Review Article

Agronomic Biofortification of Vegetable Crops–A Systematic Review

ABSTRACT

The diets of more than two-thirds of the world's population are deficient in one or more essential mineral elements. This problem can be remedied by diversifying the diet, supplementing minerals, enriching foods or increasing the concentration and/or bioavailability of mineral elements in products (i.e., biofortification).Vegetables represent the backbone of good nutrition as they provide important phytochemicals such as dietary fibre, vitamins, antioxidants and minerals, and biofortification offers a promising strategy to increase the content of these compounds. Since minerals play an important role in human diet and metabolism, the possibility of enriching fresh vegetables, by applying specific agronomic methods, has been considered. This review examines in detail, the latest findings on agronomic biofortification of vegetables, aimed at increasing the content of important micronutrients, such as iron, zinc, molybdenum and copper, in the edible parts, focusing on the direct and indirect effects of this strategy. Although agronomic biofortification is considered a feasible technique, the approach is complex 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

The global population is expected to reach 8.54 and 9.74 billion by 2030 and 2050, respectively. Concomitantly, worldwide emergencies such as climate change [1,2] and pandemics are making agriculture vulnerable, which are further exacerbating the challenges for global food security [3]. Human malnutrition has severe socio-economic implications, especially in the developing and underdeveloped countries, where people cannot afford to eat a balanced diet. Most of the diets (cereal-based) are rich in carbohydrates; however, the question of 'hidden hunger' still persists due to our inability to satisfy micronutrient necessities [4]. To maintain good health, people need certain mineral nutrients that must be included in their diet. The essentiality of minerals is evident by the fact that vitamins cannot be absorbed individually, or work in the absence of certain minerals, which are important in many physicochemical processes. The lack of specific mineral elements affects two-thirds of the world's population, in both industrialised and underdeveloped countries [5-7], and malnutrition can have a negative impact on human health [8]. For example, in Europe and Central Asia, malnutrition problems associated with diets low in micronutrients increase the number of women and children with anaemia. In addition, a study carried out in southern Italy showed that the population had a low intake of calcium and potassium [9].

There exist several approaches to address malnutrition (Fig. 1.), viz. dietary diversification, food supplementation, food fortification and biofortification, each of which has its own advantages and disadvantages. Dietary diversification and food supplementationare attractive options in terms of protein, mineral and vitamin intake, but they are not possible in many socio-economic contexts [10]. For socio-economic groups with limited access to expensive and commercially-marketed fortified foods, biofortification of edible crops is one of the most promising, efficient, sustainable and cost-effective strategies in combating mineral malnutrition in humans [11-14]. Biofortification refers to increasing the levels of bioavailable micronutrients using methods such as conventional breeding, biotechnological tools or agronomic approaches. Agronomic biofortification of crops is achieved through the application of mineral fertilisers to increase the concentrations of essential nutrients in edible parts of plants.

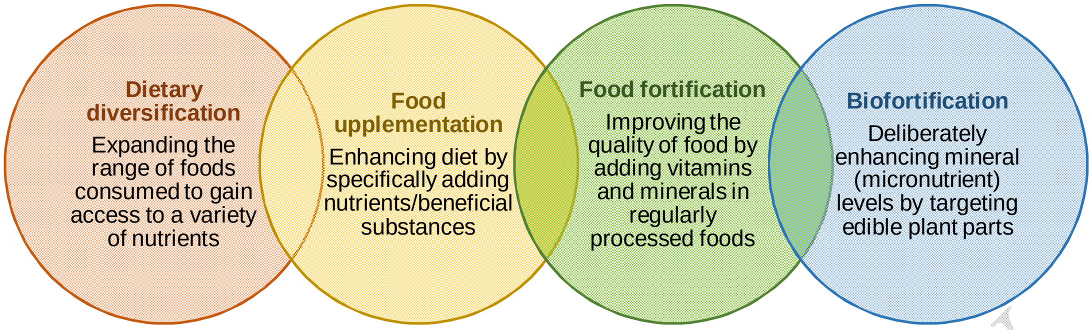

Fig. 1.Different approaches to alleviate malnutrition

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 only way to enhance the nutrient content before harvest is to use improved genotypes/varieties, or to adopt specific agronomic methods [15].

The growing interest in enriching fresh vegetables with mineral elements has prompted intensive research efforts focused on the development of appropriate application methods. This review describes the advances in agronomic biofortification of vegetables with reference to some mineral elements that are absent or not adequately present in human diets, such as iron, zinc, molybdenum and copper. After considering their role in human nutrition and plant physiology, this review aims to discuss the most successful agronomic strategies to increase the amount of the considered minerals in the edible portion of vegetables.

2. VEGETABLES, HUMAN HEALTH AND BIOFORTIFICATION

Plant foods make up 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 are one of the pillars of a good plant-based diet, especially providing dietary fibre, phytochemicals such as vitamins and antioxidants, and minerals [16,17]. Minerals are considered essential nutrients–humans cannot synthesise them themselves, and must get them from food. Mankind has evolved through a diet rich in fruits and vegetables, and not eating enough vegetables is one of the reasons for many non-communicable diseases. Moreover, vegetables play a crucial 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 religious, ethical or medical reasons [6,7,19]. Health authorities have established dietary reference intakes (DRI) based on the recommended daily allowance (RDA) and tolerable upper limits (UL). In general, strategies to address vitamin or mineral deficiencies should aim to achieve the RDA for each component without exceeding the UL [20]. However, the actual contribution of minerals to the human diet is not related solely to their concentration in a given plant tissue. They must be released from the food matrix as they pass through the gastrointestinal (GI) tract, 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 phytochemicals and minerals in plants is a promising target of agronomic strategies to improve the nutritional quality of vegetables [21]. Vegetable consumption is expected to increase in the coming years due to growing concerns about health and sustainability. To cope with the growing global population, more sustainable food sources will be required [22]. The most important vegetables in the global economy today are tomato, cucurbits (cucumber, pumpkin and squash), bulbs (onion, shallot and garlic), pepper (hot and sweet), brassicas (cabbage and cauliflower), spinach and carrot. Hence, it is reasonable to focus biofortification efforts on these species.

Biofortification is an effective crop-based approach to address the problem of hidden hunger 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 specific 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 content 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 other hand, breeding programmes, even when effective, can have their effects eliminated due to high rates of varietal turnover. Therefore, biofortification programmes carried out through the agronomic approach are the best option, as they involve simple methods to accumulate or stimulate the production of specific compounds at the plant level.

3. AGRONOMIC BIOFORTIFICATION OF VEGETABLE CROPS

The use of NPK fertilisers to increase crop yields is essential to feed the growing global population and address the problem of hunger in underdeveloped countries. However, it is also known that trace elements such as Zn, Fe and Mo have important role in plant growth and human health [25]. A large portion of these micronutrients are easily accessible to plants and thus, become 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 useful trace elements [27,28].Accordingly, agronomic biofortification is particularly useful in developing countries as a strategy to increase crop performance and stimulate tissue concentration of trace elements. 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].

Agronomic approaches to increase mineral concentration in edibleplant organs are usually based on the supply of mineral fertilisers and/or improving the mobilisation and dissolution of mineral elements in the rhizosphere. Vegetables are usually grown in high-input agricultural systems characterised by a high intensification of production processes, and where food supply is largely based on fertigation, seed soaking, soilless cultivation and foliar fertilisation. These capabilities offer different opportunities 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].

One alternative strategy to overcome the low availability of soil minerals to the plant is soilless cultivation, in which continuous root contact with nutrient solution enhances 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 main advantage of agronomic biofortification over genetic approach is that the forms and application methods of fertilisers are not crop-specific.

4. NUTRIENT DYNAMICS IN BIOFORTIFICATION PROGRAMMES

The effects of agronomic biofortification on crops are direct and/or indirect. The direct effect corresponds to an increase in the concentration of mineral elements in the plant tissues, whereas crop yield and quality constitute the indirect effects. These two effects must be taken into consideration when going for agronomic biofortification programmes. Information regarding reaction of the plant to the consumption of nutrients forms a very crucial aspect in planning the dosage, method and time of application. Moreover, knowledge of the direct and indirect effects of biofortification can be useful in determining specific results in terms of crop yield and quality by optimising the biofortification process itself. Discussed below are the direct and indirect effects of fouressential micronutrients, viz. iron, zinc, molybdenum and copper, which are most commonly used in agronomic biofortification programmes.

4.1 Iron (Fe)

Iron is the second most abundant metal present in the earth's crust. The main function of Fe in human health is related to the synthesis of haemoglobin and myoglobin [36]. In addition, it is essential for many metabolic processes such as oxygen transport, deoxyribonucleic acid (DNA) synthesis and electron transport [37]. It also plays an important role in the nervous system, immune cell functioning and homeostasis, and is required for energy metabolism and exercise [38]. The symptoms of Fe deficiency usually include weakness, fatigue, 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 of Fe ranges between 8 and 18 mg day^{-1}, whereas the UL for adults 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, and for several vital cellular processes such as respiration, chlorophyll biosynthesis, nitrogen fixation and photosynthesis. Even though Fe is very important to plants, it is estimated that about 30% of arable land do not have the optimal pH and aeration conditions to promote its uptake by plant. After absorption, Fe is transported from the roots to the plant organs through the xylem, driven by the transpiration pull and root pressure, mainly in a citrate complex [42]. If the plant is deficient in Fe, its leaves develop chlorosis. To overcome Fe deficiency, plants have developed two strategies to acquire Fe from the growth substrate, based either on its reduction (Strategy I plants), or chelation with organic ligands (Strategy II plants)[43]. In non-graminaceous species (Strategy I plants), such as most of vegetable crops, organic acids and phenolic compounds released by roots chelate ferric ion (Fe^{3+}) on the root surface, which is subsequently reduced to its ferrous form (Fe^{2+}) to transport the element across the plasmalemma of root epidermal cells. Inside the plant, Fe cannot move freely because of its poor solubility, high reactivity and excess cytotoxicity [41], and therefore, must be linked to a chelating molecule. The transportation of Fe within the plant system occurs in chelated forms, mainly with citrate and malate in the xylem, and nicotinamine and its derivatives in the phloem [44-46]. Chloroplasts represent the main pool of Fe within the cell, as they gather approximately 80-90% of cellular Fe [25]. Even though the range of Fe in leaves is between 50 and 150 mg kg^{-1} DW, Fe requirement is highly variable among species.

In vegetable crops, the knowledge concerning Fe enrichment, and specifically biofortification, is still poor. Although the earth's crust is rich in Fe, its phytoavailable concentration (10^{-17} M) does not reach the optimal range for plant growth (10^{-9}-10^{-4} M) [47]. Particularly in alkaline, calcareous and aerated soils, once applied through fertilisation, Fe quickly becomes unavailable to root absorption due to the formation of compounds such as hydroxides, oxyhydroxides and oxides, as a result of precipitation and oxidation [48]. For this reason, when Fe is supplied to crops, it is preferred to use a chelate form, which protects the Fe ion from oxidation and, consequently, from insolubilisation [25]. Alternatively, Fe can also be also supplied via the leaves through foliar spray even if, on adopting chelated or sulphate-salt form, a high degree of fixation by cuticle can be observed [49]. However, in this case, it is important not to use Fe-EDDHA chelate because of its photosensitivity [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. However, Kromann et al. [52] did not observe any positive relationship between foliar spray of Fe with Fe-EDTA and its concentration in potato tubers, and hypothesised that the limited effect was related to the form of Fe used. The use of Fe-EDDHA up to 2.0 mM (112 mg L^{-1}) was effective in soilless cultivation of lettuce in increasing the Fe content of the leaves from 2.31 mg kg^{-1} FW (control) to 4.30 mg kg^{-1} FW [53]. However, a 25% reduction in yield was observed, which increased proportionally with the amount of Fe added to the nutrient solution. On the other hand, Fe toxicity, reported in concentrations above 500 mg kg^{-1} DW, is usually related to production of reactive oxygen species (ROS) and, consequently, to the production of antioxidative enzymes, such as ascorbic acid peroxidase, and Fe-binding proteins [54], besides causing damages to membrane and irreversible impairment of cellular structure, DNA and proteins. Giordano et al. [54] reported that application of Fe at a concentration higher than 0.5 mM significantly reduced leaf area, fresh biomass, dry biomass and radiation use efficiency of lettuce plants cultivated in a soilless system. Likewise, Buturi et al. [55] stated that Fe application significantly reduced total dry biomass and increased dry matter content, chlorophyll, total phenols, anthocyanins, flavonoids, carotenoids, ascorbic acid, antioxidant activity, proline and malondialdehyde in lettuce plants compared to the control.

Overall, the research work on Fe biofortification is going on, and has not been explored enough to draw a strong conclusion. Factors such as (i) significant insolubilisation in the soil, (ii) limited translocation into the plant and accumulation into edible organs, (iii) association of Fe fortification with antinutritional factors (ANFs) such as tannins, phytic acid and phenolic compounds, which are difficult to remove from the plants, and (iv) negative effects on yield are the main constraints in Fe biofortification [56].

4.2 Zinc (Zn)

Zinc is the second most abundant transition metal in organisms after iron. 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 (the only mineral nutrient involved in all enzyme classes), and it also plays a key role in the synthesis of nucleic acids and proteins. It affects cell differentiation, glucose utilisation and insulin secretion [57], and is also associated with reproductive health, immune system functioning, neurotransmitter signalling and egg fertilisation [58,59]. The RDA for Zn ranges from 9 to 14 mg day^{-1}, while the UL for adults is 40 mg day^{-1} [60].

Zn is important in plant metabolism, because it plays a key role in the development and functioning of chloroplasts and the repair of photosystem I, in addition to participating 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}), Zn availability to plants is often limited by root uptake, so it is estimated that approximately one-fifth of the world's population actually suffers from Zn deficiency [62]. Zn toxicity is less spread than Zn deficiency; however, plants begin to experience symptoms of Zn toxicity, such as chlorosis, stunting and oxidative stress, when Zn concentration in leaf ranges between 100 and 700 mg kg^{-1} DW [25]. Zn toxicity is common in anthropogenically polluted soils resulting from activities like mining, smelting and sewage sludge application, especially if they have a low pH [63,64]. Symptoms of Zn toxicity are mostly related to secondary Fe or Mn deficiency due to competition between Zn and other metals for transport and protein-binding sites, resulting in impaired Fe absorption and disrupted Fe allocation in the body [65-67]. Similarly, Zn nutrition has been shown to affect Cu, S, PO_4^{3-} and Ni homeostasis, as well as Cd concentration and transport [68,69]. Therefore, it is appropriate to consider Zn nutrition in the multidimensional context of mineral nutrient balancing.

Zn is absorbed by the plant roots in the form of Zn^{2+} ions or as organic acid chelates, then it is transported via the xylem to the above-ground organs [70]. Inside the plant, xylem loading occurs either through symplast or apoplast, while in xylem sap, Zn is transported in ionic form or in the form of metal complexes with asparagine, histidine, organic acids and nicotinamine [71]. Similarly, the redistribution of phloem Zn to different organs is believed to be associated either as Zn^{2+} or in a complex with nicotianamine, malate or histidine. Due to low phloem mobility, Zn-applied plants show a decrease in Zn concentration in the order: shoot ≈ root > fruit, seed, tuber, suggesting a penalty for phloem-fed organs [72]. Therefore, root vegetables and leafy vegetables are thought to have greater potential to increase dietary intake of Zn [62]. Common inorganic Zn fertilisers include $ZnSO_4$, ZnO and synthetic chelates such as Zn-EDTA, Zn-DTPA or Zn-HEEDTA.

The ability of plants to accumulate Zn in tissues varies widely, but in general, most crops require leaf Zn concentrations above 15-30 mg kg^{-1} DW for maximum yield. Plants affected by Zn deficiency show interveinal chlorosis, root apex necrosis, internode shortening, epinasty, leaf curling and reduction of leaf area. However, symptoms of phytotoxicity are usually observed at concentrations greater than 0.1-0.7 g kg^{-1} DM, depending on the species and exposure time [62]. When toxic levels are reached, plants show various heavy metal stress responses, such as 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] found that foliar application of Zn is more effective than soil application in enhancing the Zn concentration in potato tubers. 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 studied and applied in biofortification programmes. This form is preferred due to its high absorption efficiency, as it is highly water soluble and easily removed by plants. In this regard, Solanki and Laura [76] reported that granular $ZnSO_4$ is less effective than the corresponding nano form. In fact, Zn biofortifications aim to reduce the particle size of Zn and thus, increase its absorption efficiency. There is evidence supporting that spinach [63], beetroot [77] and pak choi [78] are hyperaccumulators of Zn.

Leafy cabbages have been widely studied in biofortification protocols for their ability to over-accumulate Zn. In kale, de Sousa Lima et al. [79] reported up to 28-fold increase in Zn concentration when 300 mg Zn kg^{-1} soil was applied to the crop. After soil application of 22.7 kg Zn ha^{-1} (as zinc sulphate, $ZnSO_4.7H_2O$), Mao et al. [80] detected a significant increase (200%)in Zn content in the edible parts of cabbage. Zn biofortification by foliar spray was successfully performed on arugula using 1.5 kg ha^{-1} $ZnSO_4.7H_2O$, resulting in a 94% increase in foliar Zn concentration [81]. Among leafy vegetables other than brassicas, Barrameda-Medina et al. [82] recorded 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. Recommended daily intakes vary throughout the world, with Mo primarily obtained from the diet. Mo 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 typically found in soils at relatively high concentrations (0.2-6.0 $mgkg^{-1}$) 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 programmes[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 crucial Mo-related enzymes are nitrate reductase and aldehyde oxidase. Nitrate reductase plays a key role in converting nitrate to nitrite, so without sufficient Mo, nitrogen assimilation would be impossible. Aldehyde oxidase partakes in the biosynthesis of hormones such as abscisic acid and indole-3-acetic acid, which are crucial for controlling plant growth and development [85,91]. Additionally, Mo is involved inthe 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 an increasednitrate content in plant tissues and a decrease in plant growth and yield. Therefore, increased supply of Mo can presumably reduce the nitrate content, especially in leafy vegetables. Plants grown in acidic soils with excessive watering often show 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.However, farmers rarely incorporate this micronutrient in standard fertilisation programmes for open field cultivations. As a result, Mo concentration in plants could be quite low and not sufficient enough to assure beneficial effects on human health [86,94], especially when plants are cultivated in soilless systems. In that case, agronomic biofortification is a potential and alternative method for increasing Mo content in the edible plant organs, to obtain a positive effect on human health [95-98].

Sabatino et al. [84] observed that Mo biofortification improved 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. Moncada et al.[99] reported that 100 g of leaves of lettuce, curly endive and escarole grown in hydroponic floating system with nutrient solutions containing Mo provided a maximum of 50, 268 and 402 µg Mo 100 g^{-1}, respectively, without any impact on their yield, morphological traits and colour. Likewise, La Bella et al. [100] observed that biofortification with 8µmol Mo L^{-1}, in form of sodium molybdate (Na_2MoO_4), through foliar spray, notablyincreased 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 biofortificationcombined 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 Mobiofortification by 12.8%. With respect to the effect of the type of application, soil (banding) or foliar, Mondy and Munshi [102] observed that plots treated with foliar spray of Na_2MoO_4 efficiently increased the Mo concentration inpotatoes, but did not reach levels that would be toxic 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 functionsof enzymes, and also helps to maintain cardiovascular integrity, lung elasticity, normal development of connective tissue and nerve coverings. It has also neuroendocrine and immune functions, and is involved in the Fe metabolism [103].The RDA of Curanges between 1.0 and 1.6 mg day^{-1}, while the UL for adults is 10 mg day^{-1} [60]. Cu deficiency in early part of pregnancy can causeserious 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 tovarious pathological conditions and, in severe cases, can lead to death.

Cu is a redox-active transition metal that, under physiological conditions, is found in two forms–the reduced Cu^+ (cuprous) state and the oxidised Cu^{2+} (cupric) state [104,105]. Plants use Cu as a cofactor for a wide range of proteins involved in several physiological processes, including photosynthesis, mitochondrial respiration, carbohydrate metabolism, formation of phenolics in response to pathogen attack, superoxide scavenging, cell wall remodelling and ethylene perception [106-108]. The majority of Cu proteins (≈90%) found in nature function as oxidoreductases [109]. The most abundant Cu protein in plants is plastocyanin, a protein essential for photosynthetic electron transport in chloroplasts, that transfers electrons from the cytochrome b6f complex to PSI [110,111]. Cu is involved in metabolic pathways that supply energy for cellular processes [112]. As a trace element, an optimumamount of Cu is required to ensure proper cellular function, but in excess, it induces harmful impact on the production and survival of plants [107,114].

Cu occurs naturally in soils with contents ranging from 60 to 125 mg kg^{-1} [115], the worldwide average concentration being 14 mg kg^{-1} [116]. It is mobile in soil and its absorption is directly related to its concentration in the soil solution. Plants can absorb Cu in huge amounts by roots and in minor 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 occurs through transporters present in the plasma membrane of root cells–P-type ATPase copper transporters, COPT copper transporters, ZIP (zinc-iron-regulated transporter-like proteins) family transportersand NRAMP (natural resistance-associated macrophage proteins) family transporters. After being absorbed by the roots, Cu can be transported in the xylem to the shoot in the form of Cu^+ and Cu^{2+}.Normally, crop species can tolerate a maximum of 20-30 mg kg^{-1} DW of Cu in leaves, but Cu-tolerant species can accumulate as much as 1000 mg kg^{-1} DW of Cu in leaves [25].

With respect to Cu biofortification, Obrador et al. [117] conducted a study with ‘Viroflay Esmeralda’ spinach, applying eight different liquid fertilisers to the soil surface, with the irrigation water,at concentrations ranging from 0 to 3 mg Cu kg^{-1} soil. They observed that 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}, when plants were subjected to 3 mg Cu kg^{-1} soil (as Cu-EDTA); however, there was a 10% reduction in the dry matter yield. On the other hand, a 153%increase in Cu content was recorded with 1 mg Cu kg^{-1} soil, along with a yield increase of 71% compared to the control. Regarding the chemical form, the results showed that the best fertilisers to increase Cu content in the edible part of spinach were Cu-DHE and, especially, Cu-EDTA. Fortis-Hernández et al. [118] observed a positive trend between the increase in the concentration of the applied copper nanoparticles (NPs Cu) and the increase in the concentration of Cu in melon fruit pulp grown in hydroponic system. 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, the highest Cu concentration (5.39 mg kg^{-1}) in the melon fruit pulp was recorded with 9.0 mg L^{-1}NPs Cu.Likewise, in lettuce, Fortis-Hernández et al. [119] reported the highest leaf Cu content with 20 mgL^{-1}, with an average of 9.93 $µgkg^{-1}$ DW.On the contrary, Xiong et al. [120] reported that the foliar uptake, biotransformation and effects of NPs-CuO in lettuce, where the Cu content in the leaves and root of the plant increased with the foliar application of 100 and 1000 mgL^{-1} concentrations, could be toxic for human consumption. When Cu biofortification is concerned, attention must be given to the release of Cu in the soil substrate in relation to crop rotations and soil biological properties.

5. RESEARCH GAPS AND FUTURE PROSPECTS

Agronomic biofortification is relatively simpler than other methods, and is likely to be suitable for immediate results.The evidences discussed above make it clear that biofortification should be considered a promising strategy to combat malnutrition in many cases. 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 considered in this review, which are important in human nutrition, e.g., Fe, information is still lacking.On the other hand, even when experimental evidence for biofortification shows a significant increase in the concentrations of mineral elements, biofortification is not economically beneficial. Furthermore, an effective biofortification process depends on frequent and regular applications, and negative environmental impacts cannot be excluded.

The application of biofortification factors raises certain issues related to interactions with other factors at the soil level (e.g., phytoavailability) and at the plant level (e.g., competition with other elements). In many studies, traditional fertigation methods are applied instead of foliar application, which can be more cost-effective and ecofriendly. Also, there are only a few biofortified products on the market. In the future, in addition to a diverse choice of vegetables, the market is expected to offer biofortified products with more than one mineral. Therefore, research including simultaneous or combined biofortification is essential.

According to the results of the literature, biofortification is not expected to completely control or eliminate mineral deficiencies, but it complements other interventions aimed at providing micronutrients to humans. To be effective, a biofortification programme must rely on very appropriate planning involving health and nutrition surveys, nutritional habits, design and validation of sustainable biofortification processes, as well as estimation of the positive impact on human health. In the reviewed literature, most of the specific elements present in the edible part of plants have received attention, but key concepts such as bioaccessibility and bioavailability have rarely been considered.To modulate mineral bioavailability, special attention should be paid to substances that stimulate or inhibit bioavailability.

6. CONCLUSION

Although the main goal of current agriculture is to maximise crop yields, many diseases that affect human health are related to mineral and nutritional deficiencies. It is to overcome this drawback that the agricultural industry is increasingly interested in biofortification programmes. Agronomic biofortification provides an economical and rapid solution to addressing dietary deficiencies of many nutrients. The main challenges of agronomic biofortification in the immediate future will depend on 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 environmental or health impacts arising from new agronomic protocols (e.g., in the case of Cu). As reported in this review, promising results have been achieved with biofortification of various trace elements in many vegetables; however, the results are not entirely consistent and coherent. Considering this, and regardless of the specific scientific relevance, future achievements should be planned from a broader perspective, adopting an approach 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.

REFERENCES

1. De Pascale S, Rouphael Y. Chemical eustress and biofortification: Targeted nutrient solution management for enhancing quality in hydroponically grown vegetables. Acta Horticulturae. 2021;1321:179-183.
2. Zandalinas SI, Fritschi FB, Mittler R. Signal transduction networks during stress combination. Journal of Experimental Botany. 2020;71(5):1734-1741.
3. Vinoth A, Ravindhran R. Biofortification in millets: a sustainable approach for nutritional security.

Frontiers in Plant Science. 2017;8:29.

4. Bouis H. Reducing mineral and vitamin deficiencies through biofortification: progress under HarvestPlus. In: Hidden hunger: strategies to improve nutrition quality. 2018;118:112-122. Karger Publishers.
5. Bailey RL, West Jr KP, Black RE. The epidemiology of global micronutrient deficiencies. Annals of Nutrition and Metabolism. 2015;66(Suppl. 2):22-33.
6. Hefferon KL. Nutritionally enhanced food crops; progress and perspectives. International Journal of Molecular Sciences. 2015;16(2):3895-3914.
7. O'hare TJ. Biofortification of vegetables for the developed world. Acta Horticulturae. 2015;1106:1-8.
8. Tardy AL, Ballesta AA, Yilmaz GC, Milana DA, Ramírez DM, Lam HY, Azais-Braesco V, Pouteau E. Adult's dietary intakes of selected vitamins & minerals essential for energy metabolism and cognition: A comparison across countries & genders (FS10-04-19). Current Developments in Nutrition. 2019;3:nzz039-FS10.
9. Castiglione D, Platania A, Conti A, Falla M, D'Urso M, Marranzano M. Dietary micronutrient and mineral intake in the mediterranean healthy eating, ageing, and lifestyle (MEAL) study. Antioxidants. 2018;7(7):79.
10. Nair MK, Augustine LF, Konapur A. Food-based interventions to modify diet quality and diversity to address multiple micronutrient deficiency. Frontiers in Public Health. 2016;3:277.
11. Baldassano S, Polizzi MR, Sabatino L, Caldarella R, Macaluso A, Alongi A, Caldara GF, Ferrantelli V, Vasto S. A new potential dietary approach to supply micronutrients to physically active people through consumption of biofortified vegetables. Nutrients. 2022;14(14):2971.
12. La Bella S, Consentino BB, Rouphael Y, Ntatsi G, De Pasquale C, Iapichino G, Sabatino L. Impact of Ecklonia maxima seaweed extract and Mo foliar treatments on biofortification, spinach yield, quality and NUE. Plants. 2021;10(6):1139.
13. Sabatino L, Consentino BB, Rouphael Y, De Pasquale C, Iapichino G, D'Anna F, La Bella S. Protein hydrolysates and Mo-biofortification interactively modulate plant performance and quality of 'Canasta' lettuce grown in a protected environment. Agronomy. 2021;11(6):1023.
14. Sabatino L, Di Gaudio F, Consentino BB, Rouphael Y, El-Nakhel C, La Bella S, Vasto S, Mauro RP, D'Anna F, Iapichino G, Caldarella R. Iodine biofortification counters micronutrient deficiency and improve functional quality of open field grown curly endive. Horticulturae. 2021;7(3):58.
15. Kyriacou MC, Rouphael Y. Towards a new definition of quality for fresh fruits and vegetables. Scientia Horticulturae. 2018;234:463-469.
16. Fan, S. Ending hunger and undernutrition by 2025: The role of horticultural value chains. Acta Horticulturae. 2016;1126:9-20.
17. Wang G, Xu M, Wang W, Galili G. Fortifying horticultural crops with essential amino acids: a review. International Journal of Molecular Sciences. 2017;18(6):1306.
18. Ogutu SO, Ochieng DO, Qaim M. Supermarket contracts and smallholder farmers: Implications for income and multidimensional poverty. Food Policy. 2020;95:101940.
19. Sharma A, Verma RK. Biofortification: a promising approach toward eradication of hidden hunger. In: Microbial Interventions in Agriculture and Environment: Volume 1: Research Trends, Priorities and Prospects. 2019:313-327.
20. Sanahuja G, Farré G, Berman J, Zorrilla-López U, Twyman RM, Capell T, Christou P, Zhu C. A question of balance: achieving appropriate nutrient levels in biofortified staple crops. Nutrition Research Reviews. 2013;26(2):235-245.
21. D'Imperio M, Renna M, Cardinali A, Buttaro D, Serio F, Santamaria P. Calcium biofortification and bioaccessibility in soilless "baby leaf" vegetable production. Food Chemistry. 2016;213:149-156.
22. Ruini LF, Ciati R, Pratesi CA, Marino M, Principato L, Vannuzzi E. Working toward healthy and sustainable diets: The "Double Pyramid Model" developed by the Barilla Center for Food and Nutrition to raise awareness about the environmental and nutritional impact of foods. Frontiers in Nutrition. 2015;2:126479.
23. Siwela M, Pillay K, Govender L, Lottering S, Mudau FN, Modi AT, Mabhaudhi T. Biofortified crops for combating hidden hunger in South Africa: availability, acceptability, micronutrient retention and bioavailability. Foods. 2020;9(6):815.
24. Gómez-Galera S, Rojas E, Sudhakar D, Zhu C, Pelacho AM, Capell T, Christou P. Critical

evaluation of strategies for mineral fortification of staple food crops. Transgenic Research. 2010;19:165-180.

25. Marschner H. Marschner's mineral nutrition of higher plants, 3rd ed. Academic Press: Cambridge, MA, USA, 2011.
26. Buturi CV, Mauro RP, Fogliano V, Leonardi C, Giuffrida F. Mineral biofortification of vegetables as a tool to improve human diet. Foods. 2021;10(2):223.
27. de Valença AW, Bake A, Brouwer ID, Giller KE. Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. Global food security. 2017;12:8-14.
28. Prasad R, Shivay YS, Kumar D. Agronomic biofortification of cereal grains with iron and zinc. Advances in Agronomy. 2014;125:55-91.
29. Jaiswal DK, Krishna R, Chouhan GK, de Araujo Pereira AP, Ade AB, Prakash S, Verma SK, Prasad R, Yadav J, Verma JP. Bio-fortification of minerals in crops: current scenario and future prospects for sustainable agriculture and human health. Plant Growth Regulation. 2022;98(1):5-22.
30. Stangoulis JC, Knez M. Biofortification of major crop plants with iron and zinc-achievements and future directions. Plant and Soil. 2022;474(1):57-76.
31. Carvalho SM, Vasconcelos MW. Producing more with less: Strategies and novel technologies for plant-based food biofortification. Food Research International. 2013;54(1):961-971.
32. Rouphael Y, Kyriacou MC. Enhancing quality of fresh vegetables through salinity eustress and biofortification applications facilitated by soilless cultivation. Frontiers in Plant Science. 2018;9:1254.
33. Wiesner-Reinhold M, Schreiner M, Baldermann S, Schwarz D, Hanschen FS, Kipp AP, Rowan DD, Bentley-Hewitt KL, McKenzie MJ. Mechanisms of selenium enrichment and measurement in brassicaceous vegetables, and their application to human health. Frontiers in Plant Science. 2017;8:1365.
34. Li R, Li DW, Liu HP, Hong CL, Song MY, Dai ZX, Liu JW, Zhou J, Weng HX. Enhancing iodine content and fruit quality of pepper (*Capsicum annuum* L.) through biofortification. Scientia Horticulturae. 2017;214:165-173.
35. Niu J, Liu C, Huang M, Liu K, Yan D. Effects of foliar fertilization: a review of current status and future perspectives. Journal of Soil Science and Plant Nutrition. 2021;21:104-118.
36. Zoroddu MA, Aaseth J, Crisponi G, Medici S, Peana M, Nurchi VM. The essential metals for humans: a brief overview. Journal of Inorganic Biochemistry. 2019;195:120-129.
37. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. Journal of Research in Medical Sciences. 2014;19(2):164.
38. Haschka D, Hoffmann A, Weiss G. Iron in immune cell function and host defense. In: Seminars in Cell & Developmental Biology. 2021;115: 27-36.
39. Camaschella C. New insights into iron deficiency and iron deficiency anemia. Blood Reviews. 2017;31(4):225-233.
40. Gowthami V, Ananda N. Dry matter production, yield and yield components of groundnut (*Arachishypogaea* L.) genotypes as influenced by zinc and iron through ferti-fortification. Indian Journal of Agricultural Research. 2017;51(4):339-344.
41. Kobayashi T, Nozoye T, Nishizawa NK. Iron transport and its regulation in plants. Free Radical Biology and Medicine. 2019;133:11-20.
42. Zhang X, Zhang D, Sun W, Wang T. The adaptive mechanism of plants to iron deficiency via iron uptake, transport, and homeostasis. International Journal of Molecular Sciences. 2019;20(10):2424.
43. Colombo C, Palumbo G, He JZ, Pinton R, Cesco S. Review on iron availability in soil: interaction of Fe minerals, plants, and microbes. Journal of Soils and Sediments. 2014;14:538-548.
44. Durrett TP, Gassmann W, Rogers EE. The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. Plant Physiology. 2007;144(1):197-205.
45. Von Wiren N, Klair S, Bansal S, Briat JF, Khodr H, Shioiri T, Leigh RA, Hider RC. Nicotianamine chelates both FeIII and FeII. Implications for metal transport in plants. Plant Physiology. 1999;119(3):1107-1114.
46. Connorton JM, Balk J. Iron biofortification of staple crops: lessons and challenges in plant genetics. Plant and Cell Physiology. 2019;60(7):1447-1456.

47. Sperotto RA, Ricachenevsky FK, de Abreu Waldow V, Fett JP. Iron biofortification in rice: it's a long way to the top. Plant Science. 2012;190:24-39.
48. Lemanceau P, Bauer P, Kraemer S, Briat JF. Iron dynamics in the rhizosphere as a case study for analyzing interactions between soils, plants and microbes. 2009;321:513-535.
49. Ferrandon M, Chamel AR. Cuticular retention, foliar absorption and translocation of Fe, Mn and Zn supplied in organic and inorganic form. Journal of Plant Nutrition. 1988;11(3):247-263.
50. Nahim-Granados S, Oller I, Malato S, Pérez JS, Polo-Lopez MI. Commercial fertilizer as effective iron chelate (Fe^{3+}-EDDHA) for wastewater disinfection under natural sunlight for reusing in irrigation. Applied Catalysis B: Environmental. 2019;253:286-292.
51. Dukpa P, Chatterjee R, Thirumdasu R, Subba S. Performance of terrestrial water spinach (*Ipomoea reptans*) through biofortification under different mode and source of iron nutrition. Current Journal of Applied Science and Technology. 2018;27(2):1-8.
52. Kromann P, Valverde F, Alvarado S, Vélez R, Pisuña J, Potosí B, Taipe A, Caballero D, Cabezas A, Devaux A. Can Andean potatoes be agronomically biofortified with iron and zinc fertilizers?. Plant and Soil. 2017;411:121-138.
53. Giordano M, El-Nakhel C, Pannico A, Kyriacou MC, Stazi SR, De Pascale S, Rouphael Y. Iron biofortification of red and green pigmented lettuce in closed soilless cultivation impacts crop performance and modulates mineral and bioactive composition. Agronomy. 2019;9(6):290.
54. Briat JF, Duc C, Ravet K, Gaymard F. Ferritins and iron storage in plants. Biochimica et Biophysica Acta (BBA)-General Subjects. 2010;1800(8):806-814.
55. Buturi CV, Sabatino L, Mauro RP, Navarro-León E, Blasco B, Leonardi C, Giuffrida F. Iron biofortification of greenhouse soilless lettuce: An effective agronomic tool to improve the dietary mineral intake. Agronomy. 2022;12(8):1793.
56. Chatterjee R, CHOWDHURY RS, DUKPA P, THIRUMDASU RK. Iron fortification in leafy vegetables: present status and future possibilities. Innovare Journal of Agricultural Sciences. 2016;4(4):1-3.
57. Roohani N, Hurrell R, Kelishadi R, Schulin R. Zinc and its importance for human health: An integrative review. Journal of Research in Medical Sciences. 2013;18(2):144-157.
58. Que EL, Bleher R, Duncan FE, Kong BY, Gleber SC, Vogt S, Chen S, Garwin SA, Bayer AR, Dravid VP, Woodruff TK. Quantitative mapping of zinc fluxes in the mammalian egg reveals the origin of fertilization-induced zinc sparks. Nature chemistry. 2015;7(2):130-139.
59. Wessels I, Rink L. Micronutrients in autoimmune diseases: possible therapeutic benefits of zinc and vitamin D. The Journal of Nutritional Biochemistry. 2020;77:108240.
60. Trumbo P, Yates AA, Schlicker S, Poos M. Dietary Reference Intakes. Journal of the American Dietetic Association. 2001;101(3):294.
61. Sharma A, Patni B, Shankhdhar D, Shankhdhar SC. Zinc–an indispensable micronutrient. Physiology and Molecular Biology of Plants. 2013;19:11-20.
62. White PJ, Pongrac P, Sneddon CC, Thompson JA, Wright G. Limits to the biofortification of leafy brassicas with zinc. Agriculture. 2018;8(3):32.
63. Chaney RL. Cadmium and zinc. Trace elements in soils. 2010:409-439.
64. Wuana RA, Okieimen FE. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. International Scholarly Research Notices. 2011;2011(1):402647.
65. Eijkelkamp BA, Morey JR, Ween MP, Ong CL, McEwan AG, Paton JC, McDevitt CA. Extracellular zinc competitively inhibits manganese uptake and compromises oxidative stress management in *Streptococcus pneumoniae*. PLoS ONE. 2014;9(2):e89427.
66. Imlay JA. The mismetallation of enzymes during oxidative stress. Journal of Biological Chemistry. 2014;289(41):28121-28128.
67. Chandrangsu P, Helmann JD. Intracellular Zn (II) intoxication leads to dysregulation of the PerR regulon resulting in heme toxicity in *Bacillus subtilis*. PLoS Genetics. 2016;12(12):e1006515.
68. Le¡ ková A, Giehl RF, Hartmann A, Farga¡ ová A, von Wirén N. Heavy metals induce iron deficiency responses at different hierarchic and regulatory levels. Plant Physiology. 2017;174(3):1648-1668.
69. Briat JF, Rouached H, Tissot N, Gaymard F, Dubos C. Integration of P, S, Fe, and Zn nutrition signals in *Arabidopsis thaliana*: potential involvement of PHOSPHATE STARVATION RESPONSE 1 (PHR1). Frontiers in Plant Science. 2015;6:290.

70. Stanton C, Sanders D, Krämer U, Podar D. Zinc in plants: Integrating homeostasis and biofortification. Molecular Plant. 2022;15(1):65-85.
71. Gupta N, Ram H, Kumar B. Mechanism of Zinc absorption in plants: uptake, transport, translocation and accumulation. Reviews in Environmental Science and Bio/Technology. 2016;15:89-109.
72. White PJ, Broadley MR. Physiological limits to zinc biofortification of edible crops. Frontiers in Plant Science. 2011;2:80.
73. Tsonev T, Cebola Lidon FJ. Zinc in plants-an overview. Emirates Journal of Food & Agriculture (EJFA). 2012;24(4):322-333.
74. Pandey R, Behera SR, Pradhan PP, Pradhan SS. Zinc application enhances tuber quality and zinc bioavailability in potato (*Solanum tuberosum* L.) in the *tarai* region of Uttarakhand. Journal of Experimental Agriculture International. 2024;46(5):399-405.
75. Rivera-Martin A, Broadley MR, Poblaciones MJ. Soil and foliar zinc biofortification of broccolini: effects on plant growth and mineral accumulation. Crop and Pasture Science. 2020;71(5):484-490.
76. Solanki P, Laura JS. Biofortification of crops using nanoparticles to alleviate plant and human Zn deficiency: A review. Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences. 2018;4(5):364-385.
77. Boawn LC, Rasmussen PE. Crop response to excessive zinc fertilization of alkaline soil^1. Agronomy Journal. 1971;63(6):874-876.
78. Long XX, Yang XE, Ni WZ, Ye ZQ, He ZL, Calvert DV, Stoffella JP. Assessing zinc thresholds for phytotoxicity and potential dietary toxicity in selected vegetable crops. Communications in Soil Science and Plant Analysis. 2003;34(9-10):1421-1434.
79. de Sousa Lima F, Nascimento CW, da Silva Sousa C. Zinc fertilization as an alternative to increase the concentration of micronutrients in edible parts of vegetables. Revista Brasileira de Ciências Agrárias. 2015;10(3):403-408.
80. Mao H, Wang J, Wang Z, Zan Y, Lyons G, Zou C. Using agronomic biofortification to boost zinc, selenium, and iodine concentrations of food crops grown on the loess plateau in China. Journal of Soil Science and Plant Nutrition. 2014;14(2):459-470.
81. Rugeles-Reyes SM, Cecílio AB, Lopez Aguilar MA, Silva PH. Foliar application of zinc in the agronomic biofortification of arugula. Food Science and Technology. 2019;39(4):1011-1017.
82. Barrameda-Medina Y, Blasco B, Lentini M, Esposito S, Baenas N, Moreno DA, Ruiz JM. Zinc biofortification improves phytochemicals and amino-acidic profile in *Brassica oleracea* cv. Bronco. Plant science. 2017;258:45-51.
83. Ierna A, Mauro R, Mauromicale G. Improved yield and nutrient efficiency in two globe artichoke genotypes by balancing nitrogen and phosphorus supply. Agronomy for Sustainable Development. 2012;32(3):773-780.
84. Sabatino L, D'Anna F, Iapichino G, Moncada A, D'Anna E, De Pasquale C. Interactive effects of genotype and molybdenum supply on yield and overall fruit quality of tomato. Frontiers in Plant Science. 2019;9:1922.
85. Vatansever R, Ozyigit II, Filiz E. Essential and beneficial trace elements in plants, and their transport in roots: a review. Applied Biochemistry and Biotechnology. 2017;181:464-482.
86. Kaiser BN, Gridley KL, Ngaire Brady J, Phillips T, Tyerman SD. The role of molybdenum in agricultural plant production. Annals of Botany. 2005;96(5):745-754.
87. Schwarz G, Mendel RR, Ribbe MW. Molybdenum cofactors, enzymes and pathways. Nature. 2009; 460(7257):839-847.
88. Tejada-Jimenez M, Chamizo-Ampudia A, Galván A, Fernandez E, Llamas A. Molybdenum metabolism in plants. Metallomics. 2013;5(9):1191-1203.
89. Leimkuhler S, Iobbi-Nivol C. Bacterial molybdoenzymes: Old enzymes for new purposes. FEMS Microbiology Reviews. 2016;40(1):1-18.
90. Mendel RR, Kruse T. Cell biology of molybdenum in plants and humans. Biochima et Biophysica Acta (BBA)-Molecular Cell Research. 2012;1823(9):1568-1579.
91. Seo M, Peeters AJM, Koiwai H, Oritani T, Marion-Poll A, Zeevaart JAD, Koornneef M, Kamiya Y, Koshiba T. The Arabidopsis aldehyde oxidase 3 (AAO3) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. Proceedings of the National Academy of Sciences. 2000;97(23):12908-12913.

92. Min Y, Hu CX, Wang YH. Effects of molybdenum on the intermediates of chlorophyll biosynthesis in winter wheat cultivars under low temperature. Agricultural Sciences in China. 2006;5(9):670-677.
93. Min YU, Hu CX, Sun XC, Wang YH. Influences of Mo on nitrate reductase, glutamine synthetase and nitrogen accumulation and utilization in Mo-efficient and Mo-inefficient winter wheat cultivars. Agricultural Sciences in China. 2010;9(3):355-361.
94. Manuel TJ, Alejandro CA, Angel L, Aurora G, Emilio F. Roles of molybdenum in plants and improvement of its acquisition and use efficiency. In: Plant micronutrient use efficiency 2018:137-159. Academic Press.
95. Baldassano S, Di Gaudio F, Sabatino L, Caldarella R, De Pasquale C, Di Rosa L, Nuzzo D, Picone P, Vasto S. Biofortification: Effect of Iodine fortified food in the healthy population, double-arm nutritional study. Frontiers in Nutrition. 2022;9:871638.
96. Consentino BB, Ciriello M, Sabatino L, Vultaggio L, Baldassano S, Vasto S, Rouphael Y, La Bella S, De Pascale S. Current acquaintance on agronomic biofortification to modulate the yield and functional value of vegetable crops: A review. Horticulturae. 2023;9(2):219.
97. Vasto S, Di Gaudio F, Raso M, Sabatino L, Caldarella R, De Pasquale C, Di Rosa L, Baldassano S. Impact on glucose homeostasis: is food biofortified with molybdenum a workable solution? A two-arm study. Nutrients. 2022;14(7):1351.
98. Vasto S, Baldassano D, Sabatino L, Caldarella R, Di Rosa L, Baldassano S. The role of consumption of molybdenum biofortified crops in bone homeostasis and healthy aging. Nutrients. 2023;15(4):1022.
99. Moncada A, Miceli A, Sabatino L, Iapichino G, D’Anna F, Vetrano F. Effect of molybdenum rate on yield and quality of lettuce, escarole, and curly endive grown in a floating system. Agronomy. 2018;8(9):171.
100. La Bella S, Consentino BB, Rouphael Y, Ntatsi G, De Pasquale C, Iapichino G, Sabatino L. Impact of Ecklonia maxima seaweed extract and Mo foliar treatments on biofortification, spinach yield, quality and NUE. Plants. 2021;10(6):1139.
101. Vultaggio L, Allevato E, Sabatino L, Ntatsi G, Rouphael Y, Torta L, La Bella S, Consentino BB. Modulation of cherry tomato performances in response to molybdenum biofortification and arbuscular mycorrhizal fungi in a soilless system. Heliyon. 2024.
102. Mondy NI, Munshi CB. Effect of soil and foliar application of molybdenum on the glycoalkaloid and nitrate concentration of potatoes. Journal of Agricultural and Food Chemistry. 1993;41(2):256-258.
103. Karlin KD, Tyeklár Z. Bioinorganic chemistry of copper. Springer Science & Business Media. 2012.
104. Bost M, Houdart S, Oberli M, Kalonji E, Huneau JF, Margaritis I. Dietary copper and human health: Current evidence and unresolved issues. Journal of Trace Elements in Medicine and Biology. 2016;35:107-115.
105. Ogunkunle CO, Bornmann B, Wagner R, Fatoba PO, Frahm R, Lützenkirchen-Hecht D. Copper uptake, tissue partitioning and biotransformation evidence by XANES in cowpea (Vigna unguiculata L) grown in soil amended with nano-sized copper particles. Environmental Nanotechnology, Monitoring &Management. 2019;12:100231.
106. Reed ST, Martens DC. Copper and zinc. Methods of Soil Analysis: Part 3 Chemical Methods. 1996;5:703-722.
107. Kumar V, Pandita S, Sidhu GP, Sharma A, Khanna K, Kaur P, Bali AS, Setia R. Copper bioavailability, uptake, toxicity and tolerance in plants: A comprehensive review. Chemosphere. 2021;262:127810.
108. Lange B, van Der Ent A, Baker AJ, Echevarria G, Mahy G, Malaisse F, Meerts P, Pourret O, Verbruggen N, Faucon MP. Copper and cobalt accumulation in plants: a critical assessment of the current state of knowledge. New Phytologist. 2017;213(2):537-551.
109. Shabbir Z, Sardar A, Shabbir A, Abbas G, Shamshad S, Khalid S, Murtaza G, Dumat C, Shahid M. Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. Chemosphere. 2020;259:127436.
110. Andresen E, Peiter E, Küpper H. Trace metal metabolism in plants. Journal of experimental botany. 2018;69(5):909-954.
111. Aguirre G, Pilon M. Copper delivery to chloroplast proteins and its regulation. Frontiers in Plant

Science. 2016;6:1250.

112. Zhang D, Liu X, Ma J, Yang H, Zhang W, Li C. Genotypic differences and glutathione metabolism response in wheat exposed to copper. Environmental and Experimental Botany. 2019;157:250-259.
113. Orłowska R, Zimny J, Bednarek PT. Copper ions induce DNA sequence variation in zygotic embryo culture-derived barley regenerants. Frontiers in Plant Science. 2021;11:614837.
114. Printz B, Lutts S, Hausman JF, Sergeant K. Copper trafficking in plants and its implication on cell wall dynamics. Frontiers in Plant Science. 2016;7:601.
115. Kabata-Pendias A. Trace elements in soils and plants. CRC Press; 2000.
116. Alloway BJ. Sources of heavy metals and metalloids in soils. In: Heavy metals in soils: trace metals and metalloids in soils and their bioavailability. 2013:11-50.
117. Obrador A, Gonzalez D, Alvarez JM. Effect of inorganic and organic copper fertilizers on copper nutrition in *Spinacia oleracea* and on labile copper in soil. Journal of Agricultural and Food Chemistry. 2013;61(20):4692-4701.
118. Fortis-Hernández M, Lopez JO, Rangel PP, Valencia RT, Fortiz EL, Andrade-Sifuentes A, PUENTE EO. Biofortification with copper nanoparticles (Nps Cu) and its effect on the physical and nutraceutical quality of hydroponic melon fruits. NotulaeBotanicae Horti Agrobotanici Cluj-Napoca. 2022;50(1):12568.
119. Fortis-Hernández M, González-Rodríguez T, Espinosa-Palomeque B, Preciado-Rangel P, Gallegos-Robles MA, Rueda-Puente EO. Foliar biofortification with copper nanoparticles and its effect on phytochemical quality and enzymatic activity in lettuce. Horticultura Brasileira. 2024;42:e2617.
120. Xiong T, Zhang T, Xian Y, Kang Z, Zhang S, Dumat C, Shahid M, Li S. Foliar uptake, biotransformation, and impact of CuO nanoparticles in *Lactuca sativa* L. var. *ramosa* Hort. Environmental Geochemistry and Health. 2021;43:423-439.