Iron and Zinc Biofortification Strategies in Dicot Plants by Intercropping with Gramineous Species: A Review

Y. Zuo and F. Zhang

Abstract The lack of micronutrients such as iron and zinc is a widespread nutrition and health problem in developing countries. Biofortification is the process of enriching the nutrient content of staple crops. Biofortification provides a sustainable solution to iron and zinc deficiency in food around the world. Reports have highlighted the current strategies for the biofortification of crops, including mineral fertilization, conventional breeding and transgenic approaches. Any approach which could increase root growth and result in a high transfer of Fe and Zn from the soil to the plant is crucial for biofortification. In addition to these approaches, we draw attention to another important aspect of Fe and Zn biofortification: intercropping between dicots and gramineous species. Intercropping, in which at least two crop species are grown on the same plot of land simultaneously, can improve utilization of resources while significantly enhancing crop productivity, whereas monocropping is a traditional cropping system of only one crop growth. Monocropping has maintained crop productivity through heavy chemical inputs including the application of fertilizers and pesticides. Monocropping has therefore resulted in substantial eutrophication, environmental pollution, a food security crisis and economic burdens on farmers. Monocropping has also reduced the plant and microorganism diversity in the ecosystem. Compared with monocropped plants, intercropped plants can use nutrients, water and light better due to the spatial and temporal differences in the growth factors and a variety of species-specific mechanisms of physiological response to environmental stress. Intercropping is common in developing countries such as China, India, Southeast Asia, Latin America and Africa. In particular, interspecific interaction facilitates the iron and zinc nutrition of intercropping systems such as peanut/maize, wheat/chickpea and guava/sorghum or maize. Intercropping also increases iron and zinc content in the seeds. In a peanut/maize case study, the Fe concentrations in peanut shoots and seed were 1.47–2.28 and 1.43 times higher than those of peanut in monocropping, respectively. In intercropping of chickpea and wheat, the Fe contents in wheat and chickpea seed were increased 1.26 and 1.21 times, respectively, and Zn concentration in chickpea seed was 2.82 times higher than that in monocropping. In this review, we focus on exemplary cases of dicot/gramineous species intercropping that result in improved iron and zinc nutrition of the plants. We present the current understanding of the mechanisms of improvement of iron and zinc in intercropping. The available literature shows that a reasonable intercropping system of nutrient-efficient species could prevent or mitigate iron and zinc deficiency of plants. Here, we propose that intercropping can potentially offer an effective and sustainable pathway to iron and zinc biofortification.

Keywords Biofortification • Dicots • Gramineous species • Intercropping • Iron and zinc

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1 Introduction

The World Health Organization states that the lack of micronutrients such as iron and zinc represents a major threat to the health and development of populations in the world. Two billion people are anemic, many due to iron deficiency (WHO 2007). Billions of individuals are also at risk for zinc deficiency (Prasad 2003). Although food supplementation or fortification efforts have been effective in some countries, their overall success remains limited in developing countries. Biofortification, the process of enriching the nutrient content of crops as they grow, provides a sustainable solution to malnutrition in the world (Jeong and Guerinot 2008). Biofortification can be achieved by utilizing crop and soil management with plant breeding to increase micronutrient concentrations in the edible parts of crops. The concept of biofortification is attractive not only for improving the growing conditions of crops but also for exploiting a plant's potential for micronutrient mobilization and utilization. There have been several recent reviews on the current strategies for the biofortification of crops, including mineral fertilization, conventional breeding and transgenic approaches (Zhu et al. 2007; Mayer et al. 2008). In addition to these approaches, we would like to draw attention to another important aspect of Fe and Zn biofortification: intercropping between dicots and gramineous species, which are strategy I and Strategy II plants, respectively, in their response to iron deficiency. Relatively little attention has been paid to the effects of intercropping on crop micronutrient status. However, considering the importance of intercropping systems in nutrient acquisition and crop production processes, the management of intercropping would be the key to Fe and Zn biofortification.

Intercropping, which is the intermingled growth of two or more crops, is practiced in >28 million hectares of annually sown area in China (Liu 1994) and is also common in other parts of the world, such as India, Southeast Asia, Latin America and Africa (Vandermeer 1989). Multiple cropping, i.e., intercropping or intercropped cropping, plays an important role in agriculture because of the effective utilization of resources, significantly enhancing crop productivity compared with that of monocultured crops (Li et al. 1999, 2007). Facilitative root interactions in mixed cropping systems are most likely of importance for the nutritional improvement of crops grown in nutrient-poor soils and low-input agroecosystems (Zhang and Li 2003; Li et al. 2004). Recently, some research groups have reported that interspecific root interactions and rhizosphere effects could be linked to improved Fe and Zn nutrient uptake in dicot plants by intercropping with graminaceous species in pairings which included maize/peanut, guava/sorghum or maize and chickpea/wheat. For instance, maize/peanut intercropping was shown to improve Fe and Zinc nutrition of peanut (Kamal et al. 2000; Zuo et al. 2000; Gunes et al. 2007; Inal et al. 2007). In particular, as one of the important staple crops with high consumption, any increase in mineral nutrient content might have a significant effect on human nutrition (Graham et al. 1998; Graham and Welch 2001; Cakmak 2002). Presumably, if the nutritional quality of such staple crops can be improved by intercropping it would benefit human nutrition, particularly for the important micronutrients iron and zinc.

In this article, we concentrate on reviewing the literature on how intercropping dicots and gramineous species has been applied to advancing our knowledge specifically related to iron and zinc improvement in plants, and speculate on its future potential impact on biofortification. Hopefully, it will provide a significant component of integrated approaches, which include conventional plant breeding, transgenic approaches and mineral fertilization. The combined use of multiple strategies for iron and zinc improvement will offer a more effective and sustainable pathway to alleviating micronutrient malnutrition.

2 Improvement of Fe and Zn Uptake by Intercropping

2.1 Improvement of Fe and Zn Uptake in Peanut by Rhizosphere Effects from Maize in Intercropping

Iron deficiency is a common nutritional disorder in crop plants in China, particularly in northern China where aerobic and calcareous soils are widespread. Peanut (*Arachis hypogaea* L.) is the major oilseed crop in China, accounting for 30% of the total oilseed production in the country. Iron chlorosis is one of the most common yield-limiting nutrient problems in peanut grown in monocropping systems in the calcareous soils

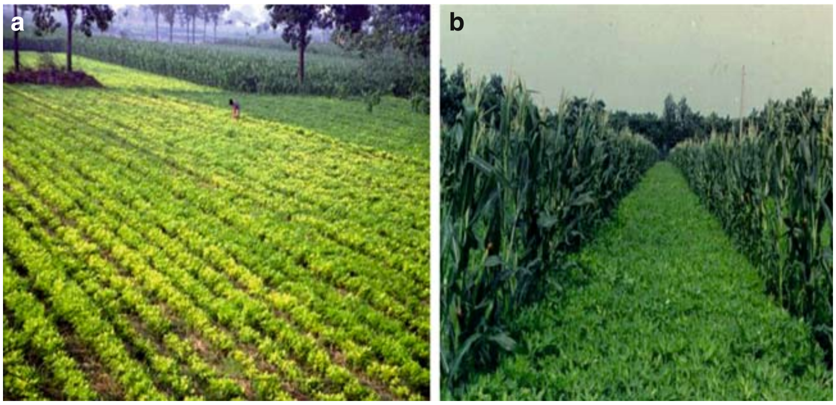

Fig. 1 Peanut growing in monoculture in the field with symptoms of Fe deficiency chlorosis (**a**), Peanut intercropped with maize in the field without symptom of Fe deficiency chlorosis in particular in the vicinity of maize (**b**)

Table 1 The effects of peanut intercropped with maize plants on Fe and Zn contents (mg kg^{-1} DW) in the shoot of peanut at the flowering stage in the field

Cropping systems	Distance from maize (Rows)	Fe	Zn
Monocropping peanut		205.8 ± 23.3^{c}	43.6 ± 5.2^{b}
Peanut/maize (danyu13)	1	302. 6 ± 21.4^{a}	54.8 ± 5.3^{a}
	2	290.0 ± 29.3^{a}	52.1 ± 6.1^{b}
	3	279.8 ± 40.2^{a}	50.6 ± 4.2^{b}

Columns with the same letter are not significantly different at 0.05, using the LSD multiple range test

of northern China (Zuo et al. 2000). In about 50% of these soils, the DTPA-extractable Fe concentration is lower than 5.5 mg kg^{-1} (DTPA: diethylenetriaminepentaacetic acid). For example, in most parts of Henan province iron chlorosis is very severe in peanut grown in monocropping systems in calcareous soil. (Fig. 1a). Calcareous soils are characterized by low organic matter, high pH (7.5–8.5) and high levels of bicarbonate. Soil amendment and foliar application of Fe fertilizers are usually ineffective or uneconomic measures for correction of Fe deficiency chlorosis. There is therefore considerable interest in devising practical approaches for the correction or avoidance of Fe deficiency in crops in Chinese agriculture. Chlorosis in peanut was much less pronounced when this species was intercropped with maize. This is a much more common cropping system than peanut monoculture in the region (Fig. 1b). The extent of improvement in the Fe nutritional status of intercropped peanut was found to be closely related to the distance between the peanut plants and the neighboring maize plants. The nearer the peanut plants to the maize, the less Fe chlorosis in peanut plants was observed. The severity of iron deficiency chlorosis in young leaves of peanut in the intercropping systems was closely related to the distance of the peanut plants from the maize roots when treatments were assessed during the peanut flowering period. In the unrestricted intercropping treatment, where neighboring roots of peanut and maize intermingled freely, the young leaves of peanut plants in rows 1–3 from the maize grew without visible symptoms of iron deficiency (Table 1), while those in rows 5–10 showed variable degrees of chlorosis. These results indicated that the comprehensive rhizosphere effects of maize played an important role in the improvement of the Fe nutritional status of peanut intercropped with maize under field conditions.

Based on the phenomena and evidence from the field, a greenhouse experiment was designed to test whether interaction between roots of maize and peanut has any effect on the Fe nutritional status of peanut in rhizoboxes. The only difference between the monocropping and intercropping systems in the rhizobox experiment was due to separation versus interaction between maize and peanut roots. The taller maize plants would have shaded the peanuts in both treatments, with or without root barriers, but chlorosis developed only in the former treatment. Since the

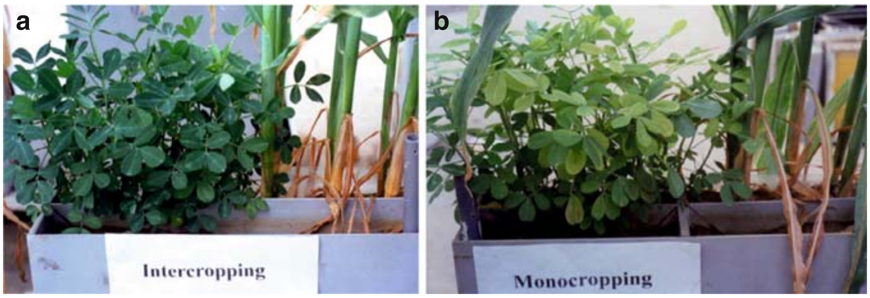

Fig. 2 Peanut grown in intercropping with root interaction of maize and peanut without symptom of Fe deficiency chlorosis. (**a**) Peanut grown in monocropping without root interaction of maize and peanut with symptom of Fe deficiency chlorosis (**b**)

Table 2 The effects of intercropping peanut with maize on Fe and Zn concentrations in peanut (mg kg^{-1} DW) grown on calcareous soil

Plant tissue	Monocropping	Intercropping	References
Fe			
Shoots	28.0 ± 7.0^b	65.5 ± 8.9^a	Zuo et al. 2000
Roots	159.5 ± 13.1^b	203.1 ± 16.8^a	
Seeds	22.2 ± 2.9^b	31.8 ± 3.9^a	
Zn			
Shoot	10.4	26.2	Inal et al. 2007
F values	14.01*		

All data were analyzed using SAS software, expressed as means of three replicates with standard deviation, and the means were subjected to another test by using the least significant difference (LSD) method at the 5% probability level (Zuo et al. 2000). Statistical significance of difference was determined by analysis of variance (ANOVA) and the LSD test at $P \leqslant 0.05$ for multiple comparisons (Inal et al. 2007) $^*P < 0.05$

peanut plants in both the monocropping and intercropping systems shared the same lighting conditions, it seems unlikely that the major interaction between the two species in rhizoboxes can be explained by a shading effect. The younger leaves of peanut plants remained green when the roots of maize and peanut interacted in the intercropping system (Fig. 2a), whereas chlorosis appeared on the youngest leaves when root interaction between the two species was prevented using a PVC barrier in the monocropping system (Fig. 2b). This indicates that maize could markedly improve the Fe nutrition of peanut plants. A more likely explanation for enhanced Fe nutrition was root interaction between maize and peanut. The Fe concentrations in various parts of peanut plants whose roots were allowed to mix with those of maize were generally higher than those whose roots were kept separate (Table 2). The Fe concentration in roots, shoots and seeds of peanut plants grown in the intercropping system without root barriers were 1.3, 2.3, and 1.4 times higher, respectively, than those of peanut plants grown with root barriers (Table 2). The chlorophyll concentration increased about threefold and the HCl-extractable Fe concentration doubled in the intercropping system (Zuo et al. 2000).

It was noteworthy that the maize not only improved the Fe status of peanut in the intercropping system, but intercropping also enhanced Zn content in the peanut (Table 2): this indicates that agronomic intercropping helps mobilize and uptake the limiting nutrient elements Fe and Zn as well as providing benefits through effects on plant growth, development and adaptability to adverse environments.

2.2 *Improvement of Fe and Zn Uptake in Plants in Intercropping of Chickpea/Wheat by Interspecific Root Interactions*

Malnutrition and micronutrient deficiencies, especially Fe and Zn, can be prevalent in many chickpea- and wheat-consuming regions, even though chickpea and wheat seeds are good sources of essential mineral nutrients (Graham et al. 1999; Welch and Graham 1999; Wang et al. 2003; Cakmak et al. 2004). However, in regions where wheat and chickpea are a significant component of the human diet, there appears to be considerable variation in the Fe and Zn concentrations present in the edible portions of the two crop species and their cultivars.

The ranges in Fe and Zn concentrations of wheat germplasm seeds grown in Mexico were 28.8–56.5 mg kg^{-1} and 25.2–53.3 mg kg^{-1}, respectively (Graham et al. 1999). Reported Fe and Zn concentrations (mg kg^{-1}) of chickpea seeds for more than 20 cultivars varied between Fe 39–98 and Zn 25–35 (Williams and Singh 1987). A breakthrough study was published in 2007 in which intercropping of wheat and chickpea improved the concentrations of Fe in wheat seeds, and Fe and Zn in chickpea seeds in the field experiment (Gunes et al. 2007). The concentrations of Fe and Zn in intercropped wheat shoots were significantly higher than in monocrop wheat. In chickpea, the Zn concentration was higher in intercropped chickpea than in the monocropped chickpea (Table 3). Intercropping could overcome potential Fe and Zn nutrient deficiencies, particularly in harvested seeds.

In another chickpea/wheat study under a different P supply (Li et al. 2004), the Fe content in wheat shoots was significantly increased by the complete interspecies root interactions (intercropping) between wheat and chickpea, compared with treatments without the root contact (roots being separated by a solid root barrier, monocropping). The Fe content was increased by the free interspecies root interactions compared with the treatment with the root barrier and inorganic P, suggesting mobilization of Fe from inorganic $FePO_4$ by chickpea (Table 4). Zinc content in wheat and chickpea shoots supplied with inorganic P was increased by interspecies root interactions. Compared with the treatment with the root barrier, however, complete interspecies root interactions (no barrier) between two species increased Zn content in wheat shoots, but decreased Zn content in shoots of chickpea supplied with organic P (Table 4). This study indicated that the Fe and Zinc concentrations vary in response to both genetic and environmental factors. The results from this glasshouse study need to be verified in the field.

According to those studies, we propose that wheat/chickpea intercropping can potentially offer a more effective and feasible method both for increasing Fe and Zn in grain/seed of wheat and chickpea and producing human foods with a higher micromineral nutrient content than when the two species are grown as monocrops. However, considering the importance of different intercropping systems in nutrient

Table 3 Shoot and seed iron and zinc concentrations per dry mass of wheat and chickpea grown as monocropping and intercropping in field conditions (modified from Gunes et al. 2007)

Cropping system	Fe (mg kg^{-1})	Zn (mg kg^{-1})
Shoot		
Wheat	28.69	5.71
Wheat intercropped	40.31	9.45
F test	**	**
Chickpea	70.65	5.01
Chickpea intercropped	80.11	13.63
F test	ns	**
Seed		
Wheat	36.58	25.09
Wheat intercropped	46.13	27.10
F test	*	ns
Chickpea	18.75	10.67
Chickpea intercropped	22.75	30.05
F test	**	**

$^{**}P < 0.01$, $^{*}P < 0.05$, and *ns* nonsignificant. Means within each column followed by different letters are significantly different by Duncan's multiple range test at $P = 0.05$

Table 4 Iron and zinc content of wheat and chickpea grown with two P sources in monocropping and intercropping treatments (modified from Li et al. 2004)

Treatments		Wheat		Chickpea	
		Fe (μg Fe pot^{-1})	Zn (μg Zn pot^{-1})	Fe (μg Fe pot^{-1})	Zn (μg Zn pot^{-1})
Organic P	Monocropping	218b	209b	286a	138a
(phytate)	Intercropping	475a	339a	193b	97b
Inorganic P	Monocropping	240b	327b	321a	168b
($FePO_4$)	Intercropping	340a	440a	343a	204ab

Mean values of the three intercropping treatments with the same *P* source followed by different letters (a, b) are significantly different ($P \leqslant 0.05$)

acquisition and crop production processes, the management of intercropping would be the key to Fe and Zn biofortification.

3 Strategies for Fe and Zn Uptake in Plants

3.1 Physiological Responses to Increase Fe and Zn Uptake in Plant Species

In general, plant species develop physiological responses to increase iron uptake under a Fe-deficient environment which are characterized as "Strategy I" and "Strategy II" systems (Curie and Briat 2003; Hell and Stephan 2003; Schmidt 2003; Grotz and Guerinot 2006). It is well known that peanut and maize have distinctly different response mechanisms to Fe deficiency stress. Peanut and chickpea are 'strategy I' plants, while maize and wheat belong to the 'strategy II' group. Specifically, peanut displays Strategy I mechanisms, under conditions of Fe deficiency, where reductase activity is increased and release of protons and reductants is enhanced from the roots. Furthermore, plants use the reduction strategy to mobilize iron from the rhizosphere and ferric chelate reductase activity has been shown to be the rate-limiting step for iron uptake (Guerinot 2007). In calcareous soils, the high pH and large bicarbonate buffering capacity may render this strategy ineffective in the peanut due to decreased expression of a ferric chelate reductase (Bienfait 1988; Guerinot 2007). This strategy might not succeed even if Fe-efficient varieties of peanut were used in an attempt to overcome the iron chlorosis problem.

Strategy II plants are characterized by a higher Fe acquisition efficiency in soils with high pH and, in particular, high bicarbonate content through the excretion of phytosiderophores (PS) into the rhizosphere (Römheld and Marschner 1986), and thus have a high resistance to Fe deficiency stress compared with strategy I plants. Graminaceous plant species respond to Fe and Zn deficiency by exudation of phytosiderophores to increase the availability of Fe and Zn and transport PS-Fe(III) or PS-Zn from the rhizosphere to the root cell for uptake (Marschner et al. 1989; Cakmak et al. 1994). Grasses, which exude phytosiderophores in response to Fe deficiency, may also use this chelation strategy in order to obtain Zn from the soil. The mugineic acid family phytosiderophores (MAs) play a major role in iron (Fe) acquisition, and may also contribute to the acquisition of Zn and other metal nutrients by graminaceous plants (Römheld 1991; Welch 1995; Wirén et al. 1996). Therefore, exudate phytosiderophores from gramineous species have important ecological significance in calcareous soil.

3.2 Molecular Regulation of Fe and Zn Homeostasis in Plants

Significant progress on molecular aspects of Fe and Zn homeostasis in plants has been made in recent years in our understanding of how metals are obtained from the soil and distributed throughout the plant. For instance, in strategy I plants, Fe is first reduced on the root surface from ferric to ferrous ion by a plasma membrane-bound Fe(III) chelate reductase (FRO gene family) and subsequently translocated across the rhizodermal plasma membrane barrier by a high-affinity Fe(II) transporter such as the IRT gene family into the root cell (Varotto et al. 2002; Vert et al. 2002; Connolly et al. 2003; Mukherjee et al. 2005). Other Fe transporter genes, such as AtNRAMP3, AtNRAMP4, and AtVIT1, are all expressed in the vascular system of the roots and shoots, and the proteins that these genes encode appear to play a role in vacuolar Fe homeostasis (Thomine et al. 2003; Lanquar et al. 2005). Vacuolar Fe storage is also critical for seedling development that will ultimately aid to increase the chances of obtaining a nutrient-rich seed, benefiting both human health and agricultural productivity (Kim et al. 2006). Recent results confirmed that the FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient Fe translocation; this process is important for the translocation of Fe to the leaves. FRD3 transports a small, organic iron-chelator that is necessary for the correct localization of Fe throughout the plant into the xylem (Durrett et al. 2007).

In strategy II plants, the first YS1 gene was identified from maize roots that could transport PS-Fe(III) from the rhizosphere (Curie et al. 2001). Recently, 18 YSL genes have been identified in rice, many of which are expressed in both the roots and the shoots

(Koike et al. 2004). In fact, the YSL genes also play a role in Strategy I plants. The YSL family, consisting of eight members in *Arabidopsis*, has been implicated in the intercellular transport of Fe chelates, specifically Fe complexed into nicotianamine (NA), which regulates Fe and Zn homeostasis and plays a role in Fe and nicotianamine seed loading (Le Jean et al. 2005; Schaaf et al. 2005). A number of small, organic molecules have been implicated in metal ion homeostasis as metal ion ligands to facilitate uptake and transport of metal ions with low solubility and also as chelators implicated in sequestration for metal tolerance and efficient storage of metals in fruits and seeds (Haydon and Cobbett 2007). A fuller understanding of the role of mugineic acid, nicotianamine, organic acids (citrate and malate), histidine and phytate as ligands for iron (Fe), zinc (Zn), copper (Cu), manganese (Mn) and nickel (Ni) in plants could make a significant contribution to our understanding of metal homeostasis in plants.

Although the molecular mechanisms for Zn efficiency are not understood, it has been suggested that an increased secretion of phytosiderophores by Zn-efficient plants would be involved. Furthermore, several ZIP proteins have been characterized in the Strategy II rice plant, suggesting that this protein family plays a role in the grasses (Ramesh et al. 2003). Genetic engineering approaches have been applied to increasing plant tolerance to low-Zn soils. At present, knowledge of the genes controlling specific steps of the Zn network in soil–plant systems is still rudimentary, but increasing rapidly. Transformation and overexpression of known Zn transporters from Arabidopsis to barley (Hordeum vulgare cv. Golden Promise) can increase plant Zn uptake and seed Zn content (Ramesh et al. 2004). Recently, a NAC gene was identified in wheat, which can accelerate senescence and increase Zn and Fe remobilization from leaves to developing grains (Uauy et al. 2006). These results clearly show the contribution of molecular genetic tools to manipulating Zn and Fe efficiency in crops and the potential for enrichment of the food supply with Zn and Fe. Novel breeding strategies, combined with improved agronomy practice, have been developed based on these genetic findings. However, there is no information on how Fe and Zn nutrient content of seeds is affected by intercropping at a molecular level. The fact that many of the molecular and biochemical changes in response to Fe and Zn deficiency occur in synchrony suggests that genes involved in Fe or Zn uptake and translocation are co-ordinately expressed in intercropping. Therefore, systematic studies are needed to understand the molecular mechanisms of improvement of Fe and Zn content in the seeds of staple crops.

4 The Mechanism of Improvement of Fe and Zn Uptake in Intercropping

4.1 The Potential Role of Phytosiderophores from Graminaceous Plants in Improvement of Fe and Zn Nutrition of Dicot Plants

Cropping systems such as intercropping may have numerous advantages in terms of increasing availability of micronutrients such as Fe and Zn. In the peanut (*Arachis hypogaea* L.)/maize (*Zea mays* L.) intercropping case, the excretion of phytosiderophores by maize into the rhizosphere plays an important role in improving Fe nutrition of peanut crops (Zuo et al. 2000). For strategy II plants, iron accumulation can be enhanced by the production of higher levels of phytosiderophores (Suzuki et al. 2006). There seems to be some cross-talk between the iron and zinc transport pathways because transgenic plants and mutants with overexpressed iron transporters also show increased zinc accumulation (Schaaf et al. 2005). Therefore, the possible reason for such differential effects on Zn concentrations of peanut plants caused by intercropping could be root exudates from gramineous species. Specifically, production and release of phytosiderophores from gramineous species may improve solubility of Fe and Zn by chelation, which helps plants obtain those essential elements from the soil (Rengel 2002; Schmidt 2003; Inal et al. 2007). In a study of peanut intercropping with different gramineous species, it was clearly shown that the incidence of chlorosis of peanut could be eliminated in intercropping with gramineous species such as two maize genotypes, barley, oats and wheat (Fig. 3). Moreover, there

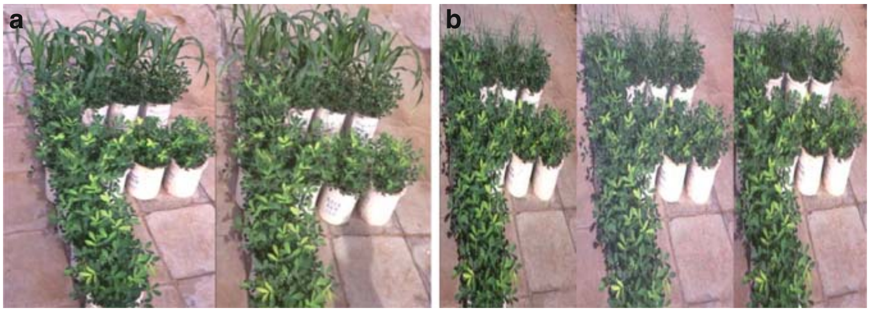

Fig. 3 The effects of six cropping systems on iron deficiency chlorosis symptoms in the young leaves of peanut plants at 60 days growth. (**a**) Peanut intercropping with two maize genotypes from left to right (danyu13, zhongdan2). (**b**) Peanut intercropping with barley, oats, or wheat from left to right. There are seven pots of monocropped peanut and four pots of intercropped peanut and gramineous species in each picture

is a strong, positive correlation between the amounts of phytosiderophores and the resistance of plants to iron deficiency (Mori et al. 1987; Takagi et al. 1988; Zhang et al. 1990).

Generally speaking, the release rates of phytosiderophores of barley, oats and wheat are much higher than those of maize under solution culture conditions (the order is barley > oats > wheat > maize). Plants that released more phytosiderophores positively correlated with improved growth in alkaline soils (Awad et al. 1994; Marschner and Römheld 1994; Shen et al. 2002). The results indicated that the effect of improved iron nutrition of the peanut by the two genotypes of maize (danyu13 and zhongdan2) is similar to that of barley, oats and wheat in intercropping, and the iron content in shoots of peanut plants intercropped with maize was lower than that of peanut plants intercropped with barley, oats or wheat (Table 5). In a greenhouse study, peanut intercropping with different gramineous species not only improved the iron nutrition of the peanut, but also enhanced zinc content in the peanut shoot. This suggests that the lower phytosiderophore levels produced by maize could be enough to improve iron nutrition of peanut in calcareous soil. It was not technically feasible to determine in the field the rates of synthesis and release of phytosiderophores of those gramineous species in different cropping setups, mostly because they cannot be recovered after release into the rhizosphere in soil conditions. It is difficult to answer directly the question of whether phytosiderophores play an important role in the improvement of iron nutrition in peanut, but there is some evidence to support the hypothesis.

Table 5 The effects of peanut intercropping with five gramineous plants on Fe and Zn contents (mg kg^{-1} DW) in the shoot of peanut at 60 days' growth in the greenhouse experiment

Treatments	Iron and zinc concentration in peanut
	Fe content
Monocropping peanut	190.5 ± 13.1^{c}
Peanut/maize(danyu13)	313.6 ± 16^{ab}
Peanut/maize(zhongdan2)	280.8 ± 24.3^{b}
Peanut/barley	330.3 ± 10.5^{a}
peanut/oats	345.7 ± 24.0^{a}
Peanut/wheat	362.9 ± 30.3^{a}
	Zn content
Monocropped peanut	109.8 ± 3.1^{c}
Peanut/maize(danyu13)	124.4 ± 7.2^{b}
Peanut/maize(zhongdan2)	119.9 ± 4.3^{b}
Peanut/barley	121.0 ± 6.9^{b}
peanut/oats	132.3 ± 2.6^{a}
Peanut/wheat	122.3 ± 10.3^{ab}

Columns with the same letter are not significantly different at 0.05, using the LSD multiple range test

4.2 Ferric Reductase Capacity for Improvement of Fe and Zn Uptake in Intercropped Dicot Plants

For strategy I plants, the inducible activity of Fe^{3+} chelate reductase reduces $Fe^{3+}$to Fe^{2+}, which is the rate-limiting step for Fe acquisition from soil (Ishimaru et al. 2007), so enhancing the Fe^{3+} chelate reductase activity of peanut plants renders those plants resistant to Fe deficiency. In a maize/peanut study in China (Zuo et al. 2003), the reducing capacity of peanut roots in monoculture increased in conjunction

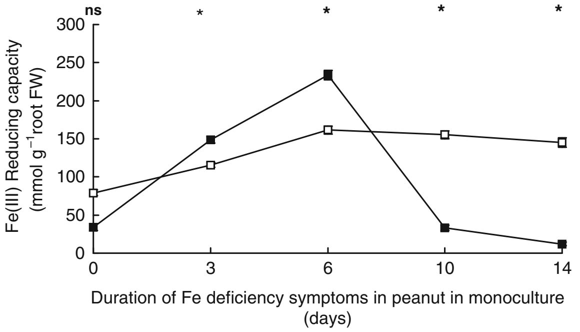

Fig. 4 Reducing capacity of peanut roots in monocropping and intercropping during the period of Fe deficiency symptoms in peanut in monocropping. Significance of difference between monocropping and intercropping by paired *t*-test: $*P < 0.05$; *ns* not significant. cropping type: *filled square*, monocropping; *open square*, intercropping. *Bars*: standard error of the mean ($n = 4$)

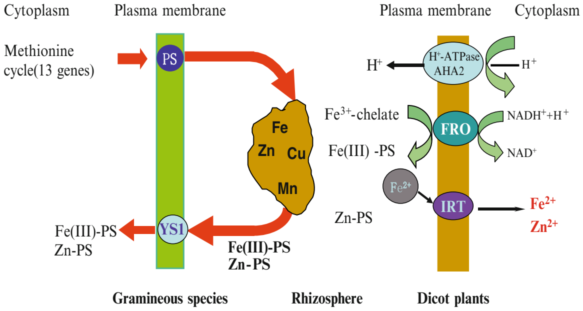

Fig. 5 Summary of the possible molecular and physiological mechanisms of improvement in iron and zinc nutrition of dicot plants intercropped with gramineous species

with the appearance of Fe deficiency chlorosis symptoms in young leaves. The maximum Fe(III)-reducing capacity of roots in monoculture occurred at 6 days and subsequently decreased rapidly. By the fourteenth day, when peanut showed severe Fe deficiency in monoculture, the reducing capacity of the roots was lower than that of peanut that had no Fe deficiency symptoms from the intercropped culture system. In contrast, the reducing capacity of peanut roots grown in intercropping with maize increased very slowly, and was greater than that of peanut roots from monoculture after the appearance of Fe deficiency chlorosis in monoculture at 10 days (Fig. 4). In another maize/peanut case in Turkey, the results also indicated that the root Fe(III)-reducing capacity of peanut was found to be significantly higher in intercropping (0.56 mmol Fe g^{-1} FW h^{-1}) than that of monocropped peanut (0.29 mmol Fe g^{-1} FW h^{-1}) (Inal et al. 2007). Those studies confirmed that maize/peanut intercropping could keep a higher ferric reduction capacity of peanut roots for a longer time period than that of monocropping, indicating that intercropping could enhance Fe^{3+}chelate reductase of peanut, which helps peanut plants tolerate Fe deficiency in calcareous soil.

Based on available research evidence, Fig. 5 shows the possible mechanisms of improvement of iron and zinc nutrition of dicot plants in this review. In dicot plant/gramineous species intercropping systems in calcareous soil, the release of phytosiderophores by strategy II plants not only acquires Fe to meet their demand, but also improves Fe and Zn uptake of strategy I plants. Compared with monocropping dicot plants, one possible explanation is that gramineous species released phytosiderophores into the rhizosphere of dicot plants and helped to make much more phytosiderophore-Fe available to dicot plants in intercropping; however, there is no phytosiderophore-Fe available to dicot plants in monoculture. Although peanut does not produce phytosiderophores (PS) under Fe deficiency, phytosiderophore-Fe chelates from maize should exist in the rhizosphere of peanut intercropped with maize. For strategy I peanut plants, it has to reduce solubilized Fe(III) by a membrane-bound Fe(III) chelate reductase and subsequent transport of the resulting Fe(II) into the plant root cell by a Fe(II) transporter. Phytosiderophore-Fe is one of the Fe(III) states which are more easily reduced and taken up by dicots than other Fe(III) forms (Hopkins et al. 1992; Jolley and Brown 1994), We infer from those studies that intercropping provides more phytosiderophore-Fe, which is easily reduced and absorbed by peanut. Furthermore, a higher ferric reduction capacity of dicot plant roots for a longer time period in intercropping may have assisted in the mobilization of sparingly soluble Fe(III) compounds from the rhizosphere so that the dicot plants remained green. A noteworthy advance in Fe and Zn research in plants is that there seems to be cross-talk between the iron and zinc transport pathways, because transgenic plants and mutants with overexpressed Fe(III) reductases and iron transporters also show increased zinc accumulation (Zhu et al. 2007). Those combined factors may thus have contributed to the improvement in Fe and Zn nutrition of dicot plants in intercropping with gramineous species.

5 Conclusion

Biofortification of iron and zinc content in plants is an emerging international research area of plant nutrition. Anemia as a result of iron deficiency afflicts more than two billion people worldwide, especially in developing countries including China (http://www.harvestPlus.org/iron.html). Biofortification of iron and zinc content and availability in plant foods could be an economical solution to this problem (Nestel et al. 2006; Yan et al. 2006). Enriching the nutrition contribution of staple crops through plant breeding, transgenic crops and mineral fertilization are significant tools in the fight against human malnutrition. Micronutrient-dense crop varieties are being developed using the best traditional breeding and modern biotechnology methods to achieve increases in nutrient concentrations. However, feasible and cost-effective approaches are needed, especially to reach the rural poor in developing countries. In this review, maize/peanut, chickpea/wheat and guava/sorghum or maize intercropping could overcome iron and zinc nutrient deficiencies, particularly in harvested seeds. The development of ecologically and economically viable strategies to prevent iron zinc deficiency represents the goal of the biofortification of crops.

The studies suggest that a rational intercropping system of nutrient-efficient species should be considered to prevent or mitigate iron and zinc deficiency of plants in agricultural practice. It will be one of a number of approaches to produce more biofortified crops. More researchers are becoming aware that increasing bioavailability of micronutrients in the edible parts of staple crops through agricultural management is a cost-effective and sustainable way to alleviate micronutrient malnutrition. Although significant progress has been made in recent years in our understanding of how metals are obtained from the soil and distributed throughout the plant, there is still a lack of knowledge of how Fe and Zn micronutrients behave in intercropping systems of strategy I and strategy II plants. Substantial efforts are being made aimed at increasing plant Fe and Zn nutrient efficiency in intercropping at the molecular, cellular and whole-plant levels. This requires a multidisciplinary research approach, a willingness among scientists to communicate across disciplinary boundaries, and innovative funding strategies to support the research and ultimate dissemination of the biofortified seeds. Strategies for intercropping dicot plants and gramineous species could potentially contribute to iron and zinc biofortification in a more practical, effective and sustainable manner in developing countries.

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