

Phytoferritins - Implications for Human Health and Nutrition

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ABSTRACT

Ferritin, a metalloprotein, is rich in iron, and ubiquitous in all organisms. Plant ferritins play a pivotal role in many important redox reactions. Iron is an essential element for all forms of life and its limitation of oxidizing to ferric form has a profound impact on the productivity of organisms. The function of ferritin in plants is storage of Fe for short or long periods to protect the cell against the toxic effects of free Fe, thus serving as a primary antioxidant. Iron homeostasis in organisms is regulated at the level of iron uptake. If iron absorption is not tightly regulated, iron overload and associated toxicity occurs. The bioavailability of non-heme ferritin iron has been demonstrated by recent experiments and may provide a model for novel, utilizable, plant-based forms of iron for populations making it a potential target for biofortification. Information on the bioavailability of different forms of iron in the diet would foster research to design balanced diet and appropriate supplementation of required iron to ameliorate a wide variety of genetic background related to iron bioavailability and biosorption. The possible role of ferritin acting against biotic and abiotic stresses, accumulating heavy metals and as a protector of the genome is also reported. Plant ferritin genes have been obtained from many different plants, mostly from legumes. Fortifying plants with ferritin through a transgenic approach would aid in corroborating the existing iron fortifying programmes. Understanding the molecular, biochemical and physiological aspects of the ferritin molecule would be a significant accomplishment to construct plants overexpressing ferritin that require reduced applications of fertilizers, that can grow on marginal lands, and accumulate bioavailable iron.

Keywords: biofortification, ferritin, gene family, genetic engineering, iron bioavailability, overexpression

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INTRODUCTION

Iron is an important constituent of many proteins and enzymes that take part in vital processes and therefore is fundamental for human food supply. However, most diets based on cereals are insufficient for an adequate supply of iron. Lack of this micronutrient causes a number of severe health problems like iron deficiency that globally affects about 3 billion people and can result in anemia in women and irreversible impairment of children's learning capability (World Health Organization, WHO 2001). Many programs have aimed at supplementing food with additives. These programs have shown that supplementation is a difficult but feasible task (Trowbridge and Martorell 2002) and they have contributed to a large amount of knowledge about the nutritional availability of added compounds, their interaction with other food ingredients, and their effects on human and animal health (King 2002). Improvement of the intrinsic nutritional value of plant foods by biofortification would provide a more efficient and sustainable solution to the problem and would greatly benefit human nutrition. Efficacy of biofortified foods for improving human nutrition

and health has been promising (Haas *et al.* 2005). Classical breeding and genetic engineering could contribute together to the required improvement (Grusak and DellaPenna 1999). A more detailed understanding of the molecular and cellular processes involved in uptake, transport, storage or synthesis of iron will offer new possibilities to alter these processes by breeding and/or genetic engineering. A number of approaches to modify the iron content of various plants have been pursued world-wide by over-expressing ferritin genes (Goto *et al.* 1999; Drakakaki *et al.* 2000; Lucca *et al.* 2001) (Table 1).

FERRITIN

Ferritin, a ubiquitous class of iron storage nuclear encoded protein plays a major role in eukaryotic iron homeostasis (Harrison and Arosio 1996). It is composed of 24 sub-units, which can store up to 4000 iron atoms in the central cavity as a solid oxo mineral in a soluble bio-available form. Ferritin is the only protein capable of solving iron/oxygen chemistry with cellular concentration requirements of $\sim 10^{-4}$ M compared to the 10^{-18} M solubility of the iron, a gradient of

Table 1 Ferritin genes expressed in transgenic crops.

Gene	Gene source	Plasmid used	Gene introduction method	Promoter used	Level of expression	Result of transformation	Reference
Lettuce							
<i>Soybean ferritin</i> cDNA	<i>Glycine max</i>	pBG1	<i>Agrobacterium</i> -mediated transformation	<i>CaMV</i> 35 S promoter	1.2 to 1.7 times in leaves	Enhanced growth, high photosynthesis rates	Goto <i>et al.</i> 2000
Rice							
<i>Soybean ferritin</i> cDNA	<i>Glycine max</i>	pGPTV	<i>Agrobacterium</i> -mediated transformation	<i>GluB-1</i> Glutelin promoter	3-fold increase in seeds	Normal growth and development	Goto <i>et al.</i> 1999
<i>Soy ferH-1</i>	<i>Glycine max</i>	pGPTV	Biolistic method	<i>GluB-1</i> Glutelin promoter	3-fold increase in seeds	Iron concentrations increased even after polishing; Zinc also detected	Vasconcelos <i>et al.</i> 2003
<i>Soy ferH-1</i>	<i>Glycine max</i>	pGPTV	<i>Agrobacterium</i> -mediated transformation	<i>GluB-1</i> Glutelin, Globulin promoters	3-fold increase in seeds	No significant morphological changes; increase of iron accumulation did not parallel ferritin mRNA	Qu <i>et al.</i> 2005
<i>pfe</i> + Phytase (<i>phyA</i>) and the cysteine-rich protein metallothionein (<i>rgMT</i>)	<i>Phaseolus vulgaris</i>	pCAMBIA 1390	<i>Agrobacterium</i> -mediated transformation	<i>Gt1</i> glutelin promoter	2-fold increase in seeds	Normal growth and development	Lucca <i>et al.</i> 2001
<i>soybean ferritin</i> cDNA	<i>Glycine max</i>	pSF1	Particle bombardment	Constitutive maize <i>ubiquitin-1</i> promoter 2-10	2-fold in leaves but not in seeds	Chlorotic, reduced fertility	Drakakaki <i>et al.</i> 2000
<i>soybean ferritin</i> cDNA	<i>Phaseolus limensis</i>	pCAMBIA 1301	<i>Agrobacterium</i> -mediated transformation	Glutelin <i>GluB-1</i> promoter	64% increase in seeds	Normal growth and development No zinc content traced	Liu <i>et al.</i> 2004
Wheat							
<i>soybean ferritin</i> cDNA	<i>Glycine max</i>	pSF1 and pACH20	Particle bombardment	Constitutive maize <i>ubiquitin-1</i> promoter 2-10	50% in leaves but not in seeds	Ferritin mRNA and protein levels decreased during seed maturation, normal growth and development	Drakakaki <i>et al.</i> 2000
<i>soybean ferritin</i> cDNA + <i>Aspergillus phytase A</i> -encoding phytase	<i>Glycine max</i>	pSF2	Particle bombardment	Rice seed-specific <i>Gt1</i> promoter	20-70% in seeds	Increased level of iron, and bioavailability of iron in transgenic maize seeds	Drakakaki <i>et al.</i> 2005

100 trillion fold (Theil 2000; Liu and Theil 2005). Intracellularly most of the metabolic iron is sequestered in ferritin (Marentes and Grusak 1998). Besides enzymatic scavenging, ferritin controls the concentration of transition metals, which have a prime role in oxygen activation (Rama Kumar and Prasad 1999a). It has been demonstrated that ferritin plays a key role in alleviating oxidative damage and pathogens (Deak *et al.* 1999; Rama Kumar and Prasad 1999a; Mata *et al.* 2001). Mata *et al.* (2001) reported a reduction of infection and ROS in the *Phytophthora infestans*-infected leaves of *Solanum tuberosum*, upon addition of the iron chelator deferroxamine. Ferritin mRNA accumulated in response to pathogen attack in the leaves and upon treatment with the elicitor eicosapentaenoic acid in tubers, suggesting role of ferritin iron chelation in pathogen attack. Pro-oxidant (H_2O_2) and ABA treatment resulted in induction of ferritin in *Vigna mungo*. Pre-treatment of iron deficient de-rooted seedlings with free radical scavengers and antioxidants followed by co-treatment with ferric citrate inhibited ferritin induction indicating the antioxidant role of ferritin (Rama Kumar and Prasad 1999a). Iron sequestration in ferritins was found to be a part of an iron-withholding defense system induced in response to bacterial invasion, when *Arabidopsis thaliana* was used as a susceptible host for the pathogenic bacterium *Erwinia chrysanthemi* (Dellagi *et al.* 2005). Ferritin in legumes is one of the dietary non-heme iron sources in human nutrition (Goto *et al.* 1998, 1999; Murray-Kolb 2003). Because ferritin iron is separated from chelating components such as phytates by its protein coat, it is more stable rendering iron bioavailability (Theil and Briat 2004). The role of ferritin as a transient iron buffer has been documented in the developmental processes of plants (Strozycki *et al.* 2003). Developmental degradation of ferritin has been previously described for different parts of germinating pea seeds; degraded fragments were observed in the radicle, but not in other parts of the seed (Lobreaux and Briat 1991). Ferritin is accumulated in seed maturation and degraded during germination indicating its role

as a transient buffer iron supply. Ferritin levels increase in developing leaves (Theil and Hase 1993), indicating that ferritin synthesis in leaves is developmentally controlled. Plant ferritin mRNA has been shown to accumulate during the early stages of nodule development (Kimata and Theil 1994). In senescing nodules of *Lupinus luteus*, ferritin is re-synthesized through the expression of two out of the three lupine ferritin genes (Strozycki *et al.* 2003). Deleting ferritin genes is detrimental to life in animals (Ferreira 2000) and its importance is indicated by the presence even in strictly anaerobic bacteria (da Costa *et al.* 2001). In humans, diseases related to ferritin mutations were discovered and are relatively benign or appear late in life (Cazzola *et al.* 1997).

AMELIORATION OF IRON DEFICIENCIES

Plant products that deliver increased levels of essential minerals or vitamins are termed "fortified" foods. The introduction of genes that code for trace elements, binding proteins or storage proteins produce fortified foods. Biofortification is a sustainable approach to alleviate malnutrition (Foyer *et al.* 2006). A notable example of biofortification was the creation of iron-fortified rice and "Golden Rice" (vitamin A-fortified) (Goto *et al.* 1999; Ye *et al.* 2000).

About two thirds of the world's population is at risk of iron-deficiency induced anemia (<http://www.who.int/nut/ida.htm>). Iron deficiency is probably the most wide-spread micronutrient deficiency in humans. The bioavailability of iron is fairly low in the vegetable foods almost about 10%. It has been estimated by the WHO that nearly 3.7 billion people are iron-deficient and the problem is severe enough to cause anemia in 2 billion people. Among them, 40% are non-pregnant women and 50% were pregnant women. It has also been estimated that 31% of children fewer than 5 years are anemic, with mostly iron-deficiency anemia.

Various strategies have been used to combat these deficiencies including supplementation, food fortification and modification of food preparation and processing me-

thods. All of these strategies are of paramount importance, but availability of infrastructure and health care expertise might be a limitation. Nonetheless, application of all of these methods would contribute to iron nutrition. One possible strategy is through the introduction of genes that code for trace element binding proteins, storage proteins already present and/or increased expression of proteins that are responsible for trace element uptake into plants (Lucca *et al.* 2002, 2006). However, even very high levels of expression may not substantially increase the iron content unless many atoms of trace elements are bound per protein molecule. So introducing a protein that specifically enhances absorption even in the presence of naturally occurring inhibitors, may improve bioavailability. Introducing ferritin in plants which can bind as many as 4500 atoms of iron may prove beneficial (Murray-Kolb *et al.* 2003; Lönnedal 2003).

CONSERVED PROTEIN

The structure appears to have evolved as a patchwork of other proteins such as non-heme di-iron oxygenases that share with ferritin the binding of Fe and O₂ (Liu and Theil 2003). Conservation of the ferritin protein sequence, folding, tertiary and quaternary structure among plants and animals is very high, emphasized by the use of an animal sequence (frog) to clone the plant (soybean) ferritin gene (Ragland 1990; Theil 2003). The structural conservation is limited to secondary, tertiary and quaternary structure (four helix bundles, assembled in a spherical protein cage with a large nanaocavity) (Theil and Briat 2004). Plant ferritin subunits share a 40% homology with the mammalian H subunit (Goto and Yoshihara 2001). Structures of plant and animal ferritin are super-imposable (Lobreaux *et al.* 1992b). Ferritin in contemporary bacteria diverges considerably in sequence, but not in secondary, tertiary and quaternary structure (Theil 2000), suggesting evolutionary convergence with eukaryotic ferritins. However, except in higher plants and animals – where even the amino acid sequence is conserved – the amino acid sequence is highly variable, suggesting convergent evolution with selection for the higher order structure.

PHYTOFERRITINS

Plant ferritins are more likely than animal ferritins to be the source of ferritin in natural foods, and their mineral has a higher ratio of phosphate to iron (usually 4:1) than does that of animal ferritins (usually 1:8; Davila-Hicks *et al.* 2004). Studies of plant ferritins have revealed several important differences in the introns/exons organization, structure, localization and regulation of plant ferritins as compared to animal ferritins. Two different ferritin subunits, H and L, encoded by different genes have been described in animals. The H subunits contain conserved amino acids defining a ferroxidase site responsible for rapid Fe (II) oxidation, leading to a rapid uptake of iron inside the protein cavity; L subunits lack this site but are enriched in E residues facing the central cavity of the protein, thus enabling better nucleation of Fe (III) for its long-term storage (Harrison and Arosio 1996; Connolly and Guerinet 2002). One type of plant ferritin subunit has been described, sharing the characteristics of both the H and L subunits, namely a ferroxidase centre and additional E residues facing the protein cavity (Lobreaux *et al.* 1992b). Animal ferritins are found in the cytosol, plant ferritins contain transit peptides for delivery to specific organelles, the plastids (Proudhon 1996). N-terminal extension signal was found in all the genes cloned from *Arabidopsis thaliana* that shares characteristics with plant-specific transit peptides responsible for the targeting of precursor proteins to plastids (Petit *et al.* 2001a). More recently ferritins were reported to occur in mitochondria of both animals and plants with a possible role of protection against oxidative stress (Levi and Arosio 2004; Zancani *et al.* 2004). Moreover, while iron-regulated expression of

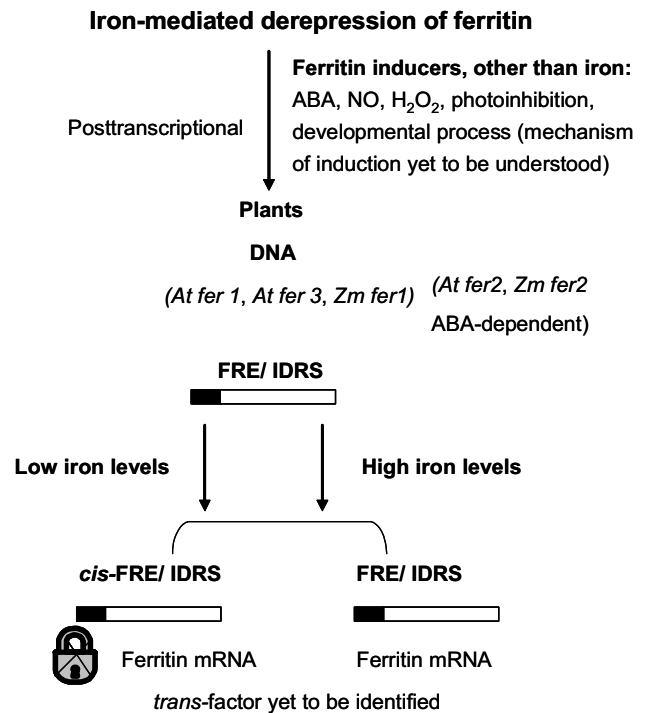


Fig. 1 Ferritin induction in plants. Iron regulates expression of plant ferritins transcriptionally through iron regulatory element (FRE in soybean) a *cis*-acting element identified in soybean ferritin gene and iron-dependent regulatory sequence (IDRS in maize and Arabidopsis). This IDRS is responsible for transcriptional repression under low iron supply conditions (indicated by lock symbol) and derepresses under high iron supply.

animal ferritin is controlled mainly at the level of translation by a system of iron-responsive elements (IREs) and iron regulatory RNA-binding proteins (IRPs) (Eisenstein 2000), experiments in soybean and maize have shown that iron regulates expression of plant ferritins both transcriptionally through iron regulatory element (FRE in soybean) a *cis*-acting element identified in soybean ferritin gene and iron-dependent regulatory sequence (IDRS in maize and Arabidopsis) (Lescure *et al.* 1991; Lobreaux *et al.* 1992a; Wei and Theil 2000; Petit *et al.* 2001b; **Fig. 1**). Post-transcriptional regulation was also reported in maize mutant *ys1* where ferritin protein and mRNA abundance does not correlate in *ys1* leaves upon iron induction, indicating that iron also controls plant ferritin accumulation post transcriptionally (Fobis-Loisy 1996). The biotechnological advancements and nutritional importance of phytoferritins is depicted in **Figs. 2, 3**.

FERRITIN GENE FAMILY AND REGULATION

Plant ferritin genes have been obtained from many different plants (**Table 2**). For example, the ferritin gene from *Lens esculenta* (Crichton 1978), *Glycine max* cell suspensions or cotyledons (Szczekan and Joshi 1987; Ragland *et al.* 1990; Lescure *et al.* 1991), *Pisum sativum* seed (Lobreaux *et al.* 1992b; van Wuytswinkel 1995), *Vigna unguiculata* (Wicks and Entsch 1993), *Zea mays* (Lobreaux *et al.* 1992b), *Phaseolus vulgaris* (Spence *et al.* 1991), maize (Lobreaux *et al.* 1992), *Medicago truncatula* (Gyorgyey *et al.* 2000), *Medicago sativa* (Deak *et al.* 1999), *Chlorella protothecoides* (Hortensteiner *et al.* 2000).

Plant ferritins are usually the products of a small gene family and all plant ferritin genes reported thus far are single-copy genes: *Zea mays* (Fobis-Loisy *et al.* 1996); *Vigna unguiculata* (Wardrop *et al.* 1999); *Arabidopsis thaliana* (Petit *et al.* 2001b); *Glycine max* (Masuda *et al.* 2001). In *Arabidopsis* and *V. unguiculata* there are four genes belonging to the ferritin family, while three were detected in *Lupinus luteus* and two in maize (Fobis-Loisy *et al.* 1995). Expression of individual family members of the known

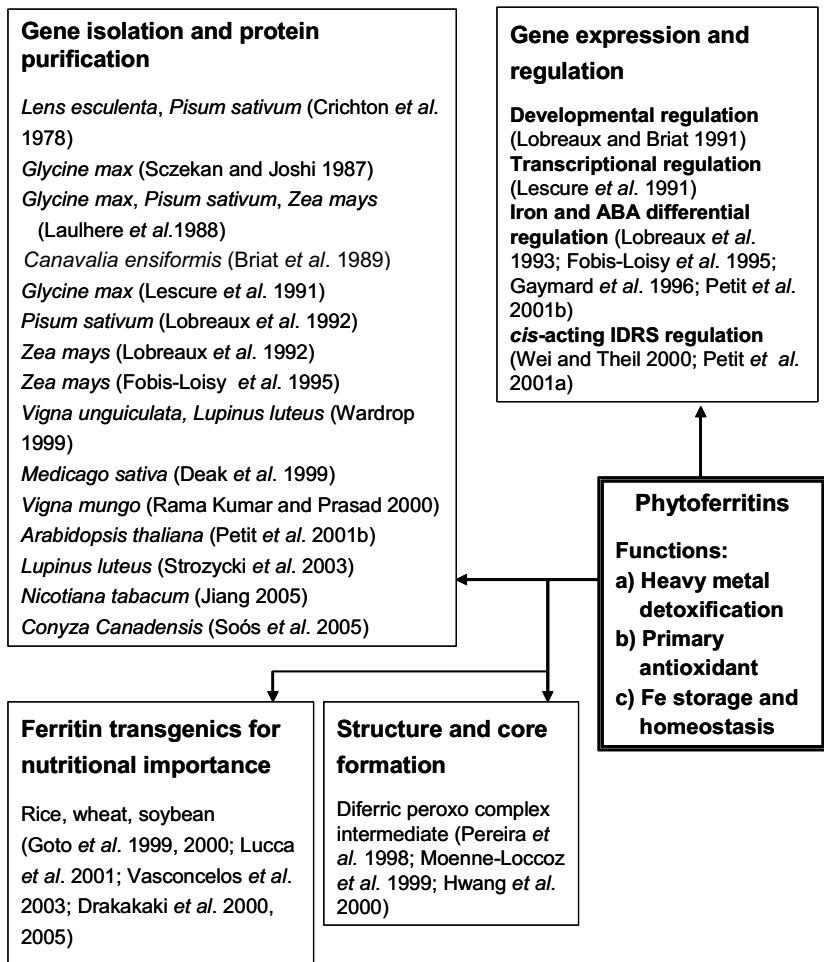


Fig. 2 Biotechnological and molecular advancements of ferritins.

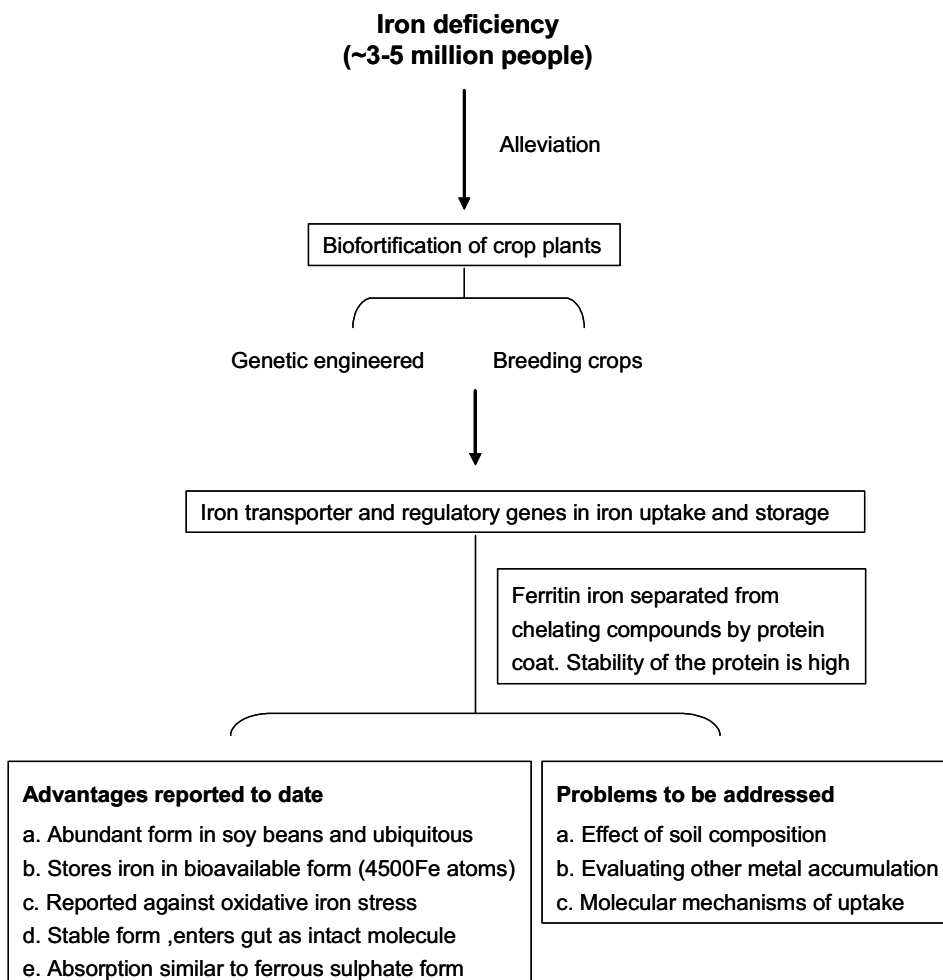


Fig. 3 Phytoferritins have gained considerable significance through biofortified foods and nutraceuticals. Advancing biotechnology of this most important metal-biomolecule would be beneficial to human health.

Table 2 Ferritin genes isolated from various sources.

Gene	Source of gene	Ferritin induction	Gene size (base pairs)	Distinct features	Gen bank accession	Reference
<i>Sof35</i>	<i>Glycine max</i> cell cultures	Iron-regulated ferritin mRNA and protein synthesis	986	Transit peptide and the extension peptide are conserved in the iron-induced mRNA	M72894	Lescure <i>et al.</i> 1991
<i>pfe</i>	<i>Phaseolus vulgaris</i> young leaves and shoot meristematic tissue	Ferritin was purified from seedlings that had been treated with 0.8 mM ferric sodium EDTA	1246	Substantial similarity with other ferritin sequences 5' untranslated region contains two out-of-frame AUG codons, a region of extreme pyrimidine composition bias and potentially stable secondary structure	X58274	Spence <i>et al.</i> 1991
<i>PeSd1</i>	<i>Pisum sativum</i> seeds	Iron induction; Fe EDTA 100 µM	1023	Lacks 5' UTR	X64417	Lobreaux <i>et al.</i> 1991
<i>FM1</i> and <i>FM2</i>	<i>Zea mays</i> roots and seeds	Iron treatment (500 µM Fe-EDTA/75 µM) Fe-citrate induced ferritin protein accumulation in roots and leaves	1292	Both were identical, except in their 3' UTRs	X61391	Lobreaux <i>et al.</i> 1992
<i>Pe Sd2</i>	<i>Pisum sativum</i> seeds	Recombinant protein was expressed in <i>E.coli</i>	1023	Δ TP/Δ EP Δ TP (Transit peptide, Extension peptide) 6base differences compared to <i>PeSd1</i> (Consensus Ferroxidase site)	X73369	van Wuytswinkel <i>et al.</i> 1995
<i>Zm fer 1</i>	<i>Zea mays</i> seedlings	Accumulation of <i>Zm fer 1</i> transcripts in response to iron	3294	Eight exons and seven introns	X83076	Fobis-Loisy <i>et al.</i> 1995
<i>Zm fer 2</i>	<i>Zea mays</i> seedlings	Accumulation of <i>Zm fer 2</i> and transcripts in response to ABA	2902	Eight exons and seven introns	X83077	Fobis-Loisy <i>et al.</i> 1995
<i>At fer 1</i>	<i>Arabidopsis thaliana</i> cell suspension	<i>AtFer1</i> transcript abundance in response to iron and not to ABA	1413	Localized on chromosome 5	X94248	Gaymard <i>et al.</i> 1996
<i>LSC30</i>	<i>Brassica napus</i> leaves	Enhanced expression during leaf senescence	977	Identified from cDNA subtractive hybridisation study in <i>Brassica</i>	U68217	Buchanan-Wollaston and Ainsworth 1997
<i>Cp2</i> <i>Cp3</i>	<i>Vigna unguiculata</i> leaves	mRNA was detected from developing leaves	958	Significantly divergent from other ferritins (only 77% identical to soybean ferritin. No similarity of transit peptide in <i>Cp2</i> . <i>Cp1</i> Transit peptide shares similarity	AF052058 AF052057	Wardrop <i>et al.</i> 1999
<i>MsFer</i>	<i>Medicago sativa</i> Somatic embryo library	Transgenic tobacco plants accumulating ferritin in their leaves exhibited tolerance to necrotic damage	1036	89% identity with pea ferritin	X97059	Deak <i>et al.</i> 1999
<i>At fer 2</i>	<i>Arabidopsis thaliana</i>	<i>AtFer1</i> and <i>AtFer3</i> transcript abundance in response to iron and not to ABA.	1006	<i>AtFer2</i> and <i>AtFer3</i> are on chromosome 3 and <i>AtFer4</i> is on chromosome 2. All 4 genes have 7 introns located at same place. <i>cis-IDRS</i> shares similarity in the four genes	AC009991	Petit <i>et al.</i> 2001b
<i>At fer 3</i>	<i>thaliana</i>	<i>AtFer3</i> transcript abundance in response to iron and not to ABA.	1042		AL163763	
<i>At fer 4</i>	Analysis of the <i>A. thaliana</i> EST database with BLASTN	<i>At fer 2</i> transcript abundance in response to ABA and not to iron, found mainly in seeds	985		AF085279	
<i>SFerH-2</i>	<i>Glycine max</i> seedlings		1135	Corresponding region in the 28-kDa soybean ferritin subunit identified in this study was not susceptible to cleavage	AB062754	Masuda <i>et al.</i> 2001
<i>StF1</i>	<i>Solanum tuberosum</i> leaves	Ferritin mRNA accumulated in response to pathogen attack	826	No presence 5'UTR.25 amino acids of the plastid transit peptide are missing	AF133814	Mata <i>et al.</i> 2001
<i>Apf1</i>	<i>Malus xiaojinensis</i>	---	771	---	AF315505	Zhou <i>et al.</i> 2001
<i>LIFer1</i> , <i>LIFer2</i> , <i>LIFer3</i>	<i>Lupinus luteus</i>	<i>LIFer2</i> class) was transcribed in response to ABA <i>LIFer3</i> gene was repressed by ABA, but up-regulated by light. <i>LIFer2</i> and <i>LIFer3</i> induced on symbiotic interaction	1032 , 1118 1039	Amino acid sequence identity of mature polypeptides (86-90%)	---	Strozycki <i>et al.</i> 2003
<i>Ferritin 2</i>	<i>Conyza canadensis</i> seedlings	Upregulated by paraquat	765	Exhibit similarity and possess all the structural characteristics of known plant ferritin genes	AJ786262	Soos <i>et al.</i> 2006
<i>NtFer1</i> <i>NtFer2</i>	<i>Nicotiana tabacum</i> seedlings	Iron loading of tobacco plantlets increased the ferritin mRNA abundance in both. <i>NtFer1</i> was expressed in both leaves and roots	1214 and 1125	Share the same characteristics as the other plant ferritins	AY083924 AY141105	Jiang 2005

plant ferritin genes is always under differential regulation (Strozycki *et al.* 2003; Jiang 2005). The differential expression of ferritin genes was detected by the induction of iron and abscisic acid (ABA) in *Lupinus* (Strozycki *et al.* 2003), and by that of iron, ABA and H₂O₂ in *A. thaliana* (Fobis-Loisy *et al.* 1996; Harrison and Arosio 1996; Petit *et al.* 2001). Paraquat-induced expression of the *ferritin 2* gene

was also reported in *A. thaliana* (Camp *et al.* 2003; Soos *et al.* 2005). Nitric oxide (NO)-mediated ferritin regulation has been shown in *Arabidopsis* (Murgia *et al.* 2002; Arnaud *et al.* 2006). NO was shown to act downstream of iron through the iron-dependent regulatory sequence (Petit *et al.* 2001b) of the *AtFer1* promoter, suggesting that NO plays an important role in the regulation of iron homeostasis in plants

Table 3 Examples of transgenic plants over expressing ferritin.

Origin of the gene (gene name)	Promoter expression	Target plant	Expressed function	Reference
Soybean ferritin cDNA	CaMV 35S	<i>Nicotiana tabacum</i>	Iron accumulation	Goto <i>et al.</i> 1998
<i>SoF 35</i>	CaMV 35S; P6 (chloroplastic); C5 (cytoplasmic)	<i>Nicotiana tabacum</i>	Iron accumulation	van Wuytswinkel <i>et al.</i> 1998
Soybean ferritin cDNA	Seed-specific <i>Glu B</i>	<i>Oryza sativa</i>	Iron accumulation	Goto <i>et al.</i> 1999
Alfalfa ferritin cDNA	Constitutive CaMV 35S	<i>Nicotiana tabacum</i>	Iron accumulation; tolerance to oxidative damage and biotic stress	Deak <i>et al.</i> 1999
Soybean ferritin cDNA	Constitutive CaMV 35S	<i>Triticum aestivum</i> and <i>Oryza sativa</i>	Increased iron levels in vegetative tissues but not in seeds	Drakakaki <i>et al.</i> 2000
Soybean ferritin cDNA	Constitutive CaMV 35S	<i>Lactuca sativa</i>	Iron accumulation and improved growth rate	Goto <i>et al.</i> 2000
Soybean ferritin cDNA	Plastid and cytoplasm expressors using CaMV 35S	<i>Nicotiana tabacum</i>	Soil dependent variability in iron accumulation	Vansuyt <i>et al.</i> 2000
<i>Phaseolus vulgaris</i> ferritin, Phytase gene (<i>Phy A</i>) from <i>Aspergillus</i> and metallothionein-like protein (<i>rgMT</i>)	Seed specific	<i>Oryza sativa</i>	Iron accumulation	Lucca <i>et al.</i> 2001
Soybean ferritin cDNA	CaMV 35S P6 (chloroplastic) C5 (cytoplasmic)	<i>Nicotiana tabacum</i>	No protection against photoinhibition and ozone stress	Murgia <i>et al.</i> 2001
Alfalfa ferritin cDNA	Constitutive CaMV 35S	<i>Nicotiana tabacum</i>	Abiotic stress tolerance	Hegedús <i>et al.</i> 2002
Soybean ferritin cDNA	Plastid and cytoplasm expressors using CaMV 35S	<i>Nicotiana tabacum</i>	Increased root ferric reductase and H ⁺ -ATPase activities and iron content, Phosphate regulated iron accumulation	Vansuyt <i>et al.</i> 2003
Soybean ferritin cDNA	Endosperm specific	<i>Oryza sativa</i>	Iron and zinc accumulation	Vasconcelos <i>et al.</i> 2003
Soybean ferritin cDNA	Constitutive CaMV 35S	<i>Nicotiana tabacum</i>	Iron and other metals accumulation	Yoshihara <i>et al.</i> 2003
Soybean ferritin cDNA	Overexpression in plastids or in cytoplasm CaMV 35S	<i>Nicotiana tabacum</i>	Heavy metal (Cd) accumulation	Sappin-Didier <i>et al.</i> 2004
Soybean ferritin cDNA	Endosperm-specific	<i>Zea mays</i>	Increased bioavailable iron	Drakakaki <i>et al.</i> 2005
<i>Aspergillus</i> phytase (<i>Phy A</i>)	Seed-specific globulin and glutelin promoter	<i>Oryza sativa</i>	Imbalance of ferritin expression and iron accumulation	Qu <i>et al.</i> 2005
Soybean ferritin cDNA	Plastid expressor CaMV 35S	<i>Nicotiana tabacum</i>	Rhizosphere bacteria of transgenics less susceptible to iron stress than wild type in spite of increased iron content in overexpressors	Robin <i>et al.</i> 2006a
Soybean ferritin cDNA	Plastid expressor CaMV 35S	<i>Nicotiana tabacum</i>	Study of structure of bacterial and pseudomonads in soil and roots in ferritin overexpressors	Robin <i>et al.</i> 2006b

(Murgia *et al.* 2002). An increase of ferritin mRNA has also been observed in *A. thaliana* leaves photoinhibited with high light or fumigated with ozone (Murgia *et al.* 2001). Transcriptional control was achieved through transcriptional repression for the *ZmFer1* and *AtFer1* ferritin genes from maize and *A. thaliana*, respectively (Petit *et al.* 2001a).

ROLE OF FERRITIN

The role of ferritin is to concentrate iron in the cells to an effective level that matches the cellular need (Goto and Yoshihara 2001). Concentration of iron leads to an iron storage function. When iron concentrations are very high, ferritin also has a protective function by sequestering the iron inside the protein (Rama Kumar and Prasad 2000; Fourcroy *et al.* 2004) thus performing a detoxification function. The possible role of ferritin acting against biotic stresses is also reported (Deak *et al.* 1999; Hegedus 2002; Dellagi *et al.* 2005). Recent reports indicate the potential role of ferritin as a protector of the genome (Surguladze *et al.* 2005).

Ferritin is, however, an abundant form of non-heme iron in many plant foods, such as legumes, that has been little considered as a nutritional iron source until recently (Davila-Hicks *et al.* 2004). The iron in pure ferritin, the major form of iron in soybeans (Ambe *et al.* 1987), can be absorbed by iron-deficient rats to correct anemia (Beard *et al.* 1996). In humans with varied iron status, iron from ferritin was well absorbed and did not differ significantly from that of iron from ferrous sulfate (a form of iron with high bioavailability) (Hallberg 1981) when given in meals with a low content of inhibitors. Ferrous sulfate, however, cannot

be used for iron fortification in most foods because it causes rancidity (oxidation) and discoloration (Hurrell 2002), which make the product unedible. Thus, ferritin iron represents a form of iron that is highly bioavailable to humans and that is not likely to affect the food in which it is consumed. Further studies are needed to evaluate the effects of inhibitors and enhancers of non-heme iron absorption on the absorption of iron from ferritin. Ferritin is very stable at a low pH and resists denaturation by heat (temperatures up to 85°C), urea, and many proteolytic enzymes (Theil 2000; Liu and Theil 2003). Ferritin also appears resistant to *in vitro* digestion (Lönnerdal 2003; Davila-Hicks *et al.* 2004). The studies on bioavailability on ferritin indicate that it might have potential impact in alleviating global iron deficiency.

OVER-EXPRESSION OF FERRITIN

Knowledge of molecular genetics obtained from one organism can be readily utilized for the improvement of another. Moreover, a large variety of techniques are available which enhance the power and speed of genetic manipulation. The mechanisms underlying iron transport and deposition in the different tissues are of particular importance since the regulatory mechanisms of iron homeostasis can be manipulated to increase the iron content of plants (Ghandilyan *et al.* 2006). Classical breeding and biotechnology could contribute together to the required improvement (Foyer *et al.* 2006).

Constitutive expression of ferritin has been done in various crops like wheat, rice and lettuce and maize (van Wuytswinkel *et al.* 1998; Deak *et al.* 1999; Goto *et al.* 1999;

Drakkaki *et al.* 2000; Goto *et al.* 2000; Drakkaki *et al.* 2005) where there was increase of iron content in the vegetative parts but not in the seed when expressed under constitutive promoter. The endosperm-specific expression of a *Glycine max* (Goto *et al.* 1999) or *Phaseolus vulgaris* (Lucca *et al.* 2001) ferritin gene in rice resulted in a three-fold increase or doubling, respectively, of the iron content in the seed (Table 1).

METAL SEQUESTRATION BY FERRITIN – HEALTH IMPLICATIONS

Knowledge of plant-metal interactions is important for socioeconomic reasons and also for reducing the risks associated with the introduction of trace metals into the food chain (Benavides *et al.* 2005). Transitional elements, like iron, copper react with reduced forms of oxygen and through Haber-Weiss and Fenton's reaction to generate free radicals and lead to oxidative stress. The transfer of one electron from the electron transport chain to oxygen (univalent reaction) generates superoxide anion (O_2^-), which then dismutates, spontaneously or enzymatically, to hydrogen peroxide (H_2O_2). The latter can react with iron (II) ion (Fenton reaction) generating the highly reactive hydroxyl radical (OH^\cdot). This metal-dependent conversion to the highly toxic $-OH$ via the Haber-Weiss reaction is thought to be responsible for the majority of the biological damage associated with these molecules. Heavy metals such as mercury, lead and cadmium have no known beneficial effect on organisms, and their accumulation over time can cause serious problems. These elements do not break down or change into other forms and therefore persist in the environment and can accumulate to toxic levels in people or plants. In order to cope with these toxic effects and to maintain the essential metals within the physiological range, plants have evolved complex mechanisms that serve to control the uptake, accumulation and detoxification of metals (Prasad 2004). Besides enzymatic scavenging, control of the concentrations of metals (known for their prime role in oxygen activation and enzyme inactivation) by sequestering them could form an important complementary way in the prevention of toxic effects. Ferritin is also capable of binding cations such as aluminum, beryllium, cadmium and zinc apart from iron in the mineral core (Szeczan and Joshi 1989; Rama Kumar and Prasad 1999b; Polanams *et al.* 2005). It is suggested that the phosphate anion in the iron core of ferritin is necessary to bind with such non-ferrous metals. Wade *et al.* (1993) showed that pea ferritin contains about one third phosphate atoms.

Genetic engineering has already been used successfully to enhance plant metal tolerance and accumulation (Lupino and Prasad 2005). This was achieved either by overproducing metal-chelating molecules such as ferritin (Goto *et al.* 1999), or by overexpression of metal transporter proteins (Hirschi *et al.* 2000). Didier *et al.* (2005) have reported increased accumulation of cadmium in the ferritin overexpressors grown in the soil containing iron and other metals along with cadmium (Table 3).

A comprehensive perspective of the chemistry and biology of ferritin would aid in creating new dimensions in engineering plants with desired characters that have potential synergies in the field of human and plant nutrition. The improvement of iron uptake efficiency will also improve the performance of plants on soils with poor iron availability (e.g., alkaline soils) and thus contribute to increased yields. Breeding for mineral content may improve disease resistance in plants; contribute to better developed root systems and boost seedling vigour, thus resulting in a beneficial situation for both farmers and consumers (Welch 2002). As promising information on bioavailability of iron from ferritin is available, the next logical step is to capitalize on this information using the ferritin as a model system to develop strategies for iron fortification determining their potential role in improving food security and nutritional value. The increasing demand for nutraceuticals and fortified foods

makes ferritin an ideal model to a beneficial effect on human health.

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