Phytoferritins - Implications for Human Health and Nutrition

M. N. V. Prasad* • N. Nirupa

Department of Plant Sciences, University of Hyderabad, Hyderabad-500 046, India
Corresponding author: * mnvsl@uohyd.ernet.in

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, utilizeable, 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

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 anaemia 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 (Trowbrigge 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 ~10^{-4}M compared to the 10^{-12} M solubility of the iron, a gradient of...
iron sources in human nutrition (Goto 2005). Ferritin in legumes is one of the dietary non-heme iron sources (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. 2001; Rama Kumar and Prasad 1999a; Mata et al. 2005). 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 deferoxamine. 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 (H2O2) 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 (Rama Kumar and Prasad 1999a). Ferritin mRNA and protein levels (Drakakaki et al. 2000) and transcript levels (Vasconcelos et al. 2000; Liu et al. 2005) are anemic, with mostly iron-deficiency anemia. It has also been estimated that 31% of children fewer than 5 years of age are anemic, with mostly iron-deficiency anemia. It has also been estimated that 31% of children fewer than 5 years of age.

### 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.

### Table 1 Ferritin genes expressed in transgenic crops

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene source</th>
<th>Plasmid used</th>
<th>Gene introduction method</th>
<th>Promoter used</th>
<th>Level of expression</th>
<th>Result of transformation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lettuce Soybean ferritin cDNA</td>
<td>Glycine max</td>
<td>pBG1</td>
<td>Agrobacterium-mediated transformation</td>
<td>CaMV 35 S promoter</td>
<td>1.2 to 1.7 times in leaves</td>
<td>Enhanced growth, high photosynthesis rates</td>
<td>Goto et al. 2000</td>
</tr>
<tr>
<td>Rice Soybean ferritin cDNA</td>
<td>Glycine max</td>
<td>pGPTV</td>
<td>Agrobacterium-mediated transformation</td>
<td>GluB-1 Glutelin promoter</td>
<td>3-fold increase in seeds</td>
<td>Normal growth and development</td>
<td>Goto et al. 1999</td>
</tr>
<tr>
<td>Soy ferH-1</td>
<td>Glycine max</td>
<td>pGPTV</td>
<td>Biologic method</td>
<td>GluB-1 Glutelin promoter</td>
<td>3-fold increase in seeds</td>
<td>Iron concentrations increased even after polishing; Zinc also detected</td>
<td>Vasconcelos et al. 2003</td>
</tr>
<tr>
<td>Soy ferH-1</td>
<td>Glycine max</td>
<td>pGPTV</td>
<td>Agrobacterium-mediated transformation</td>
<td>GluB-1 Glutelin promoter</td>
<td>3-fold increase in seeds</td>
<td>No significant morphological changes; increase of iron accumulation did not parallel ferritin mRNA</td>
<td>Qu et al. 2005</td>
</tr>
<tr>
<td>pfe + Phytase (phy4) and Phaseolus vulgaris</td>
<td>Glycine max</td>
<td>pCAMBIA</td>
<td>Agrobacterium-mediated transformation</td>
<td>G1 glutelin promoter</td>
<td>2-fold increase in seeds</td>
<td>Normal growth and development</td>
<td>Lucca et al. 2001</td>
</tr>
<tr>
<td>Ricin biosynthesis in Arabidopsis thaliana</td>
<td>pCAMBIA</td>
<td>pCAMBIA</td>
<td>Agrobacterium-mediated transformation</td>
<td>Glutelin Globulin promoter</td>
<td>64% increase in seeds</td>
<td>No zinc content traced</td>
<td>Drakakaki et al. 2000</td>
</tr>
<tr>
<td>Rice Soybean ferritin cDNA</td>
<td>Glycine max</td>
<td>pSF1</td>
<td>Particle bombardment</td>
<td>Constitutive maize ubiquitin-1 promoter 2-10</td>
<td>50% in leaves but not in seeds</td>
<td>Chlorotic, reduced fertility</td>
<td>Drakakaki et al. 2000</td>
</tr>
<tr>
<td>Wheat Soybean ferritin cDNA</td>
<td>Glycine max</td>
<td>pSF1 and pACH20</td>
<td>Particle bombardment</td>
<td>Constitutive maize ubiquitin-1 promoter 2-10</td>
<td>50% in leaves but not in seeds</td>
<td>Ferritin mRNA and protein levels</td>
<td>Drakakaki et al. 2000</td>
</tr>
<tr>
<td>Soybean ferritin cDNA + Aspergillus phytase Phy</td>
<td>Glycine max</td>
<td>pSF2</td>
<td>Particle bombardment</td>
<td>Rice seed-specific G1 promoter</td>
<td>20-70% in seeds</td>
<td>Increased level of iron, and bioavailability of iron in transgenic maize seeds</td>
<td>Drakakaki et al. 2000</td>
</tr>
</tbody>
</table>

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). 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 related to ferritin mutations were discovered and are relatively benign or appear late in life (Cazzola et al. 1997).
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 (Luca et al. 2002; de la Vina et al. 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; Lönnerdal 2005).

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, following 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 nanocavity) (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 Arasoio 1996; Connolly and Guerinot 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 and plastids. They contain transit peptides delimiting 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 Arasoio 2004; Zancani et al. 2004). Moreover, while iron-regulated expression of 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 ysl where ferritin protein and mRNA abundance does not correlate in ysl 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), Pismum sativum seed (Lobreaux et al. 1992b; van Wuytswinek 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 (Gyrgeyce et al. 2000), Medicago sativa (Deak et al. 1999), Chlorella protothecoides (Hottenstein 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.

Advantages reported to date
a. Abundant form in soy beans and ubiquitous
b. Stores iron in bioavailable form (4500Fe atoms)
c. Reported against oxidative iron stress
d. Stable form, enters gut as intact molecule
e. Absorption similar to ferrous sulphate form

Problems to be addressed
a. Effect of soil composition
b. Evaluating other metal accumulation
c. Molecular mechanisms of uptake

Gene expression and regulation
Developmental regulation (Lobreau and Briat 1991)
Transcriptional regulation (Lescure et al. 1991)
Iron and ABA differential regulation (Lobreau et al. 1993; Fobis-Loisy et al. 1995; Gaymard et al. 1996; Petit et al. 2001b)
cis-acting IDRS regulation (Wei and Theil 2000; Petit et al. 2001a)

Phytoferritins
Functions:
a. Heavy metal detoxification
b. Primary antioxidant
c. Fe storage and homeostasis

Structure and core formation
Diferric peroxo complex intermediate (Pereira et al. 1998; Moenne-Loccoz et al. 1999; Hwang et al. 2000)
Gene expression and regulation
Developmental regulation (Lobreau and Briat 1991)
Transcriptional regulation (Lescure et al. 1991)
Iron and ABA differential regulation (Lobreau et al. 1993; Fobis-Loisy et al. 1995; Gaymard et al. 1996; Petit et al. 2001b)
cis-acting IDRS regulation (Wei and Theil 2000; Petit et al. 2001a)

Iron transporter and regulatory genes in iron uptake and storage
Ferritin iron separated from chelating compounds by protein coat. Stability of the protein is high

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a. Abundant form in soy beans and ubiquitous
b. Stores iron in bioavailable form (4500Fe atoms)
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e. Absorption similar to ferrous sulphate form

Problems to be addressed
a. Effect of soil composition
b. Evaluating other metal accumulation
c. Molecular mechanisms of uptake

Gene isolation and protein purification
Lens esculenta, Pisum sativum (Crichton et al. 1978)
Glycine max (Szczekan and Joshi 1987)
Glycine max, Pisum sativum, Zea mays (Lauhere et al. 1988)
Canavalia ensiformis (Briat et al. 1991)
Pisum sativum (Lauhere et al. 1988)
Zea mays (Lobreaux et al. 1992)
Zea mays (Lobreaux and Briat 1991)
Vigna unguiculata, Lupinus luteus (Wardrop 1999)
Medicago sativa (Deak et al. 1999)
Vigna mungo (Rama Kumar and Prasad 2000)
Arabidopsis thaliana (Petit et al. 2001b)
Lupinus luteus (Strozycy et al. 2003)
Nicotiana tabacum (Jiang 2005)
Conyza Canadensis (Soós et al. 2005)

Ferritin transgensics for nutritional importance
Rice, wheat, soybean (Goto et al. 1999, 2000; Lucca et al. 2001; Vasconcelos et al. 2003; Drakakaki et al. 2000, 2005)

Structure and core formation
Diferric peroxo complex intermediate (Pereira et al. 1998; Moenne-Loccoz et al. 1999; Hwang et al. 2000)

Iron deficiency (~3-5 million people)
Alleviation
Biofortification of crop plants
Genetic engineered Breeding crops
Iron transporter and regulatory genes in iron uptake and storage
Ferritin iron separated from chelating compounds by protein coat. Stability of the protein is high
Table 2: Ferritin genes isolated from various sources.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Source of gene</th>
<th>Ferritin induction</th>
<th>Gene size (base pairs)</th>
<th>Distinct features</th>
<th>Gen bank accession</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solf 35 Glycine max cell cultures</td>
<td>Phaseolus vulgaris young leaves and shoot meristem tissue</td>
<td>Ferritin was purified from seedlings that had been treated with 0.8 mM ferric sodium EDTA</td>
<td>1246</td>
<td>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</td>
<td>X58274</td>
<td>Spence et al. 1991</td>
</tr>
<tr>
<td>Solf 35 Glycine max cell cultures</td>
<td>Phaseolus vulgaris young leaves and shoot meristem tissue</td>
<td>Ferritin was purified from seedlings that had been treated with 0.8 mM ferric sodium EDTA</td>
<td>986</td>
<td>Transit peptide and the extension peptide are conserved in the iron-induced mRNA</td>
<td>M72894</td>
<td>Lesure et al. 1991</td>
</tr>
<tr>
<td>PeSd1 Pisum sativum seeds</td>
<td>Iron induction; Fe EDTA 100 μM</td>
<td>Recombinant protein was expressed</td>
<td>1023</td>
<td>Lacks 5’ UTR</td>
<td>X64417</td>
<td>Lobreaux et al. 1991</td>
</tr>
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<td>X64417</td>
<td>Lobreaux et al. 1991</td>
</tr>
<tr>
<td>PeSd2 Pisum sativum seeds</td>
<td>Iron treatment (500 μM Fe-EDTA/75 μM Fe-citrate) induced ferritin protein accumulation in roots and leaves</td>
<td>Recombinant protein was expressed in E. coli</td>
<td>1023</td>
<td>Δ TP/Δ EP; Δ TP (Transit peptide, Extension peptide) 6 base differences compared to PEsd1</td>
<td>X73369</td>
<td>van Wuytswinkel et al. 1995</td>
</tr>
<tr>
<td>Zm fer 1 Zea mays seeds</td>
<td>Accumulation of Zm fer 1 transcripts in response to iron</td>
<td>Eight exons and seven introns</td>
<td>3294</td>
<td>89% identity with pea ferritin</td>
<td>X83076</td>
<td>Fobis-Loisy et al. 1995</td>
</tr>
<tr>
<td>Zm fer 2 Zea mays seeds</td>
<td>Accumulation of Zm fer 1 transcripts in response to iron</td>
<td>Eight exons and seven introns</td>
<td>2902</td>
<td>89% identity with pea ferritin</td>
<td>X83076</td>
<td>Fobis-Loisy et al. 1995</td>
</tr>
<tr>
<td>Atfer1 Arabidopsis thaliana somatic embryo</td>
<td>AtFer1 transcript abundance in response to iron and not to ABA</td>
<td>Localized on chromosome 5</td>
<td>1413</td>
<td>Assigned to chromosome 5</td>
<td>X94248</td>
<td>Guymard et al. 1996</td>
</tr>
<tr>
<td>LSC30 Brassica napus leaves</td>
<td>Enhanced expression during leaf senescence</td>
<td>Identified from cDNA subtractive hybridisation study in Brassica</td>
<td>977</td>
<td>Assigned to chromosome 5</td>
<td>U68217</td>
<td>Buchanan-Wollaston and Ainsworth 1997</td>
</tr>
<tr>
<td>Cp2 Vigna unguiculata leaves</td>
<td>mRNA was detected from developing leaves</td>
<td>Significantly divergent from other ferritins (only 77% identical to soybean ferritin). No similarity of transit peptide in Cp2. Cp1 Transit peptide shares similarity</td>
<td>958</td>
<td>Assigned to chromosome 5</td>
<td>AF052058</td>
<td>Wardrop et al. 1999</td>
</tr>
<tr>
<td>Msfer Medicago sativa Somatic embryo library</td>
<td>Transgenic tobacco plants accumulating ferritin in their leaves exhibited tolerance to necrotic damage</td>
<td>89% identity with pea ferritin</td>
<td>1036</td>
<td>Assigned to chromosome 5</td>
<td>X97059</td>
<td>Deak et al. 1999</td>
</tr>
<tr>
<td>Atfer2 Arabidopsis thaliana suspension</td>
<td>AtFer1 and AtFer3 transcript abundance in response to iron and not to ABA. At fer2 transcript abundance in response to ABA and not to iron, found mainly in seeds</td>
<td>Assigned to chromosome 5</td>
<td>1006</td>
<td>Assigned to chromosome 5</td>
<td>AC009991</td>
<td>Petit et al. 2001b</td>
</tr>
<tr>
<td>Atfer3 Arabidopsis thaliana suspension</td>
<td>AtFer1 and AtFer3 transcript abundance in response to iron and not to ABA. At fer2 transcript abundance in response to ABA and not to iron, found mainly in seeds</td>
<td>Assigned to chromosome 5</td>
<td>1042</td>
<td>Assigned to chromosome 5</td>
<td>AL163763</td>
<td>Petit et al. 2001b</td>
</tr>
<tr>
<td>Atfer4 Arabidopsis thaliana suspension</td>
<td>AtFer1 and AtFer3 transcript abundance in response to iron and not to ABA. At fer2 transcript abundance in response to ABA and not to iron, found mainly in seeds</td>
<td>Assigned to chromosome 5</td>
<td>985</td>
<td>Assigned to chromosome 5</td>
<td>AFO85279</td>
<td>Petit et al. 2001b</td>
</tr>
<tr>
<td>SFERH2 Glycine max seeds</td>
<td>mRNA was detected from developing leaves</td>
<td>Significantly divergent from other ferritins (only 77% identical to soybean ferritin). No similarity of transit peptide in Cp2. Cp1 Transit peptide shares similarity</td>
<td>958</td>
<td>Assigned to chromosome 5</td>
<td>AF052058</td>
<td>Wardrop et al. 1999</td>
</tr>
<tr>
<td>StF1 Solanum tuberosum leaves</td>
<td>Ferritin mRNA accumulated in response to pathogen attack</td>
<td>No presence 5’UTR.25 amino acids of the plastid transit peptide are missing</td>
<td>826</td>
<td>Assigned to chromosome 5</td>
<td>AF133814</td>
<td>Mata et al. 2001</td>
</tr>
<tr>
<td>Apf1 Malus xiaojinensis</td>
<td>LiFer2 class) was transcribed in response to ABA LiFer3 gene was repressed by ABA, but up-regulated by light. LiFer2 and LiFer3 induced on symbiotic interaction</td>
<td></td>
<td>771</td>
<td>Assigned to chromosome 5</td>
<td>AFS1505</td>
<td>Zhou et al. 2001</td>
</tr>
<tr>
<td>LiFer1, LiFer2, LiFer3 Lupinus luteus</td>
<td></td>
<td></td>
<td>1032, 1118, 1039</td>
<td>Assigned to chromosome 5</td>
<td>AFO85279</td>
<td>Strozycki et al. 2003</td>
</tr>
<tr>
<td>Ferritin 2 Conyza canadensis</td>
<td>Uregulated by paraquat seedlings</td>
<td></td>
<td>765</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NiFer1 Nicotiana tabacum</td>
<td>Iron loading of tobacco plantlets increased the ferritin mRNA abundance in both. NiFer1 was expressed in both leaves and roots</td>
<td></td>
<td>1214 and 1125</td>
<td></td>
<td></td>
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</tbody>
</table>

Ferritin 2 Conyza canadensis was also reported in A. thaliana (Camp et al. 2003; Soos et al. 2005). Nitrile 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.

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

(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; Hegedüs 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 (Lönnrand 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 (Ghandiyan 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 Wydtswinkel et al 1998; Deak et al 1999; Goto et al 1999;
Drakaki et al. 2000; Goto et al. 2000; Drakaki 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$_2$O$_2$). The latter can react with iron (II) ion (Fenton reaction) generating the highly reactive hydroxyl radical (OH$^-$). 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 (Szczekan 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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