

Review Article

Genetic Processes of Iron and Zinc Accumulation in Edible Portion of Crops and Their Agro-Biofortification: A Review

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Abstract: Iron (Fe) and zinc (Zn) are essential micronutrient for both human and plants, but Fe and Zn deficiency is prevalent in the world especially developing countries including India and China. Biofortification is considered the most promising approach to alleviate Fe and Zn malnutrition. Thus this study was mainly conducted to review the recent progresses on the strategies of the processes affecting Fe and Zn accumulation in edible portion of crops at genetic and physiological levels. While agricultural approaches are useful to gain Fe and Zn enriched cereals, therefore agro-biofortification of Fe and Zn by agricultural approaches was also reviewed for possible solution in intensive agriculture system.

Keywords: Iron, Zinc, Gene, Homeostasis, Agro-biofortification

1. Introduction

Iron (Fe) and zinc (Zn) are essential elements for human growth, development, and maintenance of immune system. Iron is needed for psychomotor development, maintenance of physical activity and work capacity and resistance to infection [1]. Zinc is needed for growth and for maintenance of immune function, which enhances both the prevention of and recovery from infectious diseases [2]. But Fe and Zn deficiencies remain a global problem that over three-billion populations are suffering from, especially among women and children in developing countries including China [3]. Meat products are the best source of both Fe and Zn. Nevertheless, Fe and Zn deficiencies result primarily from a too narrow staple food based on cereals like rice, wheat, maize and others that are low in density and bioavailability [4].

Many approaches are used to alleviate micronutrient deficiencies such as pharmaceutical preparation, food fortification, and dietary diversification. But these strategies have proved to be unrealistic, restrained, because of many reasons [5]. As a consequence there is an increasing interest in

breeding for staple crops that have higher contents of micronutrients, naming biofortification [6]. Biotechnology and transgenic crops offer us a new strategy to solve these malnutrition problems [7]. Cereals are the primary staple food of humankind and are accordingly central in strategies at alleviating micronutrient deficiencies by biofortification. However, the genetical and physiological basis for micronutrient efficiency in crop plants and the processes controlling the accumulation of micronutrients in seeds are not understood with any certainty. There are still several barriers to overcome in genetically modifying plants to accumulate more micronutrients in their edible parts [7]. During last decades, great progresses have been gained. It is necessary to review these areas for more understanding the underlying processes. In addition, agricultural approaches are also useful to improve the micronutrient density in edible parts of field crops [8, 9], thus this concern was also reviewed in this study.

2. Review and Discussion

Increasing mineral contents of plants through increasing

understanding about how minerals are got from the soil and especially how the minerals are then transported throughout the plant. In particular, we need to ensure that the desired minerals (such as Fe, Zn) are enriched in the edible part of the plant [10].

Seeds are heterotrophic organs, totally dependent on nutrients imported (nutrient loading) from the parent plant for their growth and development [11]. One of the barriers to biofortification of seeds is the lack of knowledge of how minerals are loaded into seeds, resulting in uncertainty about the best genes or pathways to target for modification [12]. In this part, Fe and Zn homeostasis including uptake from soil, intercellular and intracellular transport, long distant transport, re-translocation from source leaf to developing seeds are described for better understanding the final grain Fe and Zn accumulation.

2.1. Fe Homeostasis

Iron is the most important yet problematic of the essential elements required by plants. It is needed for life-sustaining processes from photosynthesis to respiration, yet it can be toxic at high levels due to its propensity to form hydroxyl radicals that can damage cellular constituents [13].

2.1.1. Fe Uptake from Soil

Even if Fe is the fourth most abundant element in the earth's crust, it is the third-most limiting nutrient for plant growth primarily due to the low solubility of Fe in aerobic environments [14]. It's known that more than one third of the global soils are Fe deficient. In order to fight with this limited element, the plants have evolved several methods to get Fe from the soil [15].

The strategy I mechanism included proton extrusion to solubilize Fe (III) in the soil, reduction of the solubilized Fe (III) by a membrane-bound Fe (III) chelate reductase and transport of the resulting Fe (II) into the plant root cell by a Fe (II) transporter. For every one unit drop in pH, Fe becomes a thousand fold more soluble. Thus strategy I plants extrude protons to rhizosphere in response to Fe deficiency [16]. Although the protein responsible for releasing protons into the rhizosphere has not yet been identified, several proton-ATPase of the AHA (Arabidopsis H⁺-ATPase) family are induced in the roots of Fe-deficient plants, they may be the candidates for proton exudation [17]. Except tomato, maize (Strategy II plant) also reacts with more proteins from Fe-deficient roots than from Fe-sufficient roots and Fe-PS uptake was enhanced [18]. FRO2 is the enzyme responsible for Fe (III) reduction under Fe deficiency in the roots of Arabidopsis [19]. Furthermore, overexpression of FRO2 has demonstrated that Fe (III) chelate reductase activity is the rate-limiting step in Fe acquisition; plants over expressing FRO2 are resistant to low Fe growth conditions [20]. FRO2 belongs to an eight-member gene family and is regulated both transcriptionally and post-transcriptionally [20, 21]. There are homologs in other plants (pea, tomato). Once Fe is reduced, it is transported across the plasma membrane of root epidermal cells by IRT1 which is a member of the ZIP family [22], and it is the major root Fe

transporter in Arabidopsis [23]. Orthologs of IRT1 have also been characterized in tomato and rice; the mRNAs of both genes accumulate in Fe-deficient roots [24]. Iron appears to be the most important metal for IRT1 in plants, although IRT1 is able to mediate the transport of multiple metals in yeast, including Fe, Mn, Zn and Cd [25]. IRT2 is also expressed in the epidermal cells of Fe-deficient roots, and transport Fe and Zn [26]. However, the two proteins do not have redundant functions [25, 27].

Grasses (Strategy II plants), such as corn, wheat and rice, use the chelation-based response to Fe-deficiency. Grasses release small molecular weight compounds known as the mugineic acid (MA) family of phytosiderophores (PS). The PS has high affinity for Fe³⁺ and efficiently binding Fe³⁺ in the rhizosphere. Fe³⁺-PS complexes are then transported into the plant roots via a specific transport system. Each grass produces its own sets of MAs. Nicotianamine (NA), produced by nicotianamine synthase (NAS), is the key intermediate in the generation of MA. Grass-specific nicotianamine aminotransferase (NAAT) converts NA to the intermediate 3'-keto DMA [27]. At present, we still don't know how the MAs are secreted to the rhizosphere. Following Fe³⁺ chelation by PS, a high-affinity uptake system was created for specific Fe-PS complex and transports Fe-PS into the epidermal cells of Fe-deficient roots. YS1 (yellow-stripe gene) are the possible transporter which encodes a proton-coupled transporter for phytosiderophores and NA metal chelates. YS1 mRNA accumulates in response to Fe-deficiency showing its function on Fe uptake. In addition, YS1 is also expressed in the shoots, suggesting a role for YS1 in the intercellular transport of Fe in plant shoots [28]. Interestingly, strategy II plants can also take up Fe²⁺ like Strategy I plants. Rice, for example, in addition to having the ability to transport Fe-PS complexes, is able to transport Fe²⁺ via OsIRT1 [24]. But the adoption of a Fe²⁺ acquisition strategy can be especially advantageous for rice, since rice plants are not very efficient at Fe³⁺ uptake via Strategy II [29].

2.1.2. The Intercellular and Intracellular Transport of Fe

After Fe has been transported across the plasma membrane of the epidermal cells in roots, members of several different transport families have been implicated in the intracellular and intercellular transport of Fe, including the NRAMP family and the YSL family.

The NRAMP family is an evolutionarily conserved, ubiquitous metal transport family. In Arabidopsis, there are seven members of the NRAMP family, three of which have been implicated in Fe transport [22, 28]. AtNRAMP1, AtNRAMP3 and AtNRAMP4 all mediate the transport of Fe. AtNRAMP1 has a plastid targeting sequence, thus have a role in Fe distribution to Plastid, where are a site of Fe storage in Plants [30]. It is possible that AtNRAMP1 functions to transport excess Fe into the plastids as a means of preventing toxicity. Both AtNRAMP3 and AtNRAMP4 localizes to the vascular system of the roots and shoots of Arabidopsis and mainly play a role in the intracellular transport of Fe, specially in mobilizing Fe from the vacuole [31, 32]. At same time,

VIT1 (Vacuolar Fe Transporter), similar to CCC1 (transport Fe and Mn into vacuoles in yeast), plays an opposing role to mobilize Fe into the vacuole [13].

The YSL family, consisting of eight members in Arabidopsis, has been implicated in the intercellular transport of Fe chelates, specifically Fe complexed to NA [28]. YSL2 functions in the lateral movement of Fe(II)-NA complexes with the veins [33]. YSL1 has a role in the transport of Fe-NA complexes, especially in the delivery of Fe and NA to the Seed by a loss-of-function mutant [34]. The YSL genes also play a role in Strategy II Plants. In fact, 18 YSL genes have been identified in rice. At the tissue level, OsYSL2 is expressed in the companion cells of the phloem suggesting a role for OsYSL2 in the transport of Fe in the phloem [35].

2.1.3. Perceiving and Relaying the Fe Status and Regulation of Fe Deficiency Response

How the plant is able to perceive and signal information regarding the Fe Status of the plant as well as how Fe is distributed throughout the plant? The *frd3* mutant of Arabidopsis was thought to elucidate the mechanisms of Fe localization with the plant, but it does not. Recent result showed that FRD3, belonged to MATE family, transport citrate into the vasculature, but did have a role in efficient Fe translocation [36].

Much of what is understood about the regulation of Fe deficiency responses in Strategy I plants comes from a tomato mutant, *fer*. FER encodes a bHLH transcription factor suggesting that it may act as a Fe sensor to control Fe deficiency responses in root [37]. FIT1, similarity to FER has been characterized in Arabidopsis [38]. Both of them can directly or indirectly regulate IRT1 and FRO2 expression at transcriptional or post-transcriptional level [38]. Recently a rice transcription factor IDEF1 was identified [39]. It specifically binds the Fe deficiency-responsive cis-acting element IDE1. IDEF1 overexpression led to the enhanced expression of the Fe deficiency-induced transcription factor gene OsIRO2 and exhibit substantial tolerance to Fe deficiency in both hydroponic culture and calcareous soil.

2.1.4. Long-Distance Fe Transport

Once Fe enters the root symplast, Fe is required to be bound by chelating compounds. Fe-chelator complexes then move through intercellular connections into the stele along the diffusion gradient. The mechanism of Fe efflux from the symplast into the apoplastic space is not yet clearly understood. FPT1, a Fe-regulated transporter may involve in Fe efflux. It is generally agreed that Fe is present as Fe (III)-citrate complexes in the xylem where the pH is around 5.5-6. FRD3 was supported to have a role in citrate efflux and effective Fe location [36].

The mechanism of Fe uptake from the xylem vessels into leaf tissues is not clear. However, several FRO genes are expressed in shoots and several AtZIP genes are also expressed in shoots suggesting Fe reduction first and then Fe uptake.

2.1.5. Fe Transport in Phloem and Fe Loading of Developing Seed

Iron must also be transported through the phloem, because the transpiration flow in the xylem vessels is inefficient in developing organs such as the apex, seeds and root tips. Fe remobilization from older leaves to younger leaves also takes place via phloem transport [13]. The enhanced demand of phloem-mediated transport of metals during reproductive phases, especially under mineral deficiencies, poses the question of the identity of the associated ligands. The pH in the phloem sap is higher than 7, thus Fe needs to be bound to chelators in order to remain soluble. Studies with the castor bean *Ricinus communis* have identified an 11 kDa Fe transport protein (ITP) as a Fe-chelator in the phloem [40]. ITP specifically binds Fe^{3+} as shown by *in vivo*. Thus it is presumed that Fe is transported as a Fe (III)-ITP complex in phloem. There is a gene in Arabidopsis similar to the ITP gene and it would be interesting to see whether this mechanism of phloem transport exists in other plants [13]. In addition to ITP, NA has been proposed to function in Fe transport in the phloem, based on its ubiquitous presence and its ability to form stable complexes with Fe^{2+} at neutral and weakly pH. The tomato mutant *chln* demonstrates the role of NA in long-distance Fe transport [41]. The presence of a small amount of Fe^{2+} in the phloem sap has led to the idea that NA can act as a shuttle by chelating Fe^{2+} from Fe (III)-ITP during phloem loading and unloading. Some FRO genes, expressed in shoots prove this hypothesis by supplying $\text{Fe}^{2+}/\text{Fe}^{3+}$ conversion. YSL transporters are likely involved in the transport of Fe (II)-NA complexes. As shown before, AtYSL1, AtYSL2, OsYSL2 support the role of NA in Fe phloem transport.

Nutrient loading of seeds is a spatially and temporally dynamic process. Import of transition elements, such as Fe and Zn, is of particular interest to re-dress their deficiencies in seeds used for human consumption [42]. Minerals other than nitrogen may be remobilized from vegetative sources, although a major portion of minerals in seeds are likely supplied through continuous uptake and translocation to developing seeds [12]. As shown in this part, Fe can be loaded into phloem by source leaf and unloaded to developing seed. But the proportion of Fe in seeds from uptake and remobilization is still not clear.

2.2. Zn Homeostasis in Plant

Zinc plays important roles in numerous physiological processes in plants, serving as a cofactor for many enzymes and as the key structural motifs in transcriptional regulatory proteins. A deficiency of Zn, therefore, decrease growth, but excess Zn has significant toxicity to biological systems through metal-based cytotoxic reactions [43]. Therefore, Zn homeostasis in plant must be strictly controlled.

Unlike Fe, Zn does not need to be reduced before transport. The grasses, which extrude PS in response to Fe deficiency, may also use this chelation strategy to obtain Zn from the soil [44]. Consistent with a role in Zn uptake in the grasses, Zn-PS complexes have been demonstrated to be taken up by maize

[45]. In addition, several Zn-efficient cultivars exist. And Zn-efficient cultivars have an increased ability to obtain the limiting nutrient rather than an increased tolerance to lower levels of the nutrient [46]. There are currently conflicting results on whether YSI can mediate the transport of Zn-PS complexes [47]. Furthermore, several ZIP proteins have been characterized in Strategy II plant and rice suggesting that this family plays a role in Zn acquisition in the grasses [48]. Several gene families are participated in Zn transport and will be described below.

2.2.1. ZIP Family

There are over 100 members of the ZIP family found at every phylogenetic level. 16 of these members are from Arabidopsis [49]. The Arabidopsis ZIP1, ZIP2, ZIP3, ZIP4 proteins can all functionally complement a yeast strain defective in Zn uptake [50]. In plant, the mRNAs of ZIP1, ZIP3 and ZIP4 accumulate in response to Zn deficiency. ZIP1 and ZIP3 are root-specific while ZIP4 mRNA accumulates in both the roots and shoots of Zn-deficient plants. Several ZIP proteins have been characterized in rice [51]. OsZIP4 mRNA accumulates in Zn-deficient shoots and roots, and presumably functions to transport Zn across the plasma membrane into the cytoplasm [43]. In rice OsZIP4 may be involved in the translocation of Zn throughout the plant; in situ hybridization in the phloem cells of the stem as well as in the vascular bundles of the roots and leaves. OsZIP1 and OsZIP2 are also more highly expressed in response to Zn deficiency [48]. OsZIP1 mRNA accumulates in Zn-deficient roots and shoots while OsZIP2 mRNA accumulates primarily in Zn-deficient roots.

2.2.2. CDF Family

Members of the CDF (Cation Diffusion Facilitator) family are found in bacteria, fungi, plants and animals and function in transporting metals from the cytoplasm, either by efflux to the extracellular organelles or storage compartments [51]. MTP1, one of the twelve predicted CDF family members in Arabidopsis, has been characterized to date [52]. MTP1 mRNA is present in all tissues and the addition of Zn does not induce higher levels of expression. When MTP1 is overexpressed, however, plants become resistant to toxic levels of Zn. MTP1 has been localized to the vacuolar membrane suggesting a role for MTP1 in the transport of Zn into the vacuole [52, 53].

2.2.3. HMA Family

Two members of the HMA (heavy metal ATPase) family, HMA2 and HMA4, have recently been demonstrated to function in Zn homeostasis in Arabidopsis [54]. They belong to an eight member family of P-ATPases in Arabidopsis and have both been demonstrated to be plasma membrane proteins localized to the vascular bundles in the roots and the shoots of the plants [55]. Elemental analysis of the hma2 and hma4 shows hma2 mutants accumulate more Zn and Cd than wild type [56]; hma4 mutant plants accumulate more Zn and Cd in the roots but less in leaves [57]. The results suggest that HMA2 and HMA4 may function to transport Zn or Cd to a

specific tissue or organelle for sequestration and/or detoxification. AhMHX, another type of metal transporter in A. Halleri was a vacuolar transporter capable of exchanging protons with Mg, Zn and Fe in order to sequester these metals in the vacuole [58].

Although a large number of cation transporters have been identified, but few have been characterized with respect to substrate specificity, expression pattern and cellular localization. Further studies of Zn homeostasis should be conducted in future.

2.3. Strategies to Increase Fe and Zn Content of the Grains

Cereals are the primary staple food of human, thus it is important to know how to increase Fe and Zn content of the grains [59]. Fe and Zn are complexed with various organic compounds at all stages in the plants, from uptake through transport to deposition, in order to eliminate their reactivity. Protein storage vacuoles are ubiquitous in the aleurone layer and embryo of seeds where Fe and Zn generally are considered to deposition. Many transporter proteins such as NRAMP, VIT, MTP and MHX, have participate in the influx and efflux of minerals into the protein storage vacuole.

Several studies have attempted to increase the Fe content of the endosperm by expressing Fe-binding proteins, in particular, ferritin which is a major storage form for Fe in plants being localized in the plastids and essential for Fe homeostasis. Ferritin is therefore an ideal sink for Fe and a number of studies have focused on increasing the ferritin levels and thereby the Fe content of the endosperm [60]. But study suggested that over-expression of the soybean ferritin gene exhausts the Fe reserves in the leaves and it is necessary to improve the Fe transport from the roots. Fe uptake, transport and mobilization to the seed can accordingly be manipulated in a range of different ways. Recently, Ishimaru et al. constructed transgenic rice plants expressing the ref1/372 gene fused to the promoter of OsIRT1 and enhanced tolerance to low-Fe availability and 7.9 fold the grain yield of non-transformed plants in calcareous soils [61].

Manipulation of Zn content of the cereal grain may be less straightforward than for Fe. Proteins are a major sink for Zn as about 300 enzymes and more than 1000 transcription factors require Zn as a co-factor. Furthermore, studies of related Triticum species showed strong correlations between protein content and Zn content [62]. Distelfeld et al. proposed that the Gpc-B1 locus promoted remobilization of Protein, Zn, Fe and Mn from the leaves to the grains [63]. Another way to increase the Zn content of the cereal grain may be to manipulate the transporters involved in Zn translocation, such as P-ATPase, ZIP, CDF, NRAMP and YSL transporters [64].

2.4. Fe and Zn Manipulation by Agricultural Approaches

Balanced nutrition output, especially micronutrient (such as Fe, Zn, I, Se and Vitamins) in agricultural system is the third concern after production and environment. Evidence is growing that our global food systems are failing to deliver adequate quantities of healthy, nutritionally balanced food

especially to poor people globally [65]. A new paradigm for world agriculture is urgently needed to meeting human needs by productive, sustainable, nutritious agriculture [66]. So, crop management practices must aim both the yields and the mineral nutrient concentration [8, 9].

2.4.1. Fertilization

Over 30% arable land is calcareous soil where Fe and Zn bioavailability is low and crop productions and qualities are restricted. Fertilization is a most common agricultural practice to correct Fe or Zn deficiency for improving crop productions or qualities [8].

Soil application of Zn increased grain yields in various cereal crops, such as wheat, rice, maize, peanut, soybean [67]. Increasing grain Zn concentration by soil Zn application depended on species, soil conditions, and crop genotypes. Some results increased grain Zn concentration to some extent, 18.8% in field wheat, or 1 to 16 folds in pot wheat [9]. And some results had effect on neither yields nor grain Zn concentration. Soil Zn Application also was used in vegetable and fruits production. In contrast to Zn, soil application of inorganic Fe fertilizers to Fe-deficient soils is usually ineffective because of quick conversion of Fe into plant-unavailable Fe (III) forms. It is likely to be even more uneconomic if the aim is to increase Fe concentration of the seeds. Although some result showed synthetic Fe-chelates were effective for correction of Fe deficiency [68]. So many forms of Fe fertilizers were produced to alleviate Fe deficiency such as, FeS_2 , Fe-organo complexes, Manure and humic Fe [69]. When micronutrient fertilization is exceeded a certain limit, further increases in fertilizer application cause not only a reduction in the yield, but a decrease in micronutrient density in grain as well. Two key points should be considered: (1) most soil-applied micronutrients are quickly fixed into plant-unavailable forms; (2) soil-applied nutrients are not readily transported down the soil profile. Both of them weaken the effect by soil Fe and Zn application [8].

Micronutrients are needed for small amount by plants and foliar micronutrient application is more suitable than soil application. Plants are capable of absorbing soluble compounds and gases through leaves, phenomena that have been utilized for delivering plant nutrients by foliar spraying. Increasing interest is focus on the effects of foliar application on improving the micronutrient density and other quality characters in crop edible portions. Foliar ZnSO_4 application both increased the yield and leaf Zn concentration of hollow vegetable. Recent study reported that pea Fe and Zn concentration was improved by foliar Fe & Zn complex-fertilizer [70]. Some results increased wheat grain Zn concentration by 2-3 folds by foliar or soil Zn application [8]. Yilmaz et al. reported foliar plus soil Zn application was the best methods to improve wheat grain Zn concentration [71, 72]; Yin et al. showed that foliar Zn application increased both rice grain and brown rice Zn concentration [73]. Cakmak et al. showed that foliar Zn application indeed increased wheat grain Zn concentration, and also showed that Zn was

concentrated in embryo where proteins were also concentrated [9]. It may be used to gain both Zn and protein density wheat in future [74]. Most of the results proved that Fe or Zn can re-translocate from source leaf to grain to some extent.

NPK nutrient management and organic fertilizer application can decrease the soil pH and increase soil available Fe and Zn concentration, and finally increase the yield and total Fe and Zn content in grains [75]. More researches focused on the effect of alternating effect between different elements. The Fe or Zn bioavailability is tremendously affected by pH in rhizosphere. $\text{NH}_4^{+}\text{-N}$ application decreased pH in rhizosphere and apoplast of root, thus favor the Fe or Zn activation. But $\text{NO}_3^{-}\text{-N}$ increased pH and decreased Fe or Zn bioavailability. In sand culture, $\text{NH}_4^{+}\text{-N}$ improved Zn uptake by aerial rice root and Zn translocation to shoot. More phosphorus can decrease Zn uptake by forming insoluble phosphate and finally dilute grain Zn concentration [76].

2.4.2. Micronutrient Management in Late Growth Stage of Crops

Micronutrient management must be done under high yield crop system. High yield is an important way to solve food supply issue. There would be inconsistent between need of micronutrient by crops and supply of micronutrient by roots at crop reproductive (late) stages, finally restrict the grain yields and qualities. So micronutrient manipulation in crop late stage should be useful tool to supply enough micronutrient, especially Fe and Zn by soil or foliar application. However, little attention has been paid to this field, and unfortunately no result has got.

2.4.3. Other Agricultural Approaches

Cropping system is also important way to alleviate Fe or Zn deficiency [8]. Intercropping is a normal cropping system in China. In peanut-maize intercropping system, peanut Fe nutrition was improved by partly using PS excreted by maize [77]. Crop rotations are suitable to special areas and climate. And the beneficial effects of rotation including improving soil chemical and physical fertility, reduced weed infestations, less diseases and available micronutrients (Fe, Zn and Cu). Rotation only focuses on yield and little result was available about improving the edible portion micronutrient density [7, 8]. Soaking and planting Fe (Zn)-rich seed are easy, economical and effective agricultural practices in poor soil. Both the approaches could improve seed Fe (Zn) content, supply enough micronutrient at seedling, improve resistance in stress conditions and finally gain more high yield and good quality [78].

Cakmak summarized that the factors affecting grain Fe, Zn accumulation included soil type, nutrition availability, agricultural approaches, species, climate and crop genotypes [8]. Thus a single approach cannot get the best effect and an integrated micronutrient management is urgently needed to meet the need of the yield and Fe, Zn enriched grain.

3. Conclusion and Prospective

We therefore proposed an integrated nutrition management framework for intensive agriculture (Figure 1):

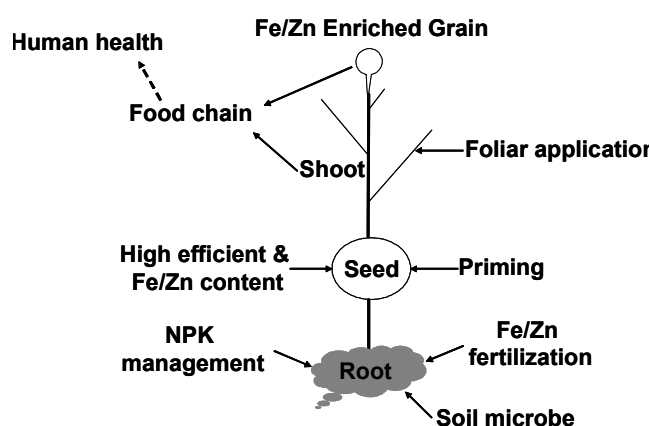


Figure 1. Integrated manipulation for Fe and Zn enriched crops in intensive agriculture.

1. Yield, environment and balanced nutrition should be considered in a sustainable agricultural system. 2. Better species and genotypes are used to improve resistance and efficiency, and harvest high yields and Fe, Zn enriched grains. 3. Balanced fertilization between NPK fertilizer and micronutrient fertilizer. 4. Manipulate micronutrient at crop late stage by foliar application of micronutrients like Zn, Fe along or combined with pesticides control, which is economically and technologically feasible [79]. 5. Combine other agricultural approaches to gain high yields, Fe and Zn enriched grains with high qualities.

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