Improvement of Micronutrient Contents by Genetic Engineering – Development of High Iron Content Crops –

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Abstract

Modification of diet by means of genetic engineering methods should be an effective way to overcome problems derived from micronutrient deficiency. Here, we review the current status of knowledge of the micronutrient contents in native foods and matters of concern for the genetic engineering of their nutritional contents, and summarize the results of experiments in the development of high iron crops by ferritin gene transformation. This means that by increasing the storage capacity of iron in plants the potential activities of the iron uptake related mechanism can be induced and thereby create high iron content crops. Finally, we also discuss the possibility of storing more iron in ferritin and the expanded application of ferritin to further breeding other than iron storage.

Key words iron accumulation, iron metabolism, ferritin, seed specific promoter, glutelin, bioavailability

Introduction

Usually the minerals in daily meals are sufficient for proper nutrition. However, some of micronutrients, especially iron, are often insufficient when the diet is inappropriate, and lack of these nutrients causes anemia-like problems. An estimated 30% of the world population is suffering from anemia due to iron deficiency (DeMaeyer and Adiels-Tegman, 1985). Especially, in the case of pregnant women and infants the requirement for iron is high and the impact of the deficiency is great. Iron deficiency anemia is the main cause of death during childbirth (approximately one-third of such deaths; Chrispeels and Sadava, 1994). Anemia also causes some physical disorders, for example, retardation of body growth, underweight, reduced immune competence, and chronic exhaustion. The negative effect on mental and motor activities decreases the capacity to work and intellectual potential. Surprisingly, iron deficiency is observed not only in developing countries but also in developed countries such as Japan. For instance, the incidence of adolescent anemia was reported to be 10% in senior high school girls in Tokyo (Akatuka and Aoki 1991).

Ferritin is one of the most important proteins for

iron storage in both plants and animals and is the only known protein to control the phase transition of ions from a solution to the solid phase. Introducing ferritin genes into crops should be an effective way to increase the iron content of foods because of their huge iron storage capacity (up to 4500 iron atoms can be stored in the shell). Plant ferritin have been identified in many species such as soybean, French bean, pea, and so forth since Hyde et al. (1963) first reported them. Whole or parts of the ferritin cDNAs have been cloned from more than ten plant species in recent years. In addition to the iron storage function, ferritin is thought to play another physical role. Free iron molecules in cells mediate the generation of an active oxygen species, known as the Haber-Weiss reaction, which is induced by various stresses, e.g., draft, low and high temperature, and infection with microorganisms (Elstner, 1987; Lamb and Dixon, 1997). Given that plant ferritin works as a scavenger of oxidative stresses, we can expect to breed new stress resistant crops.

In this review, we focus on transgenic plants expressing the ferritin gene followed by an overview of the mineral nutrients in plants and genetic engineering to produce iron fortified crops. Further work to store more iron in crops and to expand application of ferritin is also discussed.

1. Overview of the micronutrient contents in foods

Sixteen mineral elements, i.e. sodium, potassium, chloride, calcium, magnesium, phosphate, sulfur, iron, zinc, copper, manganese, cobalt, chromium, iodine, molybdenum, and selenium, are known to be essential for the human diet (Anonymous, 1992). Recent work indicates that another element, boron, also plays an important role in metabolism as a mineral element although further research is needed to determine its acceptability as an essential nutrient (Naghii and Samman, 1993). More than 100 mg per day of the first six elements, sodium, potassium, chloride, calcium, magnesium, and phosphate are required for an adult. These are called the major minerals. The necessary amounts of the rest of the elements, with the exception of sulfur, are estimated at less than 100 mg and are called minor- or micronutrients. In these micronutrients, iron, zinc, copper, and manganese, require comparably high doses (at least more than 1 mg per a day), and are especially important since they play a direct role as a cofactor or exist as the active center of some enzyme.

The content and metabolism of such micronutrients in ordinary foods can be overviewed as follows: Iron content in foods is one of the most precisely studied subjects in the field of human dietetics. The calculated need for iron is approximately 12 to 15 mg per day for an adult. Food groups containing high iron content are seaweeds, liver, egg-yolk, and beans (Naito et al., 1987). Specific leafy vegetables like spinach and the embryos of grains are also listed as high iron content food groups (over 8 mg-Fe/100g edible portion). Iron in foods is usually observed as ferric ion. Most of the iron in foods derived from animal sources is combined with heme, and is easily absorbed in the human intestine; however, iron in vegetables is mainly non-heme iron, and is difficult to absorb. One estimate showed that the availability of heme iron is 15 % to 40 % whereas that of non-heme iron is about 2 % to 10 % even though ascorbate like substances can increase the availability of nonheme iron to double or more (Schumann et al., 1998). In addition, a kind of inhibitor to absorb iron is observed in some foods. Oxalic acid and phytate in vegetal diets, especially in cereals and beans, are the typical substances that decrease the bioavailability of iron (Hurrell et al., 1992). Phosvitin is a kind of protein, which is contained in egg yolk and reduces the availability of iron (Grogan and Ta-

borsky, 1986). Inhibition of the iron intake with respect to another mineral in foods is also reported, i.e. calcium inhibits iron absorption in milk (Kalkwarf and Harrast, 1998). Even the iron holding protein in milk, lactoferrin, may possibly inhibit the availability of iron (Lonnerdal and Iyer, 1995). It is also observed that the availability of iron is reduced corresponding with the amount of the storage iron (Naito et al., 1987). Zinc is also considered as an important mineral although it is known as a toxic heavy metal. The necessary amount is comparable to the level of iron (about 6 mg for an adult per day) and exists as a conjugate with a specific storage protein, metallothionein, to reduce the toxicity (Smith et al., 1983; Sandstead, 1985). Food groups containing high zinc content are mainly recognized as of animal origin (Murphy et al., 1975); oysters, beef, dark meat of poultry, egg yolk, milk, and cheese are counted as the examples. Most vegetal diets basically contain a low amount of zinc. However, cocoa and the milling fractions of bran, germ of cereals, legumes and peanuts are comparably high in zinc. Reports concluded that although there is no need for concern about zinc deficiency in the case of an ordinary adult who takes a usual diet, there is in the case of a patient with major burns and in case of infants fed only just breast milk (Murphy et al., 1975; Naito et al., 1987). It is reported that an iron uptake inhibitor, phytate, may also prevent the uptake of zinc (Sandberg, 1991). The necessary amount of copper is one-tenth of the level of iron or zinc (about 1 mg for an adult per day). Most copper accumulates in a multicopper ferroxidase, ceruloplasmin, which is involved with not only copper, but also with iron homeostasis in cells (Mzhel'skaya, 2000). Patients of Wilson's disease are known to be deficit in ceruloplasmin and need to be careful not to take an excess copper. Food groups containing high copper content are listed as follows: oysters, sesame, liver, crab, and shrimp (Anonymous, 1992). Supplements of minerals with similar chemical characteristics can reduce copper absorption, whereas proteins and soluble carbohydrates tend to improve copper absorption and bioavailability by enhancing its solubility and availability in the intestines (Wapnir, 1998). Manganese is involved in fat metabolism in the human body. The necessary amount of manganese is estimated as a comparable or rather lower level than that of copper, though it is still being discussed (Lonnerdal, 1989). Food groups containing high manganese content are mainly recognized as of vegetal origin; seaweeds, sesame, beans, cereals, and green tea (Anonymous, 1992).

2. Genetic engineering for nutritional contents Targets and the possibility to store excess iron in plants

In the last decade, some experiments revealed the possibility for a biotechnological approach to improving the nutritive value of crops (Uzogara, 2000). Most aimed at a modification of the proteinaceous composition. Seed storage protein is the first and most reasonable target for supplementation of nutritionally deficient amino acids, i.e. that is cystein and methionine content in the dicot seed, and lysine, tryptophan and threonine content in the monocot seed, respectively (Altenbach et al., 1989). Genes for native seed storage protein, e.g. betaconglycinin (Beachy et al., 1985), beta-phaseolin (Sengapta-Gopalan et al., 1985), vicillin (Higgins et al., 1988), and glutenin (Williamson et al., 1988), were often introduced into the target crops under the control of seed specific promoter or the popular constitutive promoter of CaMV 35S. A challenge to modify the genes to contain several repeats of the deficient amino acids was also reported (Takaiwa, et al., 1995). At the present time, the mainstream of the biotechnological approach is to improve the other contents in crops, i.e. carotenoid (Schmidt-Dannert et al., 2000; Ye et al., 2000), oil or fattyacids (Somerville, 1993; Knauf and Facciotti, 1995), sugar (Geoges et al., 1999) and starch (Schwall et al., 2000). Improvement of the mineral content in crops is also underway.

Food additives or tablets for direct oral administration are the most effective ways to overcome iron deficiency. However, it is difficult to implement in developing countries due to the cost and the logistics of primary health care programs. Native highlevel iron content crops may more easily contribute to overcoming the deficiency. Actually, some crops (e.g. spinach and leguminous plants) are known to be high in iron content (Anonymous, 1992). However, this useful trait usually exists simultaneously with oxalic acid and phytate-like substances that decrease the bioavailability of iron and reduce digestibility. Several kinds of agronomic approaches through fertilization of soil and culture media or by spraying iron onto the leaf surface have been attempted to increase iron content in the crops. These are costly and it is not possible to target iron accumulation to a preferable part of the plant. In addition, the excess iron and/or the chelator in the media not only suppresses the plant growth, but also reduces commercial value and productivity (Inoue et al., 1995). Of course, ordinary breeding efforts

Targets for increase iron content in crops are mainly categorized into two ways: first, increase the amount of iron taken into the plant; and second, increase the storage capacity of iron in plants. The first way is a method for increasing the number of taps or enlarging tap diameter as it were which accelerates the stream of water into a vessel. The second way is based on a hypothesis that the establishment of a new vessel as it were for iron stimulates the metabolism related to iron intake in the meaning of "homeostasis", and increases the potential to accumulate iron in the vessel (Fig. 1). According to the findings of a few decades, two strategies are recognized in nature for iron intake depending on the plant species (Römheld, 1987; Welch, 1995; Moog and Brüggemann, 1995). Strategy I is considered to be taken mainly by dicotyledonous and non-graminaceous species. It is characterized by three steps: enhancement of net excretion of protons, reduction to ferrous ion by a plasma membrane bound reductase, and uptake into cytoplasm by a ferrous transporter (Kochian, 1991). Plants taking strategy II are confined to graminaceous species. Increase in the biosynthesis and secretion of a kind of natural chelate of ferrous ion, phytosiderophores, to the rhizosphere characterize strategy II. A group of phytosiderophores, which has only been detected, is designated as the Mugineic acid family (MAs: Takagi et al., 1984). A number of genes related to the iron acquisition in plants have been isolated and recognized as candidates for genetic engineering; for example, AHA1 (H⁺-ATPase; Harper et al., 1989), IRT1 (iron transporter; Eide et al., 1996) and FRO2 (ferric-chelate reductase; Robinson et al., 1999) have been isolated from Arabidopsis and ascertained to play major parts in strategy I. Many cDNAs related to the

1. Fortification of <u>the iron storage capacity</u> (to enlarge the water vase as it were)



2. Fortification of <u>the iron uptake ability</u> (to reinforce the water tap as it were)

Fig. 1 Two possible ways to improve the iron content in plants

biosynthesis of MAs have also been isolated; e.g., genes for S-adenosylmethionine synthethase (SAMS, Takizawa *et al.*, 1996), nicotianamine synthase (NAS, Higuchi *et al.*, 1999), nicotianamine aminotransferase (NAAT, Takahashi *et al.*, 1999), and dioxygenases, which catalyze a hydroxylation of DMA to epiHDMA, MA to epiHMA and DMA to MA (Nakanishi *et al.*, 1993; Okumura *et al.*, 1994).

On the other hand, iron holding molecules can be seen anywhere in living organisms as an important component of cells and metabolism (Table 1). For example, a kind of enzyme and cofactors (e.g. ferredoxin, SOD and cytochrome) utilize iron as the activity center. However, the highest concentration of iron is in the cytoplasm in labile form until stored or it may be changed in form to insoluble ferric hydroxides and precipitated. Iron is an essential element for all forms of life. However, freely existing iron may react with certain forms of oxygen to produce deleterious hydroxyl radicals (Briat et al., 1995). To prevent this phenomenon, living organisms have various molecules including those that scavenge for the radicals and those that serve as vessels for iron storage. Ferritin is a unique molecule, which stores the excess iron safely in its cytoplasm and releases it on demand (Theil, 1987). Sometimes an overload of iron or specific conditions under germination may induce another type of iron storage called hemosiderin (phytosiderin). However, because the amino acid conformation of hemosiderin is very similar to that of ferritin and other biochemical analyses, hemosiderin is considered to be one of the derivatives of ferritin (Laul-

here et al., 1989). Genes for such iron holding molecules have been isolated and recognized as candidate for genetic engineering. The ferritin genes shown in the next chapter are the most precisely analyzed one. Genes for other structural proteins (e.g. cytochromes, Kemmerer et al., 1991; ferredoxin, Alam et al., 1986) and/or scavengers (e.g. SOD, Cannon et al., 1987; APX, Caldwell et al., 1998; and catalase, Ni et al., 1991) are also well documented. However, ferritin has the following characteristics which made it the most suitable gene to use in our system. 1. Ubiquitous: almost all kinds of living organisms have their own ferritin molecule to store iron. If the phytoferritin gene is used for the transformation, it makes it easy to accept the transformation not only for the recipient plants but also for the consumer of the products. 2. Capacity: Ferritin can store up to 4500 iron atoms, which is the largest amount in the known proteinaceous molecules. When we take the first way to improve the iron content, the huge capacity is considerably attractive. 3. Regulation: When a large vessel is exogenously expressed in cells, the plant may behave as if it is constantly in a state of iron deficiency. Even when the cell is iron starved, ferritin may release iron on demand. 4. Role: Ferritin may not have a metabolic role other than iron storage and the prevention of active oxygen generation. 5. Availability: A recent report revealed that the bioavailability of the ferritin iron is considerably high (Beard et al., 1996). Specifically, the ferritin itself does not prevent iron uptake from molecules during digestion. On the contrary, when another way to increase iron content in plants is used, a

Substances	function	capacity*1	availability*2
ferritin	iron storage/antioxidant	up to 4500	high
hemosiderin/phytosiderin	temporal iron storage/antioxidant	up to several thousands	medium
ferredoxin	electron donor	2-8	low
phytohemagglutinin(Concanavalin A)	iron storage?	1	medium
rubredoxin	catalyze oxidation/reduction	1	low
SOD	catalyze oxidation/reduction	1	low
iron reductase	reduction/chelates	1	low
ribonucleotidereductase	electron donor	1	low
hydrogenase/nitrogenase	catalyze oxidation/reduction	1-4	low

Table 1. Examples of the iron holding substance in plant cells

*1 Theoretical iron storage capacity

^{*2} The availability for genetic improvement in plants is evaluated in the point of iron accumulation for three levels by the following reason(s): low, depends on species specific metabolism or tightly linked to the total metabolism of the cell; medium, does not store as much iron in a molecule and may not affect the total balance of metabolism or, the molecule is a degraded form and is not constituted directly (hemosiderin); high, stores or takes up much iron and may not affect the total balance of metabolism.

-FLGLSKGVG MALSCSKVLS FY-LSPVVGG GDVPKKLTFS Soybean L 1 MALAPSKVET FSGESEKPSV GGAQKNPTOS VSLSFLNEKL GERNNRV Sovbean S 1 **Extension Peptide** A-Helix ASNAPAHLAG VIFEPHOELK KOYLAVPILAH NVSLARONYA DOSESAINEO 48 VIFEPERENK KSELAVPTAP OVSLARONYA DECESAINEC 49 -IT ITG **B-Helix** YHALFAYFDR DNIALKGLAK FFKESSEEER EHAEQLUKYQ 98 INVEYNVSYV DNVALKGFAK FFKESSEEER EHAFINI INVEYNASYV YHSLFAYFDR 97 **C-Helix** NIRGGRVVLH PITSPPSEFE HSEKGDALYA MELALSLEKL TNEKLLHVHS 148 NURGGRVVLH PIKNAPSEFE HVEKGDALYA MELALSLEKL VNEKLLNVHS 147 **E-Helix D-Helix** ADFIESEFLY EQVKSIKKIA EYVAOLRLVG KGHGVWHFD 198 VADRNNDPOL 197 VADRNNDPOM ADFIESEFLS EQVEBIKKIS EYVAOLREVG KGHGVWHFDO 248 KLIHDEDHV 247

Fig. 2 The deduced amino acid sequences of soybean ferritin subunits Amino acid sequences of the L subunit (Masuda *et al.*, in submitted) and the S subunit (Regland *et al.*, 1991) are shown. Identical residues are in boxes. Residues in bold face indicate those which have been suggested to form the deduced ferroxidase center. A solid arrowhead indicates the cleavage site of the transit peptide (TP) in both the L and S subunit. The mature regions of subunits are downstream from here. An open arrowhead indicates the cleavage site in conversion from 28 kDa form to 26.5 kDa form of the S subunit.

feedback system may stop excess iron intake otherwise too much iron might injure the cells; it creates a simultaneous need to separate iron from the cell components by some way other than ferritin.

3. Practice – development of high iron crops by ferritin gene transformation

1) A genetic background of ferritin

Plant ferritin consists of 24 subunits, which have ferroxidase activity (Briat *et al.*, 1995) like the mammalian H subunit (Laulhère and Briat, 1993) and share about 40 % sequence homology. **Fig. 2** shows the amino acid sequences deduced from soybean ferritin genes. Subunits of soybean and many other leguminous ferritins are synthesized as 32 kDa precursor proteins with a unique N-terminal sequence that is composed of two domains (Ragland *et al.*, 1990). These N-terminal domains are not present in mammalian or other ferritins. The first domain is an N-terminal transit peptide (TP), that consists of 40 to 50 residues, and is presumed to facilitate transport of the ferritin precursor to plastids (Briat *et al.*, 1995). The second domain, designated the extension peptide (EP), is part of the mature protein (Laulhère *et al.*, 1988; Ragland *et al.*, 1990), however, its function is still unclear.

Plant ferritin genes have been obtained from many kind of plants since Ragland et al., (1990) cloned ferritin gene from soybean cotyledon e.g. cowpea (Wicks and Entsch 1993), pea (Lobréaux et al., 1992), French bean (Spence et al., 1991), maize (Lobreaux et al., 1992), Acacia mangium (Hoya et al., 1997), Medicago truncatula (Gyorgyey et al., 2000), loblolly pine (Li, et al., 1998), alfalfa (Deak et al., 1999) and Chlorella protothecoides (Hortensteiner et al. 2000). In addition to the genes mentioned above, ferritin genes have been registered on a database e.g., watermelon (Shin, 1999), the common ice plant (Cushman, 1997), sugar beet (de los Reyes et al., AW777187), and rice (Uchimiya, 1993). Interestingly, ferritn gene was also cloned as a senescence related gene from Brassica napus (Buchanan-Wollaston and Ainsworth, 1997). Thus far, only one polypeptide chain type has been identified as a functional subunit of plant ferritins although evidence for ferritin multigene families has been provided in maize (Lobrèaux, 1992), cowpea (Wicks, 1993; Wardrop et al., 1999), and

soybean (Wardrop *et al.*, 1999). However, our group lately revealed two types of ferritin subunits in soybean having different functions from each other (Masuda *et al.*, submitted).

The expression of plant ferritin gene seems to be regulated at the transcriptional level (Van der Mark et al., 1983a; Proudhon et al., 1989; Lobréaux et al., 1992). The existence of a novel iron regulatory element (FRE), which controls the iron-mediated depression of the ferritin gene, was reported recently (Wei and Theil, 2000). However, the possibility that plant ferritin was also controlled at the translational level like the mammalian ferritin cannot be ruled out (Lobréaux et al., 1993). It was also shown that abscisic acid (ABA) induces the transcription of the ferritin mRNA in leaves of ironstarved maize even though ferritin abundance was much lower than in the case of iron overloading. Loisy et al. (1996) also suggested the translational regulation of plant ferritin gene in the maize mutant. An iron responsive element (IRE) plays an important role in the regulation of ferritin gene expression at the translational (post transcriptional) level in mammals, even though such an element has not been found out in plant ferritin. The IRE is located at the 5' untranslated region of ferritin mRNA (Theil, 1990) and has a stem loop structure. When cellular iron level is low, iron regulatory protein binds to the stem loop of the IRE resulting in inhibition of the translation of ferritin.

2) Transgenic plants expressing ferritin

Several transgenic plants expressing soybean ferritin have been produced. At the first, we demonstrated that expressed ferritin in plants is beneficial for the accumulation of iron in plants using a tobacco system (Goto et al., 1998). The expression of soybean ferritin gene can be observed by means of RT-PCR and the exact synthesis of the product was detected by immunoblot analysis. The maximal iron content of leaves from transgenic tobacco plants was approximately 30 % higher than leaves from non-transgenic plants. Wuytswinkel et al. (1998) followed the experiment using the same experimental systems and showed its effectiveness for iron accumulation. They reported the leaf iron concentration was 2 to 3-fold higher in transgenic plants than in control plants. The different iron contents between the two results may be explained by the difference in the leaf age and/or the growing stage of the plant used for iron measurement. Goto et al. used mature leaves grown in a green house, while Wuytswinkel et al. used three-week-old plants grown in vitro. It is also possible that the richer the iron concentration in the growth media,

the greater the amount of iron stored in ferritin. Since then, we have transferred the same soybean ferritin gene into rice with an endosperm specific promoter, GluB-1 (rice seed storage protein, glutelin), to regulate the introduced ferritin gene expression in the edible part of rice (Goto et al., 1999). Rice is one of the strategically important crops in the fight to overcome iron deficiency anemia. The result clearly showed that the iron content of rice seed was significantly increased by the expression of the ferritin gene. The grains of the transformants accumulated up to 3-fold more iron than that of the control rice plant. We concluded that the iron content in a meal-size portion of transgenic "ferritin rice" would be sufficient to provide 30-50 % of the daily adult iron requirement. This suggests potential benefits for human nutrition. We also transferred the ferritin gene into lettuce (Goto et al., 2000). Lettuce is one of the most popular leafy vegetables in the world but contains significantly less iron than spinach, an iron accumulator. However, lettuce contains 100-times less oxalic acid, which is a strong inhibitor of iron uptake from the intestine and a cause of kidney stones, than spinach. From this point of view, the transgenic lettuce containing extragenous ferritin gene may also contribute in the fight against iron deficiency anemia. The transgenic lettuce plants contained 1.2 to 1.7 times more iron than the control plants. To investigate whether ferritin gene expression affects the accumulation of heavy metals other than iron, we also measured manganese content in leaves. Because manganese is known as a competitive metal with iron in plant nutrition, it is a good indicator of potential effects of ferritin expression on another metal accumulation. The result showed that the manganese content was not affected by the ferritin gene transformation. It was interesting that enhanced growth of the transgenic lettuce plants was observed in each of the transgenic plants containing the ferritin gene in addition to the increase in the iron content. In relation to this finding, a report by Deák and his colleagues (1999) provides another possibility for the practical use of ferritin gene. They showed that overexpression of alfalfa ferritin gene in tobacco leaves results in enhanced tolerance to oxidative stress and pathogens. The damage caused by excess iron or paraquat, which is an inducer of oxidative stress, was certainly less in transgenic plants than in control plants. The transgenic plants showed considerable tolerance to three microorganisms (tobacco necrosis virus, alternaria alternata and Botrytis cinnerea).

4. Future work – To store more iron ferritin and to expand application of ferritin

As mentioned above, our group identified a novel ferritin subunit recently. The function was to stabilize the ferritin shell by co-existing with a known subunit (Masuda et al., submitted). The known subunit (S subunit) was easily converted into 26.5 kDa by the cleavage of the C-terminal 16 residues, while the novel subunit kept the 28 kDa form (L subunit). We compared the rate of iron uptake and release of native ferritin containing the S and L subunits in equal amounts, and recombinant ferritin made of just the S subunit. The iron uptake of the native ferritin was much lower than that of the recombinant one, but iron release was faster. Furthermore, the recombinant ferritin degraded more easily than the native ferritin. Taken together, the novel ferritin subunit contributes to keep iron and the known subunit could release iron by cleavage of the E-helix rather than retain iron. It is possible that another transformant, which contains much more iron, can be newly developed by use of this novel ferritin subunit gene.

On the other hand, the contamination of heavy metals in soil and water is a great problem in the world. The transgenic plant expressing ferritin may be a strong tool for phytoremediation. Because Sczekan and Joshi (1989) showed that a kind of ferritin is capable of binding cations such as aluminum, beryllium, cadmium and zinc, they suggested that the phosphate anion in the iron core of ferritin is necessary to bind with such nonferrous metals. Phosphate causes eutrophication of lakes and rivers. Wade et al. (1993) showed that pea ferritin contains about one-third phosphate atoms (1800 atoms of Fe and 640 atoms of P / ferritin molecule). Waldo et al. (1995) and Barcelo et al. (1997) confirmed the existence of phosphate in the iron core of ferritin. In addition, some metals (Be, Cu, VO, Cd, Tb, Zn) were reported to bind to mammalian ferritin (Price and Joshi, 1982, 1983; Wardeska et al., 1986; Zaman and Verwilghen, 1981). Introducing animal ferritin gene into plants should be an interesting trial for phytoremediation.

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