Chapter 1

STATUS AND FUTURE DEVELOPMENTS INVOLVING PLANT IRON IN ANIMAL AND HUMAN NUTRITION

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Abstract: Iron is an essential nutrient for humans and other animals, and must be consumed in adequate amounts to ensure proper growth and development, as well as good health of the organism. Dietary sources of iron can be divided into two types: non-heme iron, mostly provided by plant foods, and heme iron, present in animal foods. Heme iron intake is usually low for the majority of humans in many developing countries because of the high cost of animal products or due to cultural constraints concerning these foods. Heme iron intake also is low in most livestock, whose major source of dietary iron comes from forages and cereal crops. For these reasons, both humans and animals rely on plants as an important source of dietary iron. However, the iron concentration of plant foods varies greatly, and low concentrations in some common food sources make it difficult for humans and animals to meet daily dietary requirements when these foods are consumed in suggested amounts. Additionally, certain food components, such as phytate or tannins, can lower the bioavailablity of the iron that is in plant foods, thereby lowering its effective concentration even more. In order to improve the iron nutritional value of crop plants and consequently to improve human and animal health, several strategies are being utilized by plant scientists. These include: cultivar evaluation, plant breeding and marker-assisted selection, alteration of pathways of iron metabolism, and modification of iron bioavailability. In this review, we present the role that plant iron plays in the diets of humans and other animals, and discuss the strategies that can be employed to improve our plant-based food supply.

Key words: animal; bioavailability; human; iron; nutrition

1. IRON ABSORPTION IN HUMANS AND ANIMALS

Iron is one of the most important micronutrients in the human diet, functioning as a component of a number of proteins, including enzymes and hemoglobin. It is crucial for optimal physical performance and cognitive development. At least four major classes of iron-containing proteins exist in the mammalian system: iron containing enzymes (hemoglobin, myoglobin, cytochromes), iron-sulfur enzymes (flavoproteins, heme-flavoproteins), proteins for iron storage and transport (transferrin, lactoferrin, ferritin, hemosiderin), and other iron-containing or activated enzymes (sulfur, non-heme enzymes) (Institute of Medicine, 2001).

Nutritional iron is usually divided into two types: heme iron, which is absorbed unaffected by other food components, and non-heme iron, which is envisioned as "free" or as weak complexes (Theil, 2004). Heme iron contributes only 10 to 15 percent of the total iron intake (1 to 3 mg/day) in diets of developed countries but may provide a substantial amount of the total absorbed iron. Where meat is consumed extensively, (e.g. Argentina and New Zealand) this contribution can rise to almost 50 percent. Heme iron intake is negligible for the majority of people in many developing countries, because of cultural constraints and the high cost of animal products (Bothwell *et al.*, 1989). For this reason, non-heme iron is the main source of dietary iron for most people in the world.

Generally, the iron content of the body is highly conserved, and iron balance is maintained by regulating absorption in the small intestine, where two principal mechanisms of iron absorption can be found on the luminal surface of enterocytes. The first mediates the absorption of heme iron, derived primarily from the proteolytic degradation of hemoglobin and myoglobin, and the second regulates the absorption of non-heme iron, extracted from plant and dairy foods (Institute of Medicine, 2001). Heme iron absorption is thought to occur via a vesicular transport process, whereby the entire heme complex is brought into the enterocyte, prior to the degradation of heme (by heme oxygenase) and the release of inorganic iron (Uzel and Conrad, 1998). The absorption of inorganic iron from non-heme sources involves a reduction and transport process. The human protein Dcytb is an iron-regulated ferric reductase that appears to utilize electrons donated from intracellular ascorbate (Atanasova *et al.*, 2005; McKie *et al.*, 2001). Ferrous ions generated by Dcytb are available for transport through the protein DMT1, which also is regulated by low iron status in the body (Gunshin *et al.*, 1997).

The total content of a given nutrient in any given food is not always a good indicator of its useful nutritional quality, because not all of the

nutrients in food are absorbed (Grusak and Dellapena, 1999). Iron in any food has a particular bioavailability, which is a function of its chemical form and the presence of food components (in that food, or in the diet) that either promote or inhibit its absorption (Abrams, 2004). Non-heme iron is generally less bioavailable than heme iron, due in part to the presence of tannins or phytate in some foods, which inhibit its absorption (Davidsson *et al.*, 1994). In fact, the bioavailability of iron in most plant sources is on the order of 5% (Consaul and Lee, 1983). On the other hand, foods also contain factors that stimulate the absorption of non-heme iron; these include organic acids (particularly ascorbic acid) and the poorly understood "meat factor" (Fidler *et al.*, 2004; Huh *et al.*, 2004).

2. IRON REQUIREMENTS

2.1 Iron requirements in humans

A large segment of the world's population does not ingest enough iron to meet daily dietary requirements. Therefore, iron deficiency and iron-deficiency anemia (IDA) are estimated to affect 30-50% of the world's population (Yip and Dallman, 1996), being especially prevalent in developing countries where food intakes can be severely low. In some populations, iron deficiency is estimated to reach 85% (Kapur *et al.*, 2002). In the United States, approximately 75% of college-aged women have low iron intake (Ramakrishnan *et al.*, 2002), and suboptimal dietary intake of iron occurs in 90% of pregnant Americans (Swenson *et al.* 2001).

The iron present in the human body is mostly in a stored form, and losses are usually minimal. However, dietary intake of iron is needed to replace the iron lost by passage of stool and urine, shedding of skin, and sweating. In fact, after exercising, a person can loose up to 1 mg of iron (Vellar, 1968), but on average, losses represent around 0.9 mg of iron per day for an adult male and 0.8 mg per day for an adult female (DeMaeyer *et al.* 1989). Infectious diseases such as intestinal parasites (Thurnham, 1997), as well as menstruation (Hallberg, 2001) can also increase iron requirements (due to elevated iron losses).

The intake of dietary iron is also linked to energy intake. In developed countries, a typical diet contains about 6 mg of iron per 1,000 kcal (equivalent to a daily consumption of 8 to 18 mg iron by most adults) with little variation from meal to meal or among persons of different economic status (Cook and Finch, 1979).

The Recommended Dietary Allowance (RDA) for a nutrient, as defined by the Institute of Medicine (1997), is "the average daily dietary nutrient

intake level sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a particular life stage and gender group". Requirements vary between individuals and every nutrient has a distribution that is described by a median and a standard deviation (SD) for different age and sex groups (Renwick *et al.*, 2004). The dietary daily iron requirements in humans are summarized in Table 1-1. For children, iron requirements vary between 7 to 11 mg per day. Adult males require 8 mg per day, and women can require up 27 mg per day during pregnancy.

Table 1-1. Daily Recommended Dietary Allowances (RDA) (mg) according to life-stage, group and sex. Adapted from Institute of Medicine (2001).

Age	Infant, Child	Male	Female	Pregnancy	Lactation
7-12 month	11				
1-3 years	7				
4-8 years	10				
9-13 years		8	8		
14-18 years		11	15	27	10
19-50 years		8	18	27	9
>50 years		8	8		

Infants, children and adolescents require iron principally for their expanding red cell mass and growing body tissue, and therefore have higher requirements than adults. Moreover, they eat less food and are thus at greater risk of developing iron deficiency (Institute of Medicine, 2001). For women, the requirements increase dramatically during the menstrual period and during the second and third trimester of pregnancy. During pregnancy, additional iron is required for the fetus, the placenta, and the increased maternal blood volume. During lactation, because menstruation usually ceases, iron requirements decline.

2.2 Iron requirements in animals

Iron has been recognized as a required nutrient for animals for more than 100 years (Pond *et al.*, 1995). Still, sub-clinical iron deficiencies occur more frequently than recognized by most livestock producers. Currently, micronutrient deficiency is a bigger problem than macronutrient deficiency, because the farmer does not readily see specific symptoms that are characteristic of a trace mineral deficiency. Instead, the animal grows or reproduces at a reduced rate, uses feed less efficiently and operates with a depressed immune system (Berger, 2000). Both iron deficiency and iron excess can compromise the immune system of farm animals. Hypoferremia is believed to be an important protective component of the acute phase response to infection (Ebersole and Capelli, 2000). It is proposed that the

decrease in red blood cells, in the acute phase following infection and inflammatory disease, is a strategy to decrease iron availability to pathogens (Ebersole and Capelli, 2000). On the other hand, anemic animals are much more susceptible to infections than those with adequate iron. Nursing pigs made anemic by withholding supplemental iron for four weeks after birth were more susceptible to the lethal action of bacterial endotoxin then their littermates that had been given iron (Osborn and Davis, 1968). Once the infection was established, iron supplementation increased the bactericidal activity of liver and splenic marcrophages. In another example, chicks inoculated with *Salmonella gallinarum* had increased survival when iron (100 μg/g of diet or more) was added to a basal diet containing 200 μg/g of iron (Hill *et al.*, 1977; Smith *et al.*, 1977). These and other data in broilers show that iron helps the immune system to destroy the invading organism. Therefore, proper iron nutrition is essential for maximal disease resistance.

Table 1-2. Dietary iron requirements (mg/kg of diet dry matter) in different animal classes (adapted from NRC 1981, NRC 1985, NRC 1989, NRC 1994, NRC 1996, NRC 1998, NRC 2001).

Class of Animal	Iron Requirements (mg/kg of diet dry matter)
Swine	
Piglets	100
Growing-finishing	40 – 80
Dairy cattle	
Calves	100
Other cattle	25
Beef cattle	50
Sheep	30 – 50
Goats	30 – 50
Horses	40 – 50
Poultry	50 – 80

Several factors influence the iron nutritional status and/or iron needs of animals. These include: 1) genetic differences amongst species, breeds, strains, stocks, sexes or individuals, 2) life cycle stage, with special emphasis on growth, pregnancy and lactation, 3) health status of the animal, 4) form of iron used in the diet (e.g. type of chelator), and 5) nutritional and anti-nutritional factors taken together with the iron source (NRC, 1989). Although there are minimally established nutrient needs for animals, designed to minimize the risk for deficiency, farm animals are usually fed to maximize their mass and/or to increase production in terms of meat, eggs, or milk (Grusak and Cakmak, 2005). Thus, mineral recommendations for production purposes are usually higher than those to prevent deficiency. Table 1-2 gives the feed iron recommendations for different classes of animals at different growth stages. When there is a range of requirements for

the same animal class, the higher value corresponds to the animal at a younger growth stage, or to females during pregnancy or lactation. The values for feed composition recommendations range from 25 mg/kg for adult dairy cattle up to 100 mg/kg for piglets or calves.

Table 1-3. Iron (mg) content of selected plant foods, raw, per common measure and per 100 g FW basis. Adapted from USDA National Nutrient Database for Standard Reference, Release 17, 2004.

Description	Common measure	Iron content (mg)	
		Per common measure	Per 100 g FW basis
Fruit			
Apple	1 apple	0.17	0.12
Avocado	1 oz	0.17	0.60
Banana	1 banana	0.31	0.26
Nectarine	1 nectarine	0.38	0.28
Strawberry	1 cup	0.70	0.42
Legumes			
Black Bean	1 cup, cooked	3.61	2.10
Chickpea	1 cup, cooked	4.74	2.89
Lentil	1 cup, cooked	6.59	3.33
Vegetables			
Cabbage	1 cup	0.41	0.58
Carrot	1 cup	0.33	0.30
Lettuce	1 cup	0.23	0.42
Potato	1 potato	2.18	1.10
Spinach	1 cup	0.81	2.70
Tomato	1 tomato	0.33	0.26
Nuts			
Almond	1 oz (24 nuts)	1.22	4.30
Cashew	1 oz (18 nuts)	1.72	6.06
Cereal Grains			
Barley	1 cup, cooked	2.09	1.33
White Rice	1 cup, cooked	0.24	0.14

3. PLANTS AS IRON SOURCES

3.1 Plants as iron sources for humans

Plant foods can contribute significantly to human nutrition and health because they contain almost all essential human nutrients (Grusak and Dellapena, 1999). Historically, humans have had an omnivorous diet. However, not all plant sources provide the same amount of iron, and the amount of iron ingested is directly proportional to the portion size that is consumed. Table 1-3 gives an example of the amount of iron (mg) in

selected raw plant sources, per 100 g fresh weight (FW) and per common serving size.

In the selected plant foods described, legumes, especially lentils, show substantially higher iron concentrations than fruit, cereal grains, and many vegetables. Based on 100 g FW, nuts such as almonds and cashews have very high iron content, whereas fruits generally have very low iron. As noted in Table 1-3, iron content varies among different plant foods, and nutrient content in a single serving rarely fulfills the RDA for any given vitamin or mineral.

Table 1-4. Iron concentration in different types of forages commonly used in the United States (adapted from Mortimer *et al.*, 1999).

Type of forage	Iron concentration (µg/g)	Sample #
Alfalfa / Alfalfa Mix	210 ± 13	196
Brome	156 ± 21	20
Bermuda	165 ± 37	120
Fescue	154 ± 22	73
Orchardgrass / Orchardgrass Mix	119 ± 15	34
Sudan	321 ± 41	61
Native grasses	179 ± 34	38
Cereal type forages (mixture of barley, oats and wheat)	174 ± 32	46
Silage / Silage grass (mixture of corn, sorghum and small grains)	252 ± 37	31
Grass (mixture of native and cultivated forages)	153 ± 18	70

Guthrie and Picciano (1995) found that the contributions of various food groups to the iron content of the North American diet were as follows: cereal products (43%); meat, fish, and poultry (22%); vegetables and beans (20%); eggs (3%); fruit (3%); and all other sources combined (9%). In a study conducted in China, it was found that grain products were the major food sources of iron (38%), with vegetables and legumes contributing 14 and 7% of dietary iron, respectively. The proportion of dietary iron acquired from meat, poultry and fish was only 13% (Liu *et al.*, 2004a). This tells us that at least 66% of the iron regularly consumed by North Americans and 59% for Chinese comes solely from plant sources.

3.2 Plants as iron sources for animals

Forage, either harvested mechanically or by grazing, is the basal dietary ingredient for beef cattle, dairy cattle, sheep, and horses. For poultry

production, the most common forages used are grains, such as corn, wheat, barley and oats. Other by-products commonly used are soybean, canola, cottonseed, and peanut. Mortimer *et al.* (1999) summarized feed analysis data from 709 forage samples, collected from 678 producers in 23 states in the US, and combined the data into 10 forage categories (Table 1-4). Values ranged from 119 μg/g in orchard grass to 321 μg/g in Sudan forage. These values were calculated from commercial forages and most likely represent samples that were contaminated with soil, dust, or other non-plant substances. Thus, the values don't necessarily reflect the plant's ability to acquire/store iron. However, these values do represent what the farmer is providing to his/her animals.

Even with some of these high levels in forage, anemia can still develop especially when an animal's total body iron stores are low. This often occurs in younger animals due to low iron content in their mother's milk (NRC, 1995). Because of this, iron supplementation is often provided during an animal's first weeks of life (Berger, 2000).

4. POSSIBLE SOLUTIONS TO INCREASE IRON STATUS IN PLANT SOURCES

We have seen that plants are essential sources of iron in the human and animal diet and that often iron concentration in plants is not enough to meet the daily dietary recommendations. In many parts of the developing world, due to elevated costs, large segments of the human population do not have access to animal sources of iron. In these cases, a commonly used strategy is iron fortification. However, iron fortification of plant foods is not always practical or economically feasible for the rural poor, and many times this fortified iron is not highly bioavailable (Boccio and Iyengar, 2003). Therefore, a more sustainable approach, that is believed relevant to both urban and rural populations, is to enhance the iron content of plant foods through biofortification. Biofortification is a process whereby the plant uses its own mechanisms to fortify or enhance the density or bioavailability of nutrients (like iron) in its edible tissues. To develop iron biofortified plants, four main strategies can be utilized: 1) cultivar evaluation, 2) plant breeding and marker-assisted selection, 3) alteration of pathways of iron metabolism, and 4) modification of iron bioavailability.

4.1 Cultivar evaluation

In the plant kingdom there is vast genetic variation that influences plant type, morphology, physiology, and plant mineral concentration. This

variation is visible at the species level, genotypic level, and even amongst individual plants. Moreover, developmental stages, stress, and environmental conditions also can influence iron concentrations. Rice genotypes, for instance, have been identified that vary from 6 to 24 µg/g iron in their grains (Gregorio *et al.*, 2000). Wheat can show a density range for iron concentration from 25 µg/g to a high of 56 µg/g, with a mean of 37 µg/g (Monasterio and Graham, 2000). Legume crops also show considerable variation in iron concentrations: wild accessions of common bean (*Phaseolus vulgaris*) can range from 60 to 95 µg/g iron in seeds, and cultivated bean ranges from 55 to 89 µg/g for seed iron (Beebe *et al.*, 2000). Table 1-5 shows how iron concentration in crops can vary not only amongst different species, but also in the same species grown at different locations. Plant breeders can utilize this genetic variation to generate or select varieties that have higher iron in the edible portions of the plant. These selections can be promoted directly as biofortified varieties, if other agronomic traits are acceptable. However, after having identified a potentially useful variety, it must be tested in multiple environments where the crop is to be grown, in order to assess whether the mineral trait will be stable in every condition.

4.2 Plant breeding and marker-assisted selection

Plant breeding has the potential to contribute to the development of cultivars with higher accumulation of nutrients, particularly iron. Until recently, however, plant breeders have focused primarily on increasing yield and improving disease resistance in crops, rather than improving micronutrient concentration in edible tissues (Frossard *et al.*, 2000). Iron concentration in plants is a quantitatively conditioned trait, showing continuous variation among individuals in a given population (Guzman-Maldonado *et al.*, 2000). Therefore, breeding for high iron plants is a viable strategy, although sure to be a difficult one. With the identification of genotypes demonstrating a high iron seed phenotype, these can be moved into a conventional breeding program to combine the iron trait with other required characteristics. In general, a useful cultivar will need to demonstrate such things as high yield, good disease resistance, tolerance to environmental stress, and/or good processing qualities. The high iron phenotype alone will not be sufficient to warrant a cultivar release. The farmer must still be able to generate a high-yielding crop in order to make a profit, and unless people are willing to eat the final product, the biofortified line will provide no nutritional benefit to the target population. When breeding for higher levels of mineral nutrients, there is no direct visual score that can be used to screen progeny for the high-iron trait. Usually, in order to find individuals with a higher accumulation of a given mineral, the breeder

must analyze plant material using techniques such as ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometry) or AAS (Atomic Absorption Spectrometry). For seed foods, this requires that all selections be grown to maturity, and that a portion of the generated seeds must be destructively analyzed. Although this approach will work, a more time- and resource-saving approach is to use marker-assisted selection (MAS) techniques, following the identification of quantitative trait loci (QTLs) that are associated with high seed iron levels. QTL analysis and MAS have proven to be valuable tools in many breeding programs, especially in wheat and rice (Steele *et al.*, 2004), but practical applications of these techniques in micronutrient breeding have been limited.

Table 1-5. Ranges in iron concentration in various staple food crops grown in different locations: CIMMYT (International Maize and Wheat Improvement Center, Mexico); IRRI (International Rice Research Institute, Philippines) and CIAT (International Center for Tropical Agriculture, Colombia).

Plant Species	Number of genotypes	Iron (µg/g)		
		Min	Max	Mean
Wheat grown at CIMMYT (Monasterio and Graham, 2000)				
Selected genotypes	170	25	56	37
Pre-breeding lines	154	32	73	43
Rice genotypes grown at IRRI (Gregorio *et al.*, 2000)				
Traditional and improved	140	8	24	13
IR breeding	350	8	17	11
Tropical japonicas	250	9	24	13
Aromatic rice	51	11	23	15
Maize grown at CYMMYT (Bänzinger and Long, 2000)				
Landraces	416	18	59	26
Germplasm pools	100	10	17	13
Breeding germplasm	100	27	57	32
Bean grown at CIAT (Beebe *et al.*, 2000)				
Wild genotypes	119	-	96	60
Cultivated genotypes	1031	34	98	55
Cassava grown at CIAT (Chavez *et al.*, 2000)				
Leaves	20	62	155	94
Roots	20	8	13	10

In rice, three loci (located on chromosomes 7, 8 and 9) were identified that are associated with high iron concentration in the seeds (Gregorio *et al.*, 2000); these explained 19–30% of the variation in seed iron concentration. However, the lines with higher iron concentration were mostly low yielding,

and therefore breeding efforts have been started to combine the high nutrient trait with higher productivity. Similarly, in common bean, two putative QTLs associated with seed iron were localized in linkage groups II and III; these explained 25% of the phenotypic variance (Guzmán-Maldonado *et al.*, 2000). Efforts must now be made to verify all of these markers.

Related QTL efforts have been undertaken with *Arabidopsis thaliana*, where the existence of a fully sequenced genome could provide an ability to identify specific genes within a QTL, rather than just a nearby marker. The mineral concentration of *Arabidopsis* seeds has been analyzed in individual lines of a recombinant inbred population (Vreugdenhil *et al.*, 2004). QTLs were identified for several minerals (Ca, Fe, K, Mg, Mn, Na and Zn), which explained up to 78% of the variation for a specific mineral. Map positions for several of the QTLs was confirmed by analysis of near isogenic lines (NILs). For Fe, two QTLs were found on chromosomes 1 and 5; these regions co-localized with a few previously identified iron-associated genes (ZIP10 and NAS1; Vreugdenhil *et al.*, 2004). However, more work is needed to confirm the role of these genes in seed iron accumulation, as opposed to whole-plant iron efficiency.

QTLs associated with other iron metabolic traits have been mapped, such as traits associated with resistance to ferrous iron toxicity in rice (Wan *et al.*, 2003; Shimizu *et al.*, 2004), or to iron deficiency chlorosis in soybean (Lin *et al.*, 2000). Theoretically, QTLs associated with iron toxicity or iron chlorosis resistance could also have an association with nutrient accumulation in seeds, but the link is not a direct one. Clearly, a plant must have sufficient internal levels of iron in vegetative tissues before this pool of iron can be partitioned to the developing seeds; however, because iron delivery to seeds also is regulated by phloem processes (Grusak, 1994), iron efficiency QTLs will presumably explain only a portion of the seed iron phenotype.

4.3 Alteration of pathways of iron metabolism

Plant sources of iron include both xylem-fed leafy vegetables and phloem-fed seeds. Increasing the iron concentration of either type of plant food will usually require increases in total iron input to the plant (Grusak and Dellapena, 1999). This can potentially be achieved through genetic manipulation, either by over-expressing endogenous genes or expressing novel transgenes associated with iron metabolism. Different processes can be targeted for genetic transformation, including: 1) root iron acquisition, 2) transport through the vascular tissues, and 3) storage in edible tissues. A complete and impressive array of genes involved in these processes has been isolated from plants, animals, and microbes (Table 1-6). Many of the initial plant iron-related genes were identified in *Arabidopsis* and in barley (Curie

and Briat, 2003). However, sequence homology has led to the identification of many more iron-related genes and homologues in other important plant species such as tomato, wheat and rice (Gross *et al.*, 2003; Koike *et al.*, 2004; Li *et al.*, 2004).

When choosing to alter a plant by targeting its iron acquisition system, it is imperative that one fully understands the processes pertinent to that plant. Higher plants utilize one of two strategies for iron acquisition (Marschner and Römheld, 1994). Strategy I involves an obligatory reduction of ferric iron prior to membrane influx of Fe^{2+}; this strategy is used by all dicotyledonous plants and the non-grass monocots. Strategy II (used by grasses) employs ferric chelators, called phytosiderophores, which are released by roots and chelate ferric iron in the rhizosphere (Curie and Briat, 2003). When plants of either strategy are challenged with Fe-deficiency stress, the processes associated with one or the other strategy is up-regulated in the plant's root system (Grusak and Dellapena, 1999).

Genes involved in Strategy I and II type processes have been cloned and transformed into different plant species in an effort to alter iron metabolism. In tobacco, the yeast FRE1 and FRE2 genes (responsible for iron reduction in yeast) have been expressed, resulting in an increase in root iron reductase activity (Samuelsen *et al.*, 1998). However, this increase was not proportional to the elevation in iron concentration in the leaves, suggesting that other factors determine the maximum amount of total mineral that can be absorbed. This can happen, for example, at the level of root absorption, in which case expression of an iron transporter such as IRT1 could result in higher iron uptake. It also is important to realize, however, that once the root cells absorb iron, this iron must be transported to the plant's aerial tissues via xylem transport. The movement of iron from root cortical cells to the apoplastic xylem pathway is thus a critical first step in this process (Stephan, 2002), and perhaps there may be a need to manipulate this transport step in order for the plant to "keep pace" with the increased influx of iron.

Once in the leaves, and prior to storage, iron appears to be reduced to Fe^{2+} before influx into leaf cells and various cellular compartments (Brüggemann *et al.*, 1993). A large portion of shoot iron is accumulated in chloroplasts, where it plays a strong role in the synthesis of chlorophyll. In chloroplasts, or other plastids, it usually is stored in the central core of the multimeric ferritin protein (Briat *et al.*, 1999). For leafy vegetables, improvement in iron concentration could be achieved by modifying ferritin levels. Tobacco transformed with a bean *ferritin* gene demonstrated a 3-fold increase in leaf iron concentration (Van Wuytswinkel *et al.*, 1998).

Table 1-6. Some of the major iron-related genes described in yeast and different plant species and their respective protein functions.

Gene	Reference	Protein function	Organism
CCC1	Li *et al*., 2001	Iron transport to the vacuole	*Saccharomyces cerevisiae*
FET1 – FET5	Dix *et al.*, 1994, Spizzo *et al*., 1997, Protchenko *et al*., 2001	Iron transport	*S. cerevisiae*
FRE1 – FRE7	Martins *et al*., 1998; Yun *et al*., 2001	Iron reduction	*S. cerevisiae*
FTR1	Stearman *et al*., 1996	Iron permease	*S. cerevisiae*
FRO2	Robinson *et al*, 1999	Iron reductase	*Arabidopsis thaliana*
IRT1 – IRT2	Eide *et al*., 1996, Vert *et al*., 2002, Varotto *et al*., 2002	Root iron transporters	*A. thaliana*
NRAMP1, 3, 4	Curie *et al.*, 2000; Thomine *et al*., 2000	Divalent metal transporters	*A. thaliana*
NaatA, NaatB	Takahashi *et al.*, 1999; Takahashi *et al*., 2001	Phytosiderophore biosynthesis	*Hordeum vulgare*
NAS	Higushi *et al*., 1999	Phytosiderophore biosynthesis	*H. vulgare*
IDS1 – IDS3	Nakanishi *et al*., 2000; Kobayashi *et al.,* 2001	Phytosiderophore biosynthesis	*H. vulgare*
MtZIP3, 5, 6	López-Millán *et al*., 2004	Iron transport	*Medicago truncatula*
FRO1	Waters *et al*., 2002	Iron reductase	*Pisum sativum*
ITP	Krueger *et al*., 2002	Peptide iron chelator	*Ricinnus communis*
YSL1 – YSL8	Basso *et al*., 1994, Curie *et al*., 2001	Phytosiderophore transport	*Zea mays*

Besides storage in leaves, iron also is transported to seeds and other terminal sinks. The peptides nicotianamine (found in all plants) and ITP (identified in *Ricinus communis*) are two putative candidates for iron transport in the phloem (Stephan and Sholtz, 1993; Krueger *et al.*, 2002; Schmidtke *et al.*, 1999). In fact, it has been speculated that nicotianamine may serve to shuttle iron to and from ITP during the loading and unloading of iron from the phloem (Curie and Briat, 2003); therefore, increasing the endogenous levels of these compounds could potentially result in higher iron

translocation to the seeds, assuming adequate iron substrate in the leaves. This could be especially important in cereal crops such as rice, whose seeds import only about 15% of total shoot iron (Grusak, unpublished). More information is needed, however, on the occurrence of ITP homologues in other species.

A possible strategy to accumulate iron in the seeds of plant species is to transform the plants with the *ferritin* gene driven by an endosperm specific promoter (Lucca *et al.*, 2002; Vasconcelos *et al.*, 2003; Liu *et al.*, 2004b). Manipulation of the *ferritin* gene and promoter sequences has proven effective in increasing the amount of iron accumulated in rice grains (Vasconcelos *et al.*, 2003, Liu *et al.*, 2004b). However, although seed iron levels were found to increase by as much as 64%, even higher levels are needed to approach the RDAs for iron, given the daily amounts of rice eaten by men, women, or children.

Finally, another important aspect of transgenic strategies will be the identification of novel *cis*-acting elements and promoters that can drive gene expression more efficiently. Recently, the IDE1 and IDE2 *cis*-acting elements of the *IDS2* gene from barley were isolated and found to confer iron deficiency-inducible gene expression (Kobayashi *et al.*, 2003). At the 12th International Symposium on Iron Nutrition and Interaction in Plants, held in Japan in 2004, several innovative strategies were described including construction of artificial promoters that are highly responsive to iron deficiency. Strategies of this sort should help the process of transformation by making it more directed and efficient.

4.4 Modification of iron bioavailability

Even if the concentration of iron in plants is increased, not all iron will be absorbed. Therefore, iron bioavailability also can be targeted, either by: 1) reducing or eliminating the presence of specific anti-nutritional factors, or 2) increasing or adding promotive compounds.

Phytate, a common compound in the seeds of many cereal grains, can lead to severe inhibition of iron absorption through its ability to complex ferric iron. To increase iron bioavailability, phytases can be introduced in order to degrade phytate, thereby liberating iron. Lucca *et al.* (2002) introduced a phytase from *Aspergillus fumigatus* into rice endosperm in an effort to enhance iron bioavailability. However, once expressed in the plant, this phytase was not found to be heat stable. More work is needed to identify a thermo-tolerant phytase that can resist the high temperatures of cooking. Alternatively, low seed-phytate mutants of some crop species have been identified (Raboy, 2001), and human nutritional studies have shown an improvement in zinc bioavailability when subjects were fed a low-phytate

maize mutant (Hambidge *et al.*, 2004). Presumably, iron bioavailability would be equally enhanced.

Tannic acid is a complex polyphenolic that can inhibit iron absorption because of its galloyl-containing group, which binds iron and thus inhibits its absorption from food (Afsana *et al.*, 2003; Afsana *et al.*, 2004). Tannins exist in both gymnosperms and angiosperm species and have a very widespread distribution, being found in buds, leaves, roots and seeds (Reed, 1995). Reduction of tannin levels in seed coats of bean is possible through breeding (Guzmán-Maldonado *et al.*, 2000), and the elimination of tannins from vegetative tissues (e.g., in forage species) could be possible through genetic engineering (Dixon *et al.*, 2005). However, the removal of tannins would have some potential drawbacks for the plant, in that tannins can provide protection against freezing, predators, and microbial pathogens (Reed, 1995). As an alternative, it could be possible to transform the plant in order to produce compounds that will prevent the anti-nutritional properties of tannins. Recent studies have shown that difructose anhydride III (DFA III), an indigestible saccharide, can partially prevent the tannic acid-induced suppression of iron absorption, at least in rats (Afsana *et al.*, 2003). DFA III can be synthesized from inulin by an inulin fructotransferase (Inulinase II) (Ushyama, 1975). Inulin is also a non-digestible dissacharide compound, and it is found in some plants. DFA III is not hydrolyzed by enzymes in the small intestine, but is metabolized by microorganisms in the large intestine. The activity of this compound should remain active even after cooking, and fortunately, evidence shows that DFA III is very stable at high temperatures, and at acidic conditions (pH 2.0 at 100°C for 30 min) (Afsana *et al.*, 2003).

Other compounds reported to have a beneficial effect on iron absorption are water-soluble soybean fiber (WSSF) (Shiga *et al.*, 2003), short-chain fructooligosaccharides (Sakai *et al.*, 2000), and 1-25 caseinophosphopeptide (beta CPP), which is obtained from the hydrolysis of beta casein (Ait-Oukhatar *et al.*, 1999). These compounds have been shown to prevent iron-deficiency anemia in rats by stimulating iron absorption in the small and large intestine. Ascorbic acid (ascorbate) also can enhance iron bioavailability (Walter *et al.*, 2004; Yun *et al.*, 2004), presumably through its ability to reduce ferric iron. Recent gene discovery in the ascorbate biosynthetic pathway (Jain and Nessler, 2000) could be leveraged to increase levels of this compound in seeds of different species. Finally, amino acids such as cysteine can act as enhancers of iron absorption (Layrisse *et al.*, 1984). Lucca *et al.* (2002) over-expressed an endogenous cysteine-rich metallothionein-like protein in transgenic rice carrying the *ferritin* gene and were able to increase the content of cysteine residues sevenfold. This strategy could be attempted in other species.

5. CONCLUSIONS

It is clear that plants play an important role as dietary sources of iron for humans and other animals. However, recommended dietary intakes of iron are not always met. Iron concentrations in some common foods are low and iron bioavailability can be poor. Thus, there is a need to enhance the iron nutritional value of edible plant products in order to improve the food supply. Efforts are underway to screen germplasm for genotypes with elevated seed iron concentrations, and quantitative loci for iron-related traits are beginning to be identified. These efforts, along with the growing body of knowledge concerning iron-related genes, should enable the sensible development of marker-assisted breeding strategies and/or transgenic approaches that will yield nutritionally improved cultivars in the coming years. Additionally, our expanding knowledge base in the area of iron bioavailability can provide opportunities for manipulating non-iron food components that also will benefit the iron nutritional quality of the edible tissues.

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