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REVIEW PAPERS

BIOFORTIFICATION – PROMISING APPROACH TO INCREASING THE CONTENT OF IRON AND ZINC IN STAPLE FOOD CROPS*

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Abstract

Micronutrient deficiencies have increased over recent decades due to the general depreciation of the quality of poor people's diet, both in developed and developing countries. The deficiencies of iron (Fe) and zinc (Zn) are a critical public health problem worldwide, with the negative impact on health, lifespan and productivity. Biofortification is an agricultural approach that can improve human nutrition on a global scale. Agronomic biofortification is considered a short-term and complementary strategy, but economic analyses suggest that genetic biofortification is the most effective strategy for increasing dietary Fe and Zn intakes of vulnerable populations. Enrichment of cereal grains by breeding is a high-priority area of research, and an effective strategy among other approaches, e.g, fortification, supplementation and food diversification. This review discusses the role of Fe and Zn in plant nutrition, the potential strategies for developing Fe and Zn biofortified crops and their importance in human nutrition.

Key words: metal homeostasis, ferti-fortification, breeding crops, micronutrient malnutrition, anaemia, bioavailability.

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BIOFORTYFIKACJA – OBIECUJĄCY SPOSÓB ZWIĘKSZANIA ZAWARTOŚCI ŻELAZA I CYNKU W PODSTAWOWYCH ROŚLINACH UPRAWNYCH

Abstrakt

Obecnie niedobory Fe i Zn stanowią istotny problem dotyczący zdrowia publicznego, są bowiem przyczyną negatywnego wpływu na zdrowie, średnią długość życia i przyrost naturalny. Biofortyfikacja agronomiczna jest to metoda, która może poprawić żywienie człowieka na całym świecie. Analizy ekonomiczne wskazują, że genetyczna biofortyfikacja jest najbardziej skuteczną strategią zwiększenia spożycia Fe i Zn, natomiast agronomiczna biofortifikacja może być podejściem komplementarnym, i to w krótkim czasie. Strategia genetyczna jest obszarem priorytetowym w badaniach zboża, i okazuje się być bardziej skuteczna niż metody fortyfikacji, suplementacji lub zróżnicowania środków spożywczych. W pracy omówiono rolę Fe i Zn w odżywianiu roślin, potencjalne strategie rozwoju upraw z wykorzystaniem biofortyfikacji Fe i Zn oraz znaczenie tych upraw w żywieniu człowieka.

Słowa kluczowe: homeostazy, biofortyfikacja, niedożywienie, mikroelementów, niedokrwistość, biodostępność.

INTRODUCTION

Iron deficiency induced anaemia and Zn deficiency are major public health problems worldwide, to which children are particularly. The major cause of malnutrition is poor quality diet, mainly lacking in animal products. Consequently, populations that consume few animal foods may suffer from a high prevalence of several micronutrient deficiencies. At the same time, the proportion of the global population suffering from micronutrient malnutrition (MNM) has increased because modern plant breeding has been historically oriented toward high agronomic yield rather than the nutritional quality, producing a lower density of minerals in many crops. It has been suggested that 4.5 billion people worldwide are affected by deficiencies of Fe, vitamin A and I; Zn is of increasing concern. MNM diminishes motivation and development, consequently impairing mental and cognitive abilities and finally reducing the productivity and potential of entire societies (Bouis, Islam 2011, Modestine et al. 2012).

Iron and zinc are essential elements for human health, required for the activity of many enzymes involved in major metabolic pathways. International research programs have been undertaken to enhance Zn and Fe nutrition; significant benefits are found through initiatives such as supplementation, fortification, food diversification and biofortification. Fortification has an important role in tackling malnutrition, but it is highly dependent on funding and restricted to urban areas. By contrast, biofortification is a prominent strategy that can increase levels of micronutrients in crops. However, to biofortify plants it is necessary to achieve a clear molecular understanding of micronutrient homeostasis (Lungaho et al. 2011).

Agronomic biofortification and genetic biofortification represent complementary agricultural approaches. It is claimed than application of Zn and Fe fertilizers is a short-term solution, complementary to plant breeding. There is convincing evidence that especially foliar application of Zn fertilizers is effective in improving Zn in grain. By contrast, Fe fertilization is less effective in the enrichment of grains, expensive and dangerous to the environment. In addition, it appears that improving the nitrogen nutritional status of plants promotes the accumulation of Fe and Zn in grain (Cakmak 2010, White, Broadley 2011, Murgia et al. 2012, Sperotto et al. 2012).

Genetic biofortification offers a sustainable and low-cost way to provide micronutrients to people in developing countries. Breeding nutrient-rich staple food crops is indeed the main goal of different international consortia, whose aim is to reduce MNM through biofortification programs. Variability is exploited to produce Fe and Zn-rich crops. Also, this study is amended by inclusion of the information on retention after processing, bioavailability from the diet, daily consumption and required amounts of plant food that have measurable effects on the nutrient status. It is thought that Fe and Zn bioavailability to a human organism can be improved by increasing the dietary factors that enhance absorption, by decreasing the factors that inhibit absorption, or by increasing the micronutrient content of a diet (Cakmak 2010, Bouis et al. 2011, White, Broadley 2011, Murgia et al. 2012).

Until now, Fe deficiency is counteracted mainly by food fortification and supplementation. With respect to Zn, there is strong evidence supporting the beneficial impact of these strategies, especially Zn supplementation of children. However, economic analyses suggest that biofortification is the most practical, lasting, and cost-effective strategy for increasing the dietary Fe and Zn intake by vulnerable populations (Hess, Brown 2009, Gibson 2012, Bhullar, Gruissem 2013). This review describes the roles of Fe and Zn in plant and human nutrition. Particular attention is paid to the importance of the molecular pathway that directs specific steps in networks of micronutrient homeostasis, to strategies for production of Fe and Zn-rich crops by biofortification, and to the importance of their increased bioavailability through diets among human populations.

THE ESSENTIAL ROLE OF IRON AND ZINC IN PLANTS

There are two criteria defined for an element to be essential for plant growth. Either a plant is unable to complete a normal life cycle in the absence of an element, or when the said element is part of some essential plant constituent or metabolite. In the traditional classification of nutrients and beneficial elements, Fe and Zn are included in the category of micronutrients essential for biological systems (Hell, Stephan 2003, Barker, Pilbeam 2007).

Like other organisms, plants require Fe to complete their life cycle. This micronutrient occurs in multiple redox states, readily accepting and donating electrons, thus being able to serve as a cofactor for several plant proteins that participate in crucial metabolic pathways. The function of Fe relies mostly on the reversible redox reaction of Fe^{2+} and Fe^{3+} ions, its ability to form octahedral complexes with various ligands and to vary its redox potential in response to different ligands of the environment. This redox potential (Fe^{2+}/Fe^{3+}) enables its use, in the form of heme or Fe sulphur clusters, in a number of protein complexes, especially those involved in electron transfer. However, excess Fe is toxic as both Fe^{2+} and Fe^{3+} can act as catalysts in the formation of noxious reactive oxygen species (ROS), which are potent oxidizing agents able to damage DNA, proteins and lipids. Therefore, Fe homeostasis in the whole organism must be balanced to supply enough Fe for cell metabolism and to avoid excessive, toxic levels. Because Fe represents one of the most versatile metals in biology with numerous cellular functions, its deficiency is among the most serious problems worldwide (Curie, Briat 2003, Hell, Stephan 2003, Bashir et al. 2011).

Zinc is the second to iron most abundant intracellular metal. Zinc is an essential micronutrient required by both animals and plants as a structural constituent of proteins or a regulatory co-factor of enzymes involved in many biochemical pathways. For example, many proteins contain Zn prosthetic groups (zinc finger, zinc twist) and around 300 enzymes require Zn as a cofactor. Actually, Zn is the only metal found in all six enzyme classes. In addition, it has been estimated that several proteins are capable of binding Zn and some of these Zn-binding proteins are transcription factors needed for gene regulation. Many cells secrete Zn as a signaling molecule, including cells in the immune and nervous systems. Zinc is also required for the structural and functional integrity of biological membranes and for the detoxification of highly aggressive free radicals. In general, Zn deficiency is a well-documented problem in food crops, causing a decrease in both yield and nutritional quality of crops. Therefore, regions with Zn-deficient soils around the world are typically characterized by a widespread Zn deficiency in humans (Broadley et al. 2006, Cakmak 2008, Gomez-Galera et al. 2010, Lee et al. 2011).

COMPLEX REGULATION NETWORK OF IRON AND ZINC IN PLANTS: MECHANISMS OF THE CONTROL OF UPTAKE AND DISTRIBUTION

Although essential minerals are generally abundant in soils, most of them largely occur in forms that are not easily available to plants. In order to satisfy the demand for minerals whilst avoiding their possible toxic effects, plants have evolved a complex regulation network controlling mineral homeostasis. Our knowledge of the molecular pathway which controls specific steps in the network of mineral homeostasis, although still undeveloped, is growing rapidly. This information is expected to improve crop yield, crop nutritional value and food safety, which are aspects of major global concern. Today, the lack of knowledge about this mechanism is an obstacle to devising approaches for biofortification, i.e. genetic engineering of staple crops to accumulate additional bioavailable nutrients in edible parts (GHANDILYAN et al. 2006, WALKER, CONNOLLY 2008).

Metal homeostasis is a function of an organism that regulates its internal metal environment (in cells and organelles) so as to maintain a stable and constant condition. This process is based on well-controlled metal uptake, translocation, redistribution and sequestration mechanisms. In general, strategies of plants to facilitate metal uptake include the modification of the environment to increase bioavailability, the up-regulation of high-affinity transporters and the activation of intra-cellular and inter-cellular pathways for subsequent distribution. Under metal excess, non-specific metal uptake from soil is unavoidable and plants activate mechanisms for intracellular chelation and compartmentalization (PUIG, PEÑARRUBIA 2009, HASSAN, AARTS 2011). The regulation of metal homeostasis is mainly mediated by membrane transporters. In many cases, transcriptional control of these transporters contributes to homeostasis. However, too little is known about post-transcriptional and post-translational control of transporter activity. In addition, whereas much is still to be learned about Zn, we are now closer to attaining the full understanding of Fe homeostasis (PILON et al. 2009).

Uptake of Fe and Zn in Plants

Soil is the main source of metals for plants. Metals can be inaccessible in soil, primarily when present in the insoluble form, which is particularly common at higher pH of alkaline soils. To overcome such inaccessibility of some metals, non-graminaceous plants rely primarily on a reduction-based strategy of uptake (strategy I), whereas gramineous plants (grasses) more commonly use a chelation-based strategy (strategy II), both depending on the environment characteristics, in order to efficiently incorporate and systemically distribute micronutrients (PALMER, GUERINOT 2009, JEONG, GUERINOT 2009).

In the case of Fe uptake, plants are classically divided into two groups, according to their strategy to obtain Fe from the environment: (1) strategy I, those acquiring Fe^{2+} after reduction by Fe^{3+} reductases and (2) strategy II, those secreting phytosiderophores (PSs) that bind Fe^{3+} for subsequent acquisition of the Fe^{3+}–chelate. However, latest results emphasize the importance of the environment in Fe acquisition (JEONG, GUERINOT 2009, PUIG, PEÑARRUBIA 2009).

Many plants use strategy I for metal uptake because metal transporters have specific affinity for a particular oxidation state. For example, whenever Zn is always found in the +2 oxidation state, under physiologically relevant conditions, Fe needs to be reduced for its uptake into the plant. The Fe uptake by Strategy I involves the release of protons to increase the solubility of Fe, via H^+-ATPases of the root plasma membrane, upon Fe deficiency. This reduction is performed by Fe-deficiency inducible plasma membrane-bound Fe^{3+}reductase. After acidification, Fe^{3+} is reduced to Fe^{2+} by membrane-bound ferric reductase oxidase (FRO). Once Fe^{3+} is reduced, Fe^{2+} is transported into the root by Fe-regulated transporter (IRT), a member of the Zn regulated transporter (ZRT)-IRT-like protein (ZIP) family (GHANDILYAN et al. 2006, JEONG, GUERINOT 2009). On the other hand, the Fe uptake by strategy II employs the release of chelators known as PSs into the rhizosphere, to bind Fe^{3+} for transport into the plant. PSs are synthesized from methionine and are compounds of the mugineic acid family (MAs) that form stable Fe^{3+}–chelates in soil. PSs, like MAs, are released to chelate Fe^{3+} and the resulting PS–Fe^{3+} complexes are then moved into the roots, via the Yellow Stripe-like (YSL) transporters, named after the YSL1 PS transporter of maize (ROBERTS et al. 2004, JEONG, GUERINOT 2009).

Although the molecular mechanisms for Zn uptake are not completely understood, it has been suggested that plants may also use Strategy II to obtain Zn from soil. Graminaceous plant species respond to Fe and Zn deficiency by exudation of PSs, increasing the availability of these metals. Thus, PS–Fe^{3+}or PS–Zn^{2+} complexes are transported from the rhizosphere to the root for uptake. The MAs family plays a major role in Fe acquisition, and contributes to the acquisition of Zn by these plants. Although, the IRT transporter has been established as the major Fe uptake system from the soil, IRT is also responsible for uptake of Zn. Genetic engineering approaches have been applied to increasing plant tolerance to low-Zn soils. For example, overexpression of known Zn transporters from Arabidopsis to barley can increase the plant Zn uptake and seed Zn content. These results show the contribution of molecular genetics tools to manipulating Zn and Fe efficiency in crops and the potential for enrichment of the food supply with these metals (VERT et al. 2002, HELL, STEPHAN 2003, ZUO, ZHANG 2008).

Distribution of Fe and Zn in plants

Metals must be transported throughout the plant, from the uptake into the roots up to the tissues where they are required. Once within the root epidermal cell, ions can move through symplastic passages, from the epidermis to the pericycle, to be loaded into the xylem. From the xylem, metals are transported to the aerial organs of the plant (via the transpiration stream), to shoot tissue or across the plasma membrane into leaf cells. Seeds are not fed by the transpiration stream and must rely on the phloem for nutrients. In addition, developing leaves do not have fully differentiated xylem and must receive the necessary metals through the phloem (KERKEB, CONNOLLY 2006, JEONG, GUERINOT 2009).

Plants have a wide range of transporters, whose exact function is not known yet. As for the Fe and Zn uptake and distribution, there are several identified transporters (Figure 1). As mentioned previously, ZRT/IRT-like

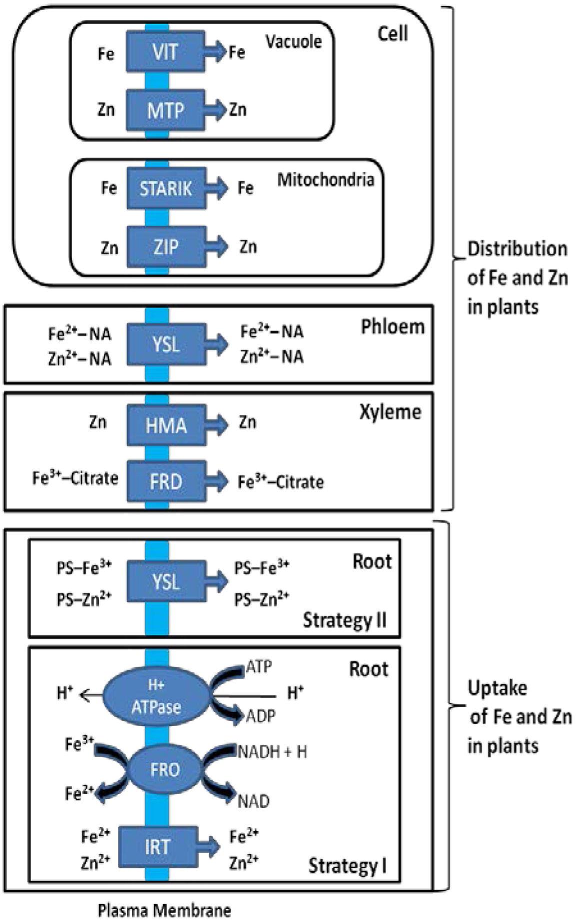

Fig. 1. Metal transports in plants. Non graminaceous plants use a reduction-based strategy I of uptake, whereas gramineous plants use a chelation-based strategy II. In non graminaceous plants, acidification of the soil by an ATPase and reduction of Fe^{3+} by FRO contributes to increase uptake of Fe^{2+} and Zn^{2+} into the root. In gramineous plants, Fe^{3+} and Zn^{2+} are also uptake as PS chelates by YSL transporters in the epidermis and Fe can also be taken up by IRT. Metals can then travel through the symplastic space to the vasculature. Transport into the xylem involves members of the HMA family and the citrate effluxer FRD. YSLs may also translocate metals to the phloem, where they can then be delivered to the above-ground organs. Intracellular Fe and Zn efflux to the mitochondria could take place by STARIK1 and ZIP transporters, respectively. Once metals have reached their target destinations, they needto be stored in vacuoles. Vacuole is an essential metal storage compartment in seeds, Zn and Fe are transported into the vacuole by the MTP and VIT1 transporter, respectively. Blue boxes represent the metals transporters. NA, nicotianamine. PS, Phytosiderophore

protein (ZIP)-like transporters are needed for the uptake of Fe and Zn into roots. With respect of the uptake by Strategy II plants, Fe is taken up as a PS-metal chelate through Yellow Stripe-like (YSL) transporters into roots, while Zn appears to be taken up through ZIP-like transporters (IRT), as in

Strategy I plants. Once in the roots, both minerals will be transferred to the xylem for further distribution towards the aerial organs by various transporters (Vert et al. 2002, Roberts et al. 2004, Ghandilyan et al. 2006).

Xylem. In order to avoid handling toxic free metals during translocation throughout the plant, they are transported in the chelated form with other molecules. The chelator candidates that load Fe into the xylem are citrate and nicotianamine (NA). Depending on the Fe-chelate complex formed, different transport systems are involved in distributing Fe throughout the plant. For example, NA may act an an Fe chelate and facilitate its symplastic translocation to the xylem parenchyma (Kerkeb, Connolly 2006). However, the pH of the xylem favors the chelation of Fe to citrate rather than NA, and it is known that Fe exists as Fe^{3+}–citrate chelates in the xylem (Durrett et al. 2007, Palmer, Guerinot 2009). Because Fe^{3+}–citrate is the major form of Fe present in xylem exudates, citrate is thought to be involved in long distance Fe transport from roots to shoots (Jeong, Guerinot 2009). Once Fe is chelated, the resulting Fe^{3+}–citrate complexes are removed into the xylem, via transporters such as FRD gene. FRD (ferric reductase defective), a citrate transporter localized in the plasma membrane of the pericycle and the vascular cylinder, has been shown to efflux citrate into the xylem and is required for Fe transport to the shoot (Durrett et al. 2007). Thus, Fe is thought to be unloaded from the vasculature into developed tissue through yet-unknown mechanisms (Ghandilyan et al. 2006, Palmer, Guerinot 2009). On the other hand, the heavy-metal ATPase (HMA) genes, which are expressed in vascular tissue, appear to be the most likely candidates to transport Zn into the xylem (Hussain et al. 2004, Ghandilyan et al. 2006). Zn is effluxed into the xylem for long-distance transport by HMA (HMA2 and HMA4), which localize to the plasma membrane of the root and shoot vasculature. The *hma2, hma4* mutants show decreased shoot Zn and increased root Zn, which supports the role of HMA2 and HMA4 in xylem loading. HMA4 is also identified as a gene with increased expression in the Zn hyperaccumulator *Arabidopsis halleri* (Hussain et al. 2004, Palmer, Guerinot 2009).

Phloem. Metal delivery to developing tissues requires transport through the alkaline phloem, with metals probably bound to chelators such as nicotianamine (NA). Fe^{2+} and Zn^{2+} are thought to be transported not only in the xylem, but especially in the phloem transport as NA chelates. The transporters involved in phloem loading are thought to include members of the YSL group (a subfamily of the oligopeptide transporter OPT family), and the NA–metal complexes are most probably transported across plasma membranes by these transporters (Ghandilyan et al. 2006). YSLs may translocate metals to the phloem, from which they can be delivered to the seed. One of more thoroughly described members of this subfamily is YSL1, which localizes to the shoot vasculature as well as the siliques, pollen grains and the developing seeds (Palmer, Guerinot 2009). In *Arabidopsis*, the YSL family (YSL1 and YSL3) as well as the oligopeptide transporter OPT3 are implicated in metal delivery from the vascular tissues to developing seeds (Stacey et al. 2008). Furthermore, it is suggested that YSL proteins could participate in both xylem-to-phloem metal transport in young growing tissues and phloem-to-xylem exchange in roots. Interestingly, the function and transport of other metal ligands including mugineic acid, histidine and phytate have been recently reviewed (Puig, Peñarrubia 2009).

Cell. Once transported to the proper tissue, metals must be distributed on the subcellular level to ensure sufficient amounts to the necessary cell compartments. Storage and buffering of Fe at the subcellular level are crucial mechanisms that allow plants to cope with Fe deficiency and toxicity. Organelles such as vacuoles and plastids play a key role in the intracellular compartmentalization or storage of Fe. In plastids, the ferritins can store an important fraction of cellular Fe, and these proteins play various roles related to Fe homeostasis during development or in response to environmental stresses (Briat et al. 2007, Palmer, Guerinot 2009).

Because electron transport chains and primary carbon metabolism require Fe, organelles such as chloroplasts and mitochondria are the most important sites for Fe utilization in the cell (Pilon et al. 2009). For example, nearly 90% of Fe in the plant is localized to the chloroplast, where it is required for use in the electron transport chain and the synthesis of chlorophyll, heme and Fe-S clusters; Fe is also transported into the mitochondria to function in same process (Kim, Guerinot 2007, Palmer, Guerinot 2009). It is believed that Fe efflux from *Arabidopsis* mitochondria could occur by the ABC-type transporter STARIK1 (STA1) protein (homolog of the yeast ATM1p: ABC Transporter Mitochondria 1 protein) located at the inner membrane (Briat et al. 2007), but not all Fe transporters involved in mitochondria and chloroplasts are identified yet (Palmer, Guerinot 2009). In addition, Zn and Fe are used in the chloroplast as cofactors for superoxide dismutases (SODs), preventing cellular damage by the reactive hydroxyl radical species. Zinc is most likely transported by a ZIP that localizes to the mitochondria, but as of yet no ZIP transporters have been assigned this function (Palmer, Guerinot 2009).

Regarding metal transports into the vacuole, this organelle is an essential metal storage compartment in seeds. The vacuole functions during early seedling development as an initial store of metals. Zinc has been shown to be transported into the vacuole by members of the MTP (metal tolerance protein) family, also referred to as CDF (cation diffusion facilitator) proteins (Gustin et al. 2009). Advances concerning Fe fluxes across the tonoplast of *Arabidopsis* seeds demonstrate than mobilization of Fe stored in the seed allow young germinating seedlings to develop during the initial heterotrophic phase. Transporters identified in both vacuolar Fe influx and efflux have been shown to be essential for germination and seedling development (Briat et al. 2007). Iron is transported into the vacuole by the transporter VIT1 (Vacuolar Iron Transport 1), which is critical for localization of Fe into the seed. Remobilization of Fe from the vacuole is thought to be mediated by the actions of NRAMP3 and NRAMP4, which are upregulated under Fe deficiency (Lanquar et al. 2005, Palmer, Guerinot 2009).

It is important to mention than the main strategies that the plant uses to combat metal toxicity are sequestration and chelation to carrier metals. Consequently, in addition to sequestration within the vacuole, Fe has been shown to be stored in plastids in ferritin, a protein nanocage that can store up to 4,500 atoms of Fe^{3+} in its interior as an Fe oxide mineral (Hintze, Theil 2009). In animals, ferritin is the primary storage form for Fe, but recent work has suggested that in *Arabidopsis* the role of ferritin is solely to deal with excess Fe and prevent oxidative damage (Ravet et al. 2009). Therefore, most plants use ferritin primarily to detoxify Fe rather than as a major storage unit. However, some plants use ferritin as a storage unit, and an exciting new study has shown that oceanic diatoms use ferritin to safely store Fe for later use (Marchetti et al. 2009, Palmer, Guerinot 2009).

PROGRESS TO IMPROVE THE IRON AND ZINC CONTENTS IN CROP PLANTS BY BIOFORTIFICATION STRATEGIES

Fe and Zn deficiencies are a well-documented public health issue and an important soil constraint to crop production. The developed world has made great attempt to alleviate MNM through diversification of diets, food fortification, improved public health care and supplementation. Whereas these strategies have been effective in industrialized countries, they have met with limited success in developing countries because these are often too expensive and difficult to sustain (Cakmak 2010, Lungaho et al. 2011). Alternatively, an agricultural approach that can be widely applied to overcome MNM is biofortification, i.e. a process of increasing the level and/or bioavailability of essential nutrients in crops. This is a relatively new strategy than involves the improvement of agronomic characteristics and the nutritional content of crops through agronomy, plant breeding and biotechnology (Petry et al. 2010, White, Broadley 2011).

Agronomic biofortification

Agronomic biofortification (ferti-fortification: fertilizer applications) and plant breeding (genetic biofortification) represent complementary agricultural approaches (Cakmak 2008, White, Broadley 2011). It is considered than application of Zn- and Fe-containing fertilizers is a short-term solution and complement to plant breeding. Published data provide convincing evidence that soil and especially foliar applications of Zn fertilizers are effective in improving grain concentration of Zn. For example, increasing bioavailable Zn levels via Zn fertilization has been shown for pea, navy bean and wheat

(Cakmak et al. 2010, Zhang et al. 2010). In addition, the Harvest Zinc Fertilizer Project has found that foliar application of Zn fertilizers to wheat can significantly increase Zn concentration in the grain; therefore, it is believed that - depending on the extent of Zn deficiency in soils - Zn fertilizers can contribute to better yield of cereal crops (Bouis, Islam 2011).

By contrast, due to the rapid conversion of Fe into unavailable forms when applied to calcareous soils and the poor mobility of Fe in phloem, soil and/or foliar Fe fertilization appears to be less effective than Zn fertilization in enrichment of grains (Cakmak 2008, Zhang et al. 2010). Moreover, some work has shown that plants did not respond to foliar Fe fertilization in terms of grain Fe concentration, such as application of various inorganic and chelated Fe fertilizers that remain ineffective for increasing grain Fe concentration. However, it appears that nitrogen (N) nutritional status of plants plays a critical role in biofortification of cereal grain. Improving the N nutritional status of plants by higher N-fertilizer applications promoted accumulation of Fe and Zn in grain (Cakmak 2010, White, Broadley 2011).

In general, agronomic biofortification of food crops (for certain essential micronutrients) can be used as an effective agricultural tool to improve human nutrition of people in the developing world. The best example of biofortification of food crops using Se fertilizers comes from Finland; this was a documented study where a whole country participated. Since 1984, the addition of selenate to NPK fertilizers for use on crops and pastures was the first method tested to increase the entire population's Se status. Conversely, ferti-fortification could not be a long-term sustainable approach in developing countries. For example, Fe fertilization is even more complicated, as Fe has a strong tendency towards insolubility, unless used in large quantities or when expensively chelated to organic molecules. Foliar applications, which improve yields of plant grown in Fe-deficient soils and increases Fe level in crops is another strategy, but when applied regularly, are costly and constitute potential dangers to the environment (Broadley et al. 2006, Bouis, Welch 2010, Murgia et al. 2012, Sperotto et al. 2012).

Genetic biofortification

On the other hand, crop biofortification by breeding offers a sustainable and low-cost way to provide micronutrients to people in developing countries. Breeding of nutrient-rich staple food crops is indeed the main goal of different international consortia, which aim to reduce MNM through different biofortification programs (White, Broadley 2011, Murgia et al. 2012). Three primary conditions have been identified to make biofortification successful. They are (1) a biofortified crop must have high nutrient density combined with high yields and high profitability, (2) this crop must be shown to be efficacious and effective in reducing micronutrient malnutrition in humans, and (3) the crop must be acceptable to both farmers and consumers in target regions where people are afflicted with MNM (Bouis, Welch 2010).

HarvestPlus, a component of the CGIAR Research Program on Agriculture for Improved Nutrition and Health, leads a global effort to develop and deliver biofortified staple food crops with most limiting nutrients in the diets of the poor: vitamin A, Zn and Fe. This interdisciplinary program works with experts in more than 40 countries. The main target crops studied are bean (Fe/Zn – DR Congo/Rwanda), cassava (Vitamin A - DR Congo and Nigeria), maize (Vitamin A – Zambia), pearl mille (Fe/Zn – India), rice (Zn/Fe – Bangladesh/India), sweet potato (Vitamin A – Uganda/Mozambique), wheat (Zn/Fe – India/Pakistan). This program also supports initial studies of the following crops: banana/plantain (vitamin A), lentil (Fe/Zn), potato (Fe/Zn) and sorghum (Zn/Fe). Most biofortified crops are still in the development pipeline. However, one biofortified staple food crop that has been successfully released is the orange sweet potato (OSP), conventionally bred to combat vitamin A deficiency in regions of Africa where sweet potato is a staple food crop (Bouis, Islam 2011).

Breeding new plant genotypes for high grain levels of Fe and Zn is the most cost-effective strategy. A broad range of Fe and Zn content in grains of major crops has been associated with genotypic variation. Such variability is exploited through breeding programs to produce Fe- and Zn-rich crop varieties. Also, research to assess and enhance genetic variability for Fe and Zn has been amended with the information on retention after processing, bioavailability from the diet, consumption per day, and amount needed in plant food to have measurable effects on nutrient status (Cakmak 2010, Murgia et al. 2012). Thus, HarvestPlus sets the preliminary "minimum" target levels for micronutrient content using gross assumptions about staple food intake (g day^{-1}); bioavailability (% of nutrient absorbed); losses of the target nutrient with processing (milling, storage and cooking); and the proportion of the daily nutrient requirement that should be achieved from the additional amount of micronutrient in the staple food (Bouis et al. 2011). Target Zn concentrations set by the HarvestPlus program are (content as dry weight: µg g^{-1}): 28 in polished rice, 38 in wheat grain, 38 in maize, 66 in pearl millet, 56 in bean, 34 in cassava root, and 70 in root of sweet potato (Bouis et al. 2011). These target concentrations are considered to be conservative, and have been exceeded in breeding lines of rice, wheat and maize (White, Broadley 2011). In the same way, target levels for Fe content of biofortified staple food crops set by this program are (content as dry weight: µg g^{-1}):15 in polished rice, 59 in wheat grain, 60 in maize, 88 in pearl millet, 107 in bean, 45 in cassava root, and 85 in root of sweet potato. The program identified five crops (pearl millet, beans, wheat, rice and potato) with the greatest potential for Zn and/or Fe biofortification (Bouis et al. 2011). It is important to mention that some otheer recent research supports the hypothesis of micronutrient dilution in cultivars over time. For example, wild and primitive wheat represent a better and more promising genetic resource for high Zn content. Collections of wild emmer wheat showed impressive genetic variation and the highest levels Zn (14-190 mg kg^{1}); also, some accessions show simultaneously both very high levels of Zn (up to 139 mg kg^{-1}) and Fe (up to 88 mg kg^{-1}) in seeds (Cakmak 2008).

Plant foods (seeds and grains) contain antinutrients [phytic acid (PA) and polyphenols (PP)] that can reduce the bioavailability of dietary Fe and Zn to humans. Dietary substances that promote/enhance the bioavailability of micronutrients [prebiotics] in the presence of antinutrients are also known, whose levels are controlled by genetic and environmental factors. These compounds, than affect Fe and Zn absorption from the human diet, must be considered when work with biofortificated crops. For example, in the last decade one of the biofortification approaches for improving Fe absorption in humans has been the isolation of low phytate (lpa) genotypes of crops to improve Fe bioavailability. But a recent study on women whose diet had a lower level of either PA or PP level in common beans, has demonstrated only a modest positive influence of the tested factors on Fe absorption. This result implies that the priority should be to breed for high Fe concentration rather than low PA content. Dietary PA and PP are associated with desirable human health benefits, PP has diverse beneficial properties (antineoplastic, antioxidant and anti-inflammation) and PA is a broad spectrum antineoplastic agent, thus it is suggested that these compounds might protect against cancer in humans (Pixley et al. 2011, Murgia et al. 2012). On the other hand, increasing Fe availability can help to treat anaemia, but there is evidence that it can also promote pathogen growth. Thus, an optimum Fe status is by and large achieved safely in developed countries. However, in developing countries, the widespread incidence of infectious diseases and the consequent host-pathogen competition for Fe complicate any effort at designing guidelines for daily Fe requirements. Crops biofortified with prebiotics (such as inulin) have the potential to avoid the ‘Fe paradox’ caused by the host-pathogen competition for Fe, by favoring amelioration of gut health and gut-associated immune defense. Fruit and vegetables have significant levels of inulin and there is some evidence of stimulatory effects of inulin on dietary Fe absorption in animals. Therefore, it is argued that an increase of the content of Fe and prebiotics in edible parts of plants is expected to improve health, whereas the reduction of PA in crops valuable for human diet might be less beneficial in developed countries than in developing countries exposed to endemic infections (Murgia et al. 2012).

Biotechnology biofortification

Finally, in the absence of genetic variation in the micronutrient content among varieties, transgenic approaches can be a valid alternative for biofortification. Two distinct approaches are used to improve mineral content: (a) increase the efficiency of uptake and transport into edible tissues and (b) increase the amount of bioavailable mineral accumulation in the plant. Engineering strategies to increase the plant mineral content are concentrated primarily on Fe and Zn, which are more frequently deficient in human diets (Palmgren et al. 2008, Hirschi et al. 2009).

Key genes of metabolic pathways, uptake, translocation and re-translocation that influence the accumulation in harvested plant parts have been identified. For example, nicotianamine (NA) is an important chelator that can control metal accumulation in seeds, thus manipulation of cellular NA levels is another approach for improving Fe and Zn contents in plants. Recent studies have shown that enhanced expression of a rice-nicotianamine synthase gene (OsNAS3) results in an increase of these metals in both vegetative tissues and mature seeds (Lee et al. 2009).

Overexpression of ferritin (Fe storage protein of bacteria, animal and plant cells) represents a possible transgenic approach to enhancing the Fe concentration in edible parts of plants. Eukaryotic ferritins consist of 24 subunits, which assemble in a cage-like structure able to sequester or release Fe upon demand, thus representing a dynamic ‘Fe-reservoir’ that can be the predominant mechanism of Fe storage in seeds of many plants (Murgia et al. 2012). The study on transgenic rice expressing an Fe storage protein, ferritin, showed up to threefold higher Fe in transgenic brown rice grains (38.1±4.5 mg kg^{-1}) compared to untransformed grains (11.2±0.9 mg kg^{-1}) (Aizat et al. 2011).

Maize has been altered using transgenes to increase Fe bioavailability, e.g. Drakakaki et al. (2005) generated transgenic maize expressing, both an Aspergillusphytase and soybean ferritin, in the kernel. In the most active transgenic line, up to 95% of the phytate was degraded and a 50% increase in the Fe level of the grain was observed. Fe bioavailability was evaluated using an *in vitro* digestion/Caco-2 cell model and demonstrated that phytase expression was directly correlated with Fe bioavailability and uptake (Lungaho et al. 2011). In addition, it was possible to increase the Fe content in polished rice more than six-fold by transferring two plant genes: nicotianamin synthase (nicotianamin) and ferritin. Their synergic action allows the rice plant to absorb more Fe from the soil and store it in the rice kernel: nicotianamin binds the Fe temporarily and facilitates its transportation in the plant and ferritin acts as a storage depot for Fe in both plants and humans. The genes are controlled in such a way that nicotianamin is expressed throughout the rice plant, but ferritin only in the rice kernel; this prototype works well in a greenhouse, without possible negative effects (Gruissem 2010).

It is important to mention that those are some examples of biotechnology showing the positive impact on human nutrition by reducing Fe malnutrition via biofortification. Most of the work being done to biofortify staple food crops relies on traditional plant breeding techniques. Only for those nutrients that cannot be bred by conventional methods, some organizations are conducting preliminary research to determine what role transgenics can play in breeding biofortified crops. However, it is claimed that only a national agricultural research program of each country can help to make dissemination decisions. For example, as a policy, HarvestPlus will not distribute transgenics ever developed under its auspices to any country that does not have biosafety regulatory systems in place or does not wish to adopt transgenic varieties (HARVESTPLUSFAQ 2012, http://www.harvestplus.org/content/faq).

IMPACT OF IRON AND ZINC BIOFORTIFICATION ON HUMAN NUTRITION AND HEALTH

Mineral nutrient deficiencies are a worldwide problem, which is directly correlated with poverty and food insecurity. MNM can affect all age groups, but young children and women of reproductive age tend to be among those most at risk of developing micronutrient deficiencies. MNM has many adverse effects on human health, not all of which are clinically evident (Benton 2008). It is estimated that 2 billion people suffer from MNM, due to the lack of critical micronutrients such as vitamin A, Zn, and Fe in the diet. This situation impairs the mental and physical development of young populations (lower IQ, stunted growth and blindness). In addition, MNM reduces the productivity of adults due to an increased risk of illness and reduced work capacity, which has profound implications for economic development (Bouis, Islam 2011). Worldwide, the three most common forms of MNM are Fe, vitamin A and iodine deficiency, followed by the shortage of micronutrients such as Zn, folate, calcium, proteins and vitamins, but Fe deficiency is the most prevalent (Benton 2008, WHO 2012. www.who.int/nutrition /topics/ida/en/index.html).

The major cause of MNM is a poor quality diet, mainly lacking in animal products (micronutrient-rich foods such as meat, fish, poultry, eggs, milk and dairy products). Consequently, populations that consume few animal source foods may suffer from a high prevalence of several micronutrient deficiencies simultaneously. Therefore, a balanced diet would be the best way to prevent MNM, but very often people have no access to appropriate food. At the same time, the proportion of the global population suffering from MNM has increased because modern plant breeding has been historically oriented toward high agronomic yield rather than the nutritional quality, producing a lower density of minerals in many crops (Rana et al. 2012).

Iron. Micronutrients are essential elements needed in small amounts for adequate human nutrition. The mineral Fe is essential to human well-being and an adequate supply of Fe helps to prevent Fe deficiency, a prevalent health concern of the entire world. Most of Fe in the human body is present in erythrocytes as haemoglobin, where its main function is to carry oxygen from the lungs to tissues. Iron is also an important component of various enzyme systems, such as cytochromes, involved in oxidative metabolism. This micronutrient is generally stored in the liver as ferritin and as haemosiderin. Iron differs from other minerals because the Fe balance in a human body is regulated by absorption only as there is no physiological mechanism for its excretion (Benton 2008, Hurrel, Egli 2010).

Iron deficiency is the result of a long-term negative Fe balance and severe stages of deficiency cause anaemia. Anaemia is defined as a low blood haemoglobin concentration; haemoglobin values that indicate anaemia have been defined for various population groups by the WHO. This is the most widespread nutritional disorder in the world and the only nutrient deficiency with significant prevalence in industrialized countries. Two billion people are anaemic (over 30% of the world's population) due to Fe deficiency, and this situation is frequently exacerbated by infectious diseases in resource-poor areas. Malaria, HIV/AIDS, hookworm infestation, schistosomiasis, and tuberculosis are important factors contributing to the high prevalence of anaemia (WHO, 2012, www.who.int/nutrition/topics/ida/en/index.html).

In general, Fe deficiency affects more people than any other condition, constituting a public health condition of epidemic proportions. Fe deficiency and anaemia reduce the work capacity of individuals and entire populations, bringing serious economic consequences and obstacles to development of affected countries. Low dietary diversity and inadequate daily intake are the main reasons for the widespread occurrence of Fe deficiency in human populations, affecting a large number of children and women in developing countries. Main consequences of Fe deficiency include mental retardation, decreased immune function, reduction of work capacity and increased mortality of mother and child at birth (WHO 2012. www.who.int/nutrition/topics/ida/en/index.html).

Based on intake data and isotope studies, Fe bioavailability has been estimated to be in the range of 14-18% for mixed diets and 5-12% for vegetarian diets, and these values has been used to generate dietary reference values for all population (HURRELL, EGLI 2010). The daily recommended Fe intake for human ranges between 8 and 18 mg day^{-1} depending on age and gender, with recommended 30 mg day^{-1} for pregnant women. However, a large number of people in the world do not have the privilege of enriching their diets enough to allow this recommended intake (ACIKSOZ et al. 2011, BHULLAR, GRUISSEM 2013).

It is important to mention than recent data refutes the use of haemoglobin as the sole indicator of Fe deficiency since this information is influenced by other factors. Therefore, prevalence of Fe deficiency can be evaluated on the basis of age, sex, race, socioeconomic status and regional variances. Newer measurements to diagnose Fe deficiency in population studies include biomarkers such as serum ferritin, transferrin saturation, free erythrocyte protoporphyrin, and C-reactive protein (BEARD et al. 2006).

The causes of Fe deficiency vary significantly during different stages of life, according to socioeconomic status, but these differences in the Fe status are also related to dietary constituents. Dietary Fe intake, as well as other dietary constituents that influence food Fe absorption such as inhibitors and enhancers play a more important role than the Fe content of the diet (HURRELL, EGLI 2010). For example, populations consuming diets rich in meat and ascorbic acid, both facilitators of Fe absorption, tend to have less Fe deficiency than populations with diets rich in inhibitors of Fe absorption (phytates and polyphenols). Diets high in fiber and/or Ca have a negative effect on Fe absorption, but to a lesser extent than those rich in inhibitors. Diets containing lower Ca content, taken with food that enhances absorption, do not have an inhibitory effect on absorption, compared to high Ca-containing diets. Until now, the interaction between all these dietary constitutes is difficult to integrate and further research is needed to gain better understanding of the relationship between dietary patterns and Fe supply (FLEMING et al. 1998, ROUGHEAD et al. 2002, HURRELL, EGLI 2010).

Improvement of Fe deficiency has been practiced mainly through food fortification and supplementation programs to help overcome the rising Fe deficiency statistics around the world (SCHONFELDT et al. 2010). Although these strategies have been in place for many years, food-based approaches to improve Fe status in the general population are considered more sustainable. Development of Fe-rich varieties of staple food crops may benefit almost every population in the world owing to the simple production and spread of Fe-rich crops, and such nutrition-rich foods are safe for consumption. Because the majority of people suffering from micronutrient malnutrition often do not have access to supplementation strategies or to diversifying their diets, biofortification could become the most sustainable approach to improvement of micronutrients nutrition across generations in resource-poor, rural households in low-income countries (BROWN et al. 2009, GIBSON 2012, BHULLAR, GRUISSEM 2013). However, more Fe studies are needed to facilitate the development of a sustainable food-based approach to combat Fe deficiency (SCHONFELDT, HALL 2011).

Some unresolved Fe bioavailability issues concern the forms of Fe found in food. It is generally accepted that of the two primary forms of Fe found in food, heme Fe is more available for absorption than non-heme Fe. Although non-heme Fe forms a greater portion of the total Fe in foods, its absorption is low and affected by many factors such as the Fe status of the host, enhancing and inhibiting substances, factors consumed prior to/or with the meal and solubility in the intestine. Heme Fe, although mostly consumed in smaller amounts, is two to three times more bioavailable (15-35%) than non-heme Fe (2-20%) and it is less affected by other dietary factors. For these reasons, the total Fe content of food needs to be further investigated and the heme- and non-heme fractions reported to facilitate development of a sustainable food-based approach to combat Fe deficiency (SCHONFELDT, HALL 2011). In addition, the mechanism by which Ca inhibits Fe absorption, the nature of the meat factor, and the influence of vitamin A, carotenoids, and nondigestible carbohydrates on Fe bioavailability from mixed diets needs clarification. The Fe status of the individual is the dominant factor that determines Fe bioavailability, and other host-related factors, such as inflammation (obesity is an inflammatory disorder related with the reduction of Fe bioavailability) may also play an important role. Actually, the consumption of Fe-fortified foods and the bioavailability of Fe-fortification compounds vary widely, so the contribution of fortification Fe to the bioavailability factors is difficult to estimate. In general, it is a priority to study the Fe bioavailability factors to combat Fe deficiency; the situation than depends on the consumption of meat, fruit, vegetables, processed foods, Fe-fortified foods and Fe-biofortificated foods (HURRELL, EGLI 2010).

Zinc. Zn deficiencies in humans occur as a consequence of an inadequate dietary intake. Factors that decrease absorption include dietary inhibitors, such as phytate or certain types of fiber, drugs, and interactions between essential nutrients. Meat is also the best food source of bioavailable Fe and Zn, so in vegetarian populations and developing countries, Fe and Zn deficiencies usually coexist (Graham et al. 2012).

The importance of Zn as an essential nutrient for adequate human health is well known. Adequate Zn nutrition is essential for human health because of zinc's critical structural and functional roles in multiple enzyme systems that are involved in gene expression, cell division and growth and immunologic and reproductive functions. As a consequence, Zn deficiency affects children's physical growth and raises the risk and severity of a variety of infections. About 30% of the world's population is Zn deficient, but infants and young children are probably the most vulnerable. Pregnant and lactating women are also likely to be very susceptible to Zn deficiency, and there is an urgent need for more information on the implications of low Zn status in these particular population groups (Lowe et al. 2009, Yakoob et al. 2011). The most important negative effects of Zn deficiency involve immune competence, subnormal growth and reproductive function. Some of the adverse health consequences of Zn deficiency vary with age: diarrhoea, dermatitis and neurobehavioural disturbances are common during infancy, whereas skin changes, anorexia, impaired taste acuity, growth retardation and recurrent infections are more frequent in children. During adolescence, delayed sexual maturation and abnormalities in skeletal growth and mineralization have been described, and among the elderly, chronic non-healing leg ulcers and recurrent infections occur (Tesan et al. 2011, Gibson 2012).

Inadequate intakes of dietary Zn can arise from low intakes of Zn *per se*, poor bioavailability, or a combination of these dietary factors. Zn is the mineral most abundant and easily absorbable from animal proteins, whereas consumption of vegetable and cereals decreases its absorption due to binding of Zn to phytates, the only substantial dietary factor that inhibits Zn absorption, especially when diets are low in flesh foods (Yakoob et al. 2011, Gibson 2012). Although mineral bioavailability is complex, phytate-to-mineral molar ratios can be used as a qualitative measure of their bioavailability in human food. Indeed, most variation in Zn bioavailability is explained by phytate:Zn contents in human diet. In fact, this ratio in food is considered to be a good indicator of Zn bioavailability. The efficiency of Zn absorption from a diet ranges from about 15% to 35%, depending largely on the amount of Zn consumed and the presence of dietary phytate (Gibson 2012). The current recommendations for dietary Zn intake in adults range from 7 mg d^{-1} (UK Reference Nutrient Intake) to 11 mg d^{-1} (US Recommended Dietary Allowance).This broad range reflects in part the variation in requirements due to differences in the bioavailability of Zn from different diets and the difficulties associated with estimating the requirements for optimal health. High levels of dietary calcium can inhibit Zn absorption, especially in the presence of phytates. Unlike Fe, Zn absorption is neither inhibited by phenolic compounds, nor enhanced by vitamin C. Until now, the influence of these risk factors for Zn deficiency has been difficult to integrate, thus further research is needed to evaluate the bioavailability of Zn from usual diets to gain better understanding of the relationship between dietary patterns and Zn supply (LOWE et al. 2009, GOMEZ-GALERA et al. 2010). Zn deficiency is now widely recognized as a leading risk factor for morbidity and mortality. Zn deficiency has been estimated to be responsible for approximately 4% of the worldwide burden of morbidity and mortality in children less than five years of age. On 2009, the IZiNCG (International Zinc Nutrition Consultative Group) Steering Committee reexamined the latest strategies to control Zn deficiency and to enhance Zn nutrition. They agrue that potential strategies to combat Zn deficiency are supplementation, fortification, dietary diversification/modification and biofortification, the choice depending on the magnitude of risk, life-stage group, and scenery. Zn supplementation is recommended for treating acute diarrhoea, and to prevent stunted growth, diarrhoea, pneumonia, and mortality in high-risk children. Zn fortified cereals are appropriate for urban households, whereas dietary diversification/modification and biofortification are suitable for the rural poor. For the maximum impact, interventions should be integrated with effective public health programs that address underlying causes of Zn deficiency (GIBSON 2012).

There is good evidence supporting the beneficial impact of Zn interventions, especially of zinc supplementation. Zn supplementation trials conducted over the last few decades in children from developing countries have demonstrated the positive benefits of improved Zn status, including better growth rates and reductions in the incidence of various infectious diseases; also, therapeutic Zn supplementation reduces the duration and severity of diarrhea (BROWN et al. 2009).

Less information is available on the impact of Zn fortification programs and of dietary intervention strategies, although the available evidence suggests that both of these approaches should enhance Zn status. Food fortification or the addition of nutrients to food in a higher level to that found originally, are considered a cost-effective strategy to improve the micronutrient status of a population. The available studies clearly show that Zn fortification can increase dietary Zn intake and total daily Zn absorption. Despite the positive effect of Zn fortification on the total Zn absorption, only a few studies have found positive impacts of Zn fortification on the serum Zn concentrations or functional indicators of Zn status. Thus, additional research is needed to determine the impact of Zn fortification, with or without other micronutrients, in populations at risk of Zn deficiency (HESS, BROWN 2009). Fortification with Zn has been limited and generally confined to infant formula milks, complementary foods, and ready-to eat breakfast cereals (HENNESSY-PRIEST et al. 2008).

Finally, dietary modification and/or diversification refer to the use of strategies to improve to access and use of Zn-rich foods. There are several strategies that can be used and although some are considered long-term strategies, they are very sustainable. Breastmilk is an important potential source of bioavailable Zn for infants, so promotion of breastfeeding programs than support adequate Zn nutrition of young children are needed. Other interventions to increase the availability, accessibility, and consumption of animal-source foods or to increase the Zn content of plant-source foods or to increase Zn absorption from these foods should all enhance the Zn statusof the consumers. However, rigorous evaluations of large-scale dietary approaches are still lacking. In the future, provided the health benefits are confirmed, biofortification could become the most sustainable approach to improve Zn nutrition across generations in resource poor rural households in low-income countries (Brown et al. 2009, Gibson 2012).

The recent WHO publication provided information on how to determine appropriate levels of fortification for conventional food fortification programs, and similar approaches can be used for establishing desirable levels of biofortification. Moreover, specific guidelines have now been published on the recommended levels of Zn and Fe fortification of cereal flours. The recommendations for Zn are based on estimates of the ability of mixed diets to meet human physiological requirements for absorbed Zn, using information on the amount of flour consumed by the population, the degree of milling commonly practiced, and the amounts of Zn and phytate consumed in the rest of the diet. By contrast, the recommendations for Fe fortification are based mainly on the experience of prior intervention programs, using different types of fortificants, because predicting Fe absorption is complicated by the fact that it is regulated according to individual Fe status and affected by multiple inhibitors and facilitators of absorption. Nevertheless, the proposed levels of conventional fortification with both Zn and Fe can be compared with proposed breeding targets for biofortification in given populations (Brown et al. 2010).

CONCLUSIONS

MNM arising from Zn and Fe deficiency is a continuing and serious public health problem in the world. The developed world has made great attempt in alleviating MNM through diversification of diets, fortification and supplementation. Although food fortification has played an important role in resolve MNM problem, this strategy has two great disadvantages: it is usually dependent on funding and restricted to urban areas. Increasing the micronutrient density of staple crops, or biofortification, can improve human nutrition on a global scale. It can be achieved by agronomic fertilization; however, this is not a long-term sustainable approach in developing countries, some fertilizers (Fe) are costly and dangerous to the environment. By contrast, genetic biofortification has multiple advantages, principally that the benefits reach the total population. Therefore, international agricultural researches are implementing programs to develop biofortified staple crops. It is well known that biofortification through plant breeding is considered as a promising and cost-effective approach for diminishing MNM, either as a stand-alone solution or in combination with supplementation and fortification. Yet some work remains to explain genetic control and molecular mechanisms affecting the accumulation of Zn and Fe in grain. Further, evidence suggests that nitrogen (N) nutritional status of plants can have a positive impact on root uptake and the deposition of Fe and Zn in seed.

Conventional breeding is the primary focus of programs to enhance staple food crops with sufficient levels of Fe and Zn, to meet the needs of at-risk populations in the world. But it is our priority to study the bioavailability factors that depend on the consumption of meats, vegetables, processed foods, fortified and biofortified foods. It is claimed that Fe and Zn bioavailability can be improved by increasing dietary factors that enhance absorption, by decreasing factors that inhibit absorption, or by increasing their content of the diet. Today, the interaction of these dietary constituents is difficult to integrate and further research is needed to understand the relationship between dietary patterns and supply of micronutrients.

Until now, the potential strategies to combat Fe and Zn deficiencies in human are fortification, supplementation, dietary diversification and biofortification, their choice depending on the magnitude of risk, life-stage group, and scenery. The recent WHO publication provided information on how to determine appropriate levels of fortification for conventional food fortification programs, and specific guidelines about the recommended levels of Zn and Fe for cereal flours. These proposed levels of fortification will help to compare and support the studies of proposed breeding targets for biofortification with both Zn and Fe, to understand the connection between dietary patterns and micronutrient supply. Furthermore, most economic analyses suggest that genetic biofortification is more effective than other programs for increasing dietary Fe and Zn intakes of vulnerable populations.

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