivation and foliar fertilisation. These capabilities provide multiple avenues for implementing targeted biofortification programmes [30]. The availability of nutrient elements to the plant can cause some interference when the mineral elements are supplied through soil fertilisation, therefore, the choice of mineral forms and their concentrations are important considerations [31].

Soilless cultivation is as alternate method of compensating for the low availability of soil minerals to the plant. This method involves continuous root contact with nutrient solution, which improves nutrient uptake, translocation and accumulation, thereby ensuring consistent results for nutritional quality [32,33]. For example, it has been observed that hydroponics can be the best option for increasing the nutrient content of plant tissues [34]. For minerals that are not easily translocated to the edible tissues, such as for crops grown on soil and/or for poorly mobile minerals, foliar fertilisation serves as an alternative [35]. The fact that fertiliser formulations and application methods are not crop-specific gives agronomic biofortification a major advantage over genetic/transgenic approaches.

4. NUTRIENT DYNAMICS IN BIOFORTIFICATION PROGRAMMES

Agronomic biofortification can have direct or indirect effects on crops. While the direct effect is associated with an increase in the concentration of mineral elements in the plant tissues, crop yield and quality constitute the indirect effects. It is important to take into account both of these effects when implementing agronomic biofortification programmes. Information regarding response of the plant to the consumption of nutrients is very important when determining the dosage, method and time of application. Furthermore, an understanding of the effects of biofortification may be helpful in determining specific results for crop yield and quality by optimising the biofortification process. The direct and indirect effects of four important micronutrients–iron, zinc, molybdenum and copper–which are most commonly utilised in agronomic biofortification programmes, are discussed below.

4.1 Iron (Fe)

Iron is the second most prevalent metal present in the earth's crust. The primary function of Fe in human health is related to the synthesis of haemoglobin and myoglobin [36]. It is also essential for a number of metabolic processes, including transport of oxygen, synthesis of DNA and transport of electrons [37]. Furthermore, it plays an important role in the nervous system, immune cell functioning and homeostasis, and is required for energy metabolism and physical activities, like exercise [38]. Fe deficiency is usually characterised by weakness, exhaustion, difficulty in concentrating, motor and mental impairment, and anaemia [39]. Fe deficiency is also one of the most responsible factors for diseases worldwide [40]. The RDA for Fe ranges between 8 and 18 mg day^{-1}, and for adults, the UL is 45 mg day^{-1} [41].

Fe is considered an indispensable element for plant growth and development, and is the third most limiting nutrient for plants. In fact, it is essential as a cofactor of many enzymes as well as for several vital cellular processes, including respiration, photosynthesis, chlorophyll biosynthesis and nitrogen fixation. Despite the fact that Fe is essential to plants, it is estimated that approximately 30% of cultivated land do not have the optimal pH and aeration conditions to promote plant uptake of Fe. Following absorption, transpiration pull and root pressure drive Fe through the xylem from the roots to the plant organs, mainly in a citrate complex [42]. In cases of Fe deficiency in plants, the leaves develop chlorosis. Plants have evolved two strategies to acquire Fe from the growth media in order to overcome Fe deficiency: Strategy I plants rely on reduction of Fe, whereas Strategy II plants chelate Fe with organic ligands [43]. In most vegetable crops (Strategy I plants), the ferric ion (Fe^{3+}) is chelated on the root surface by organic acids and phenolic compounds released by roots, which is subsequently reduced to its ferrous (Fe^{2+}) form to transport the element across the plasmalemma of root epidermal cells. Due to its poor solubility, high reactivity and excessive cytotoxicity, Fe cannot move freely inside the plant system [41], and therefore, needs to be bound to a chelating molecule. Fe is transported inside the plant system in chelated forms, mostly with citrate and malate in the xylem, and nicotinamine and its derivatives in the phloem [44-46]. Chloroplasts are the primary pool of Fe in cells, accumulating between 80 and 90% of this element [25]. While Fe requirement varies greatly among species, its concentration in leaves ranges between 50 and 150 mg kg^{-1} DW (dry weight).

There is currently a lack of knowledge regarding Fe biofortification in vegetable crops. Despite the fact that Fe is widely distributed throughout the earth's crust, its phytoavailable concentration (10^{-17} M) falls short of the ideal range for plant growth (10^{-9}-10^{-4} M) [47]. Once applied through fertilisation, Fe immediately becomes unavailable to roots for absorption due to formation of compounds such as hydroxides, oxyhydroxides and oxides, as a result of precipitation and oxidation, particularly in alkaline and calcareous soils [48]. Because of this, using a chelate form is preferred and recommended when undertaking Fe fertilisation as it shields the Fe ion from oxidation and, as a result, from insolubilisation [25]. Alternatively, even though a high degree of cuticle fixation can be observed on adopting chelated or sulphate-salt form, Fe can also be also supplied via the leaves through foliar spray [49]. However, due to its photosensitivity, Fe-EDDHA chelate should be avoided in this scenario [50].

In a comparison between different sources of Fe, viz. Fe-EDTA, $FeSO_4.7H_2O$ and $Fe_2(SO_4)_3$, Dukpa et al. [51] found $FeSO_4.7H_2O$ solution to be the best in terms of growth, yield and Fe accumulation in water spinach. On the other hand, Kromann et al. [52] did not observe any positive correlation between foliar application of Fe with Fe-EDTA and its concentration in potato tubers. They conjectured that the limited effect was related to the form of Fe applied. The Fe content of lettuce leaves grown in soilless condition was effectively increased from 2.31 mg kg^{-1} FW (control) to 4.30 mg kg^{-1} FW with the use of Fe-EDDHA at 2.0 mM (112 mg L^{-1}) [53]. Nonetheless, a 25% yield drop was noted, and this reduction increased in direct proportion to the amount of Fe added to the nutrient solution. On the other hand, Fe toxicity, which is documented at concentrations greater than 500 mg kg^{-1} DW, is typically associated with the production of reactive oxygen species (ROS) and, in turn, with the synthesis of antioxidative enzymes like ascorbic acid peroxidase, and Fe-binding proteins [54], in addition to causing damaging membranes and permanently impairing DNA, proteins and cellular structure. Giordano et al. [54] reported that when lettuce plants grown in a soilless system were subjected to Fe at concentrations more than 0.5 mM, there was significant reduction in leaf area, fresh and dry biomass, and light use efficiency. In the same way, Buturi et al. [55] observed that, in comparison to the control, there was a significant reduction in total dry biomass in lettuce, and increase in dry matter content, chlorophyll, total phenols, anthocyanins, flavonoids, carotenoids, ascorbic acid, antioxidant activity, proline and malondialdehyde.

In general, there is ongoing study on Fe biofortification, but not enough has been explored to draw a firm conclusion. The main limitations to Fe biofortification include factors such as (i) significant insolubilisation in the soil, (ii) restricted translocation into the plant and accumulation into edible organs, (iii) association of Fe fortification with antinutritional factors (ANFs) like tannins, phytic acid and phenolic compounds, which are difficult to remove from the plants, and (iv) detrimental effects on yield [56].

4.2 Zinc (Zn)

After iron, zinc is the second most abundant transition metal found in living organisms. It is a vital microelement for human and plant nutrition, and its deficiency is highly widespread in plants and humans. It is important for maintaining the structure and function of many enzymes (it is the only mineral nutrient that is involved in all enzyme classes). It affects cell differentiation, glucose utilisation and insulin production [57], in addition to being associated with reproductive health, egg fertilisation, immune system functioning and neurotransmitter signalling [58,59]. Zn has an RDA of 9 to 14 mg day^{-1} and a UL of 40 mg day^{-1} for adults [60].

Zn is essential for the development and functioning of chloroplasts and the repair of photosystem I. It also participates in the activation process of several enzymes, protein synthesis and metabolism of carbohydrates, lipids and nucleic acids [61]. Although most of the agricultural soils contain sufficient Zn to sustain its accumulation at plant-edible doses (10-100 mg kg^{-1}), root uptake often limits Zn availability to plants. Thus, it is estimated that approximately one-fifth of the world’s population is actually deficient in Zn [62]. Zn toxicity is less common than Zn deficiency, yet plants begin to exhibit symptoms of toxicity, including chlorosis, stunting and oxidative stress, when Zn concentration in leaf reaches between 100 and 700 mg kg^{-1} DW [25]. Zn toxicity is common in anthropogenically contaminated soils resulting from activities like mining, smelting and application of sewage sludge, especially if their pH is low [63,64]. Due to competition between Zn and other metals for transport and protein-binding sites, which results in impaired Fe absorption and disrupted Fe allocation in the body, symptoms of Zn toxicity are primarily associated with secondary Fe or Mn deficiency [65-67]. Similarly, it has been demonstrated that Zn nutrition influences copper (Cu), sulphur (S), phosphate (PO_4^{3-}) and nickel (Ni) homeostasis, as well as cadmium (Cd) concentration and transport [68,69]. Therefore, it makes sense to consider Zn nutrition within the multidimensional framework of mineral nutrient balancing.

Zn is taken up by the plant roots in the form of Zn^{2+} ions or as organic acid chelates, and subsequently transported to the above-ground organs via xylem [70]. Within the plant, xylem loading occurs either through symplast or apoplast, while Zn is delivered in xylem sap in ionic form or as metal complexes with asparagine, histidine, organic acids and nicotinamine [71]. Likewise, the redistribution of phloem Zn to different organs is believed to be associated either as Zn^{2+} or complexed with nicotianamine, malate or histidine. Due to limited (low) phloem mobility, plants supplied with Zn show a decrease in its concentration in the order: shoot ≈ root > fruit/seed/tuber, indicating a penalty for phloem-fed organs [72]. Therefore, it is believed that root vegetables and leafy vegetables have a higher capacity to increase dietary intake of Zn [62]. $ZnSO_4$, ZnO and synthetic chelates such as Zn-EDTA, Zn-DTPA or Zn-HEEDTA are examples of common inorganic Zn fertilisers.

Although the ability of plants to accumulate Zn in tissues varies widely, most crops generally require leaf Zn concentrations above 15-30 mg kg^{-1} DW to obtain maximum yield. Plants with Zn deficiency exhibit symptoms like interveinal chlorosis, root apex necrosis, internode shortening, epinasty, leaf curling and decreased leaf area. However, depending on the species and duration of exposure, symptoms of phytotoxicity are typically observed at concentrations greater than 0.1-0.7 g kg^{-1} DM (dry matter) [62]. When toxic levels are reached, plants exhibit various heavy metal stress responses, including inhibition of growth and yield, leaf chlorosis and necrosis, limited stomatal conductance and CO_2 fixation, and changes in chlorophyll structure and concentration [73], thus a higher threshold concentration actually represents the physiological limit of biofortification achievement.

The effects of Zn application also depend on the type of application–soil or foliar. Pandey et al. [74] observed that increased concentrations of Zn in potato tubers can be achieved more effectively with foliar treatment, compared to soil application. Likewise, Rivera-Martin et al. [75], in a study on biofortification of broccoli through soil and foliar application, revealed that the crop acquired more Zn when $ZnSO_4$ was given both topically and subsurfacely. Recently, Zn nano forms have also been researched and applied in biofortification programmes. This form is favoured because of its high absorption efficiency, high water solubility and ease of removal by plants. Regarding this, Solanki and Laura [76] established that granular $ZnSO_4$ is less effective than the corresponding nano form. In fact, the aim of Zn biofortification is to reduce the particle size of Zn and thereby improve its absorption efficiency. Research studies suggest that spinach [63], beetroot [77] and pak choi [78] are hyperaccumulators of Zn.

Since leafy vegetables can over-accumulate Zn, this ability has been extensively explored in biofortification protocols. de Sousa Lima et al. [79] reported up to 28-fold increase in Zn concentration of kale on application of 300 mg Zn kg^{-1} soil. Mao et al. [80] detected a significant increase (200%) in Zn content in the edible parts of cabbage following soil application of 22.7 kg Zn ha^{-1} (as zinc sulphate, $ZnSO_4.7H_2O$). Zn biofortification was successfully performed on arugula using 1.5 kg ha^{-1} $ZnSO_4.7H_2O$ as foliar spray, increasing the foliar Zn concentration by 94% [81]. Among leafy vegetables other than brassicas, Barrameda-Medina et al. [82] recorded a 251% increase in leaf Zn concentration in hydroponically grown lettuce plants supplemented with 100 μM $ZnSO_4.7H_2O$ in the nutrient solution. At the same time, in biofortification programmes, it should be taken into account that high Zn content in crops grown in the soil can negatively affect Fe absorption [79].

4.3 Molybdenum (Mo)

Molybdenum is an essential trace element for human health and survival. Worldwide recommendations for daily intakes of Mo vary, with diet being the primary source of Mo. It is present in foods as soluble molybdates and is needed in small amounts, typically less than 100 mg day^{-1} [83].

Mo is an essential micronutrient for plant growth and development [84,85]. It is normally found in soils at relatively high concentrations (0.2-6.0 mg kg^{-1}) to meet plant requirements; however, it is considered one of the rarest transition elements [86]. Plants take up Mo in the form of molybdate (MoO_4^{2-}), which is also the most prevalent soluble form in soils, and the most efficient form utilised in agronomic biofortification protocols [13,84]. But in plants, the MoO_4^{2-} form only serves as a component of the pterin complex called molybdopterin, which is responsible for producing the Mo cofactor (Moco) [87,88]. Mo-related enzymes play a vital role in fundamental metabolic processes, including the synthesis of phytohormones, purine metabolism, sulphite detoxification and nitrate assimilation [89,90]. The two most important Mo-related enzymes are nitrate reductase and aldehyde oxidase. Nitrate reductase is essential for the conversion of nitrate to nitrite, so without sufficient Mo, nitrogen assimilation would not be feasible. Aldehyde oxidase partakes in the biosynthesis of hormones such as abscisic acid and indole-3-acetic acid, which regulate plant growth and development [85,91]. Additionally, Mo is involved in the biosynthesis pathway of chlorophyll a and b. Without enough Mo, chlorophyll production drops, negatively affecting both crop yield and quality [92]. Furthermore, Mo nutrition has been reported to promote nitrogen use efficiency and nitrate reduction [93], and that its deficiency may result in higher nitrate levels in plant tissues and a reduction in plant growth and yield. Consequently, it stands to reason that an increased availability of Mo can presumably reduce the nitrate content, especially in leafy vegetables. Plants grown in acidic soils with excessive watering often exhibit symptoms of Mo deficiency, such as reduced growth and yield, and yellowing of the leaves, which can be difficult to distinguish from N deficiency. To improve plant health, applying Mo fertilisers and adjusting soil pH can be beneficial. Nonetheless, farmers rarely incorporate this micronutrient in their standard fertilisation schedules for open field cultivations. As a result, Mo concentration in plants could be quite low and not sufficient enough to guarantee positive effects on human health [86,94], particularly when plants are grown in soilless systems. In that case, agronomic biofortification presents a potential and alternative approach to increase the Mo content of edible plant parts, in order to benefit human health [95-98].

Sabatino et al. [84] observed that Mo biofortification enhanced plant performance, yield and quality in different tomato varieties. It also resulted in higher total yield, marketable yield, above-ground biomass, plant height, polyphenol levels, ascorbic acid, soluble solids content, and both N and Fe content in the fruit, compared to the control. According to Moncada et al. [99], 100 g of leaves of lettuce, curly endive and escarole grown in hydroponic floating system with nutrient solutions containing Mo yielded a maximum of 50, 268 and 402 µg Mo 100 g^{-1}, respectively, without no effect on their yield, morphological traits and colour. Likewise, La Bella et al. [100] observed that biofortification with 8 µmol Mo L^{-1}, in form of sodium molybdate (Na_2MoO_4), through foliar spray, notably increased the leaf Mo concentration compared to the control. According to Sabatino et al. [13], Mo biofortification through foliar spray, in form of Na_2MoO_4, was found to significantly improve the nutritional quality of 'Canasta' lettuce, leading to an increase in bioactive compounds such as ascorbic acid and the content of soluble solids. A great improvement in terms of Mo and N concentrations was recorded with the dosage of 6 µmol Mo L^{-1}. In cherry tomatoes, Mo biofortification combined with the application of arbuscular mycorrhizal fungi (AMF) inoculation resulted in enhanced fungal colonisation, increased plant height, elevated levels of lycopene and ascorbic acid, and higher concentrations of Fe, Cu and Mo in the fruit [101]. It was also observed that AMF inoculation increased efficiency of Mo biofortification by 12.8%. Regarding the effect of the type of application, soil (banding) or foliar, Mondy and Munshi [102] observed that while the Mo concentration in potatoes grown on plots treated with foliar spray of Na_2MoO_4 increased efficiently, it did not reach toxic levels for human consumption.

4.4 Copper (Cu)

Copper is a member of the 'heavy metal' group along with lead, arsenic, mercury, cadmium etc. It is an essential trace element related primarily to the functions of enzymes, and also helps to maintain cardiovascular integrity, lung elasticity, normal development of connective tissue and nerve coverings. In addition, it also has neuroendocrine and immune functions, and is involved in the Fe metabolism [103]. The RDA for Cu ranges between 1.0 and 1.6 mg day^{-1}, with 10 mg day^{-1} being the UL for adults [60]. Cu deficiency in early part of pregnancy can cause serious organ damage in the developing foetus, and if it persists, it can lead to neurological as well as immunological disorders in the newborn. Conversely, increased concentrations of Cu predispose to various pathological conditions and, in severe cases, can lead to death.

Cu is a redox-active transition metal that, under physiological conditions, exists in two states–the reduced Cu^+ (cuprous) state and the oxidised Cu^{2+} (cupric) state [104,105]. Plants use Cu as a cofactor for a variety of proteins involved in several physiological processes, such as photosynthesis, mitochondrial respiration, carbohydrate metabolism, ethylene perception, superoxide scavenging, cell wall remodelling and formation of phenolics in response to pathogen attack [106-108]. About 90% of the Cu proteins that are discovered in nature function as oxidoreductases [109]. Plastocyanin, a protein required for photosynthetic electron transport in chloroplasts, that carries electrons from the cytochrome b6f complex to PS I, is the most prevalent Cu protein in plants [110,111]. Cu is a component of metabolic pathways that provide energy for cellular processes [112]. As a trace element, an optimum amount of Cu is required to ensure proper cellular activity, but in excess, it can negatively impact plant growth, production and survival [107,113,114].

Cu occurs naturally in soils with contents ranging from 60 to 125 mg kg^{-1} [115], with an average value of 14 mg kg^{-1} globally [116]. It is mobile in soil and its concentration in the soil solution directly affect its absorption. Plants can absorb huge amounts of Cu by their roots and in smaller amounts by shoots and leaves, in the form of Cu^{2+} or Cu chelate, and despite being poorly mobile in plants, it can be translocated from old leaves to young/new leaves. Cu absorption is facilitated by transporters present in the plasma membrane of root cells, viz. P-type ATPase copper transporters, COPT copper transporters, ZIP (zinc-iron-regulated transporter-like proteins) family transporters and NRAMP (natural resistance-associated macrophage proteins) family transporters. After being absorbed by the roots, Cu can be transported via the xylem to the shoot in the form of Cu^+ and Cu^{2+}. Crop species typically have a tolerance limit of 20-30 mg kg^{-1} DW of Cu in leaves; however, Cu-tolerant species can accumulate up to 1000 mg kg^{-1} DW of Cu in leaves [25].

Obrador et al. [117] studied the effects of Cu biofortification on 'Viroflay Esmeralda' spinach, by applying eight different liquid fertilisers to the soil surface, along with irrigation water, at concentrations ranging from 0 to 3 mg Cu kg^{-1}

soil. They observed that, when plants were exposed to 3 mg Cu kg^{-1} soil (as Cu-EDTA), the total Cu concentration in the dry matter of shoots increased by up to 450%, from 9.55 mg kg^{-1} (control treatment) to 52.51 mg kg^{-1}; however, there was a 10% reduction in the dry matter yield. On the other hand, 1 mg Cu kg^{-1} soil exhibited a 153% increase in Cu concentration and a 71% increase in yield over the control. In terms of the chemical form, the results demonstrated that Cu-DHE and, particularly, Cu-EDTA were the most effective fertilisers for enhancing the Cu content in the edible portion of spinach. Fortis-Hernández et al. [118] noted a positive trend between the increase in concentration of the applied copper nanoparticles (NPs Cu) and the increase in concentration of Cu in fruit pulp of hydroponically grown melon. Among five different doses of NPs Cu (0, 1.8, 3.6, 5.4, 7.2 and 9.0 mg L^{-1}), applied as foliar spray, 9.0 mg L^{-1} NPs Cu yielded the highest Cu concentration (5.39 mg kg^{-1}) in the melon fruit pulp. Likewise, in lettuce, Fortis-Hernández et al. [119] reported the highest leaf Cu content with 20 mg L^{-1}, with an average of 9.93 µg kg^{-1} DW. However, Xiong et al. [120] revealed that the foliar uptake, biotransformation and effects of NPs-CuO in lettuce, where the foliar application of 100 and 1000 mg L^{-1} concentrations enhanced the Cu content in leaves and root of the plant, could be toxic for human consumption. In context of Cu biofortification, it is important to consider how crop rotations and soil biological characteristics affect the release of Cu in the soil substrate.

5. RESEARCH GAPS AND FUTURE PROSPECTS

Compared to other approaches, agronomic biofortification is comparatively easier to implement, and is perhaps best suited for immediate results. Based on the facts presented above, it is evident that biofortification is, in many circumstances, a promising strategy to fighting malnutrition. However, there is a lot of existing research on agronomic biofortification for only a few vegetable crops, such as tomato, lettuce, spinach and *Brassica* spp., and for some specific minerals. For some mineral elements included in this review, that are crucial to human nutrition, e.g., Fe, information is still lacking. Nevertheless, biofortification is not economically beneficial even in cases where experimental evidence for the practice indicates a notable increase in the concentrations of mineral elements. Additionally, frequent and regular applications are necessary for an effective biofortification process, and negative environmental impacts cannot be excluded.

When biofortification factors are applied, some concerns about how other factors interact at the soil level (e.g., phytoavailability) and plant level (e.g., competition with other elements) arise. In many studies, foliar application, which can be more economical and ecofriendly, is substituted for traditional fertigation methods. Furthermore, the market is limited to a few biofortified products. In the future, in addition to a diverse choice of vegetables, the market is expected to offer multi-mineral biofortified products. Therefore, research involving concurrent or combined biofortification is essential.

The findings of the literature indicate that while biofortification may not be able to completely treat or eliminate mineral deficiencies, it can enhance other interventions aimed at providing micronutrients to humans. The success of a biofortification programme depends on appropriate planning that includes health and nutrition surveys, nutritional habits, design and validation of sustainable biofortification methods, and an estimation of the beneficial effects on human health. In the reviewed literature, most of the specific elements present in the edible part of plants have received attention, but important concepts like bioaccessibility and bioavailability have rarely been brought up. Substances that stimulate or inhibit bioavailability should be specifically considered in order to modulate mineral bioavailability.

6. CONCLUSION

While maximising crop yields is the primary objective of modern agriculture, many disorders that affect human health are related to mineral and nutritional deficiencies. The agricultural industry is becoming increasingly interested in biofortification programmes as a means of overcoming this drawback. Agronomic biofortification offers a quick and affordable way to correct dietary deficiencies of many nutrients. Factors like the efficiency of fertilisation process and mineral bioavailability, the high cost of some specific chemical formulations, the possible yield losses due to changes in plant metabolism due to biofortification, and the potential environmental or health impacts arising from new agronomic protocols (e.g., in the case of Cu) will be the main challenges confronting agronomic biofortification in the near future. As this review reports, promising results have been achieved with biofortification of various trace elements in many vegetables; however, the outcomes are not entirely coherent and consistent. Considering this, and regardless of the specific scientific relevance, future achievements should be planned from a broader perspective, involving farmers, traders, extension specialists, agronomists, nutritionists and educators, assuming methodologies with the ultimate goal of positively influencing eating habits, increasing the consumption of target vegetables and improving human diet.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT etc.) and text-to-image generators have been used during writing or editing of the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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