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Isolation and Characterization of a cDNA(Fp1) Encoding the Iron Storage Protein in Red Pepper(*Capsicum annuum* L.)

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ABSTRACT

A cDNA fragment encoding iron storage protein generated by polymerase chain reaction(PCR) using highly conserved regions of ferritin related genes were used to screen a red pepper cDNA library. cDNA clone was designated as Fp1. Fp1 clone contained a 5' nontranslated region of 51bp containing stop codons. Down stream from 5' UTR, an open reading frame of 750bp was observed, followed by a 3' UTR of 272bp. The deduced amino acid sequence of red pepper protein(Fp1) showed 84%, 48% and 36% identity with soybean(SoIC), human(HuL-H) and horse spleen (HoS-L) ferritin respectively. Northern blot analysis of root and leaf RNAs, at different times after iron treatment, revealed ferritin mRNA accumulation in response to iron. Ferritin mRNA accumulation was transient and particularly abundant in leaves, reaching a maximum at 12h. The level of ferritin mRNA in roots was affected to a lesser extent than in leaves.

Key words: alignment, amino acid sequence, cDNA library, Fp1 gene, iron storage protein, Northern blot, Southern blot.

INTRODUCTION

Iron is an essential element for virtually all forms of life because of its role in fundamental processes such as respiration, photosynthesis, nitrogen fixation and cell division. However, its tendency to form insoluble salts in aqueous solutions and its potential for toxicity via free-radical formation as a result of redox reactions in the presence of oxygen led to the evolution of specific genetic systems which control iron homoestasis in cell. These systems include iron uptake, transport and storage (Crichton and Charloteaux-Wauters, 1987; Theil, 1987). Iron storage is achieved by ferritins, a class of proteins widely distributed among animals, plants and bacteria. These proteins are organized in hollow spheres able to accomondate a few thousand iron atoms inside their central cavity and they are present in all living organisms (Harrison et al., 1989). Also, ferritin are known to sequter and thus detoxify iron taken up by cells which is not utilized for metabolic requirements. Under conditions of iron need, ferritin-Fe(III) can be released by reduction for cellular use (Lanlhere et al., 1990). Therefore ferritins are key proteins acting as a buffer for iron, protecting cells from a harmful concentration of free iron and regulating their immediate need. Structure, function and synthesis of animal ferritins have been extensively studied (Theil, 1987; Harrison et al., 1989; Klausner and Harford, 1989). However, the molecular mechanisms involved in this transcription control in response to iron are unknown. It has to be noticed that the translation control of ferritin synthsis in response to iron has been well conserved during evolution since the "iron-responsive element-binding proteins" (IRE-BP) is found throughout only animal kingdom

(Rothenbergers et al., 1990).

In plants, most of the information concerning ferritins has been gained from electron microscopy studies (review of Seckbach, 1982; Proudhon et al., 1989), they are located in plastids, synthesized from ploy (A^+)RNA as a precursor, which is transported to plastids resulting in a mature 28KDa ferritin subunit able to assemble into a 24-mer apoprotein (Laulhere et al., 1989). Futhermore, the structure of gene encoding many plant ferritin have been determined sequencing soybean(Lescure et al., 1991), french bean cDNA(Spence et al., 1991), *Phaseolus Vulgaris* cDNA(Michael et al., 1991), *Pisum sativum*(Stephare LoBreaxu et al., 1992), lettuce(Goto and Yoshihaca) and maize (Stephare et al., 1992). About 50-60% of the amino acids sequence of the pea seed ferritin subunit has also been deciphered through micro-sequencing of its N-terminus and CNBr peptides(Ragland et al., 1990). NH-ferminal first part of plant ferritin protein is a transit peptide responsible for plastid tageting(Lescure et al., 1991; Ragland et al., 1990). The second part is specific for plant mature ferritin subunit and is known to be the site of free radical cleavage which occurs *in vitro* during iron exchange (Laulhere et al., 1988) and *in vivo* during germinatim(Lobreaux and Briat, 1991). Also, plant ferritins are not detectable in vegetative organs under normal iron nutrition conditions. Generally, ferritins accumulate during seed formation in the embryo axis and cotyledons in order to store iron and they are processed and disappear during the first week of germination(Lobreaux and Briat, 1991). The synthesis and degradation of ferritin in plants continuously supplied with iron, as well as iron distribution in different organs during their life cycle is developmentally regulated. However, in contrast with animal systems, regulation of this iron response in cultured soybean cell is entirely accounted for by transcription, while the major control of ferritin synthesis is translational in animals (Lescure et al., 1991).

In this paper, we report the isolation and characterization cDNAs containing the entire ferritin open reading frame from red pepper tissue and describe transient accumulation of mRNA in roots and shoots during iron stress.

MATERIALS AND METHODS

Plant cultures

Red pepper seeds were soaked for 24hr in distilled aerated water. Germination of the seeds was achieved on iron free medium into *in vitro* for 14days in the dark and were transferred to glass vials containing 200ml nutrient solution with iron mixture (500μM Fe-EDTA, 150μM trisodium citrate, 75μM $FeSO_4$). The nutrient solution was composed as described by Knop(Bergmann, 1958) with a modified micronutrient composition(van der Mark et al., 1981). The culture conditions were 16h of light at 28℃ and 8hr of dark at 20℃. Organs were harvested at different times after treatment, frozen in liquid nitrogen and stored at -70℃.

Determination of iron concentration

Plant roots were extensively washed in 1mM KCl, 10mM EDTA prior to freezing. Root and leaf samples were mineralized and total iron concentration was measured by recording absorbance of Fe^{2+} o-phenanthroline at 510nm, pH6.0, using thioglycollic acid as a reducing agent. In the case of roots, values were obtained before and after removing apoplastic iron according to Longnecker and Welch(1990). To release apoplastic iron by reduction, plants were treated as follows before mineralization. Intact plant roots were rised in 0.5mM $CaCl_2$ for 5min and incubated 1hr in 50ml of O_2-free nutrient medium containing 11.7mg of bipyridyl and 50mg of dithionite(DTT).

Cloning and Sequencing cDNA

Total RNA was isolated from the roots 24h after iron addition to the culture medium by the guanidium/caesium chloride method, and poly (A^+) RNA was purified by oligo(dT)-cellulose affinity chromatography (Maniatis et al., 1982). Complementary DNA with an EcoRI/NotI linker was synthesized with a cDNA synthesis Kit (Pharmacia, Uppasls, Sweden), cloned λgT10 (Stratagene, U.S.A.) and packaged using Gigapack Gold Extracts (Stratagene, U.S.A.). The resulting library was transferred to nylon

membrane filters (Hybond-N plus: Amersham, UK) and screened with the selected DNA fragments amplified by polymerase chain reaction(see below). For PCR amplification, the forword primer 5' -AGTGA GGAAGAAAGAGAGCA-3' and the riverse primer, 5' -AAAGTGCCAAAC ACCGTG-3', corresponding to the highly conserved SEEEREH and HGVWHFDQ, respectively. Hybridizations were performed in 6×SSPE (0.1% SDS, 0.02% PVP, 0.02% Ficoll, 50㎍/ml salmon sperm DNA) with a partial pepper ferritin cDNA labelled with ^{32}P-dCTP(Amersham 18.5 Bq/mmol) and a random priming kit from Pharmacia, at 52℃. Filters were washed twice in 6 × SSPE, 0.1% SDS at 57℃. Inserts from recombinant phage were subcloned in pUC 19 vector and their sequence were ditermined by the di-deoxychain termination method, either using a sequences 2.0 kit or a model 373A DNA Sequencer using a taq Dye primer cycle Sequencing kit(Applied Biosystems, USA).

RNA and DNA analysis

RNAs were extracted by the guanidine method with the following modification. After phenol extraction, total nucleic acids were precipitated by isopropanol. RNAs were differentially precipitated in 4M LiCl. For northern hybridizations, 20 ㎍ of total RNA were denatured and fractionated on a 1.5% formaldehyde-agarose gel. The ferritin probe was a 589 fragment which corresponds to the mature subunit coding sequence from Fp1. Gels were blotted onto nylon menbrane filter(Hybond-N plus, Amersham). For Southern hybridization, 20㎍ of total DNA extracted from seedlings of ChungYang was digested with various restriction enzymes(*XbaI*, *EcoRI*, *EcoRV*, *PstI*, *BamHI*, *HindIII* and *XhoI*), fractionated on a 0.8% agarose gel, and blotted onto a nylon membrane. After crossing with UV light, prehybridization was performed for 4hr at 50 ℃ in 50% formamide, 5 × SSC, 50mM Tris-HCl pH 7.5, 0.1% sodium pyrophosphate, 1% SDS, 0.2% PVP, 0.2% Ficoll, 5mM EDTA and 150㎍/ml denatured salmon sperm DNA. Hybridizations were achieved in the same buffer containing the probe at 50℃ for 12h. Filters were washed twice in 2 × SSC, 0.1% SDS for 15min and twice in 0.1 × SSC, 0.1% SDS for 15min at 68℃ prior to exposure at -70℃ using Kodak films.

RESULTS AND DISCUSSION

Structure analysis of red pepper ferritin gene

Ferritin from plants and animals share similar primary sequence suggesting a common evolutionary precursor. Two unique features of plant ferritin structure deserve to be discussed. Firstly, a plant specific extension of ca. 30 amino acids at the NH2 terminus of ferritin has been reported to be involved with degradation of plant ferritin by formation of radicals during iron exchange. Secondly, the region of the E-helix in animal ferritin is involved in the formation of channels supposedly important for iron uptake and release.

This experiment have used a PCR approach to identify plant cDNA which encodes ferritin proteins. Root cDNA obtained from iron treatment was prepared and used as template in PCR amplifications with degenerated oligonucleotides corresponding to the highly conserved SEEEREH and HGVWHFDQ motifs, found in members of the plant ferritin family, as primers. The PCR-amplified DNA fragments obtained, ca. 350bp long, were cloned and sequenced. Computer databank searches were then carried out with the deduced amino acid sequences. Sequences exhibited more than 95% homology to known ferritin proteins. In order to isolate and characterize full-length cDNA clones from red pepper, we screened a cDNA library from iron treated roots using a PCR-clone as a heterologous probe. Five strong immunopositive signals and numerous weaker signals were identified among approximately 40000 recombinant plaques screened. The phage producing the three strongest signals was purified and analyzed further. The longest(Fp1) was shown 1076 base pairs contained common restriction fragments(Fig.1). This insert DNA was subsequently subcloned into the Bluescript plasmids, and DNA sequence analysis of insert was initiated. The deduced amino acid sequence from the 1076bp clone was found to have substantial similarity to vertebrate ferritins when compared with the PIR database using the program FASTA. Fp1 starts with ATGGC,

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1                 CCTCACAGATGGCTTTTGATTTTTCCTCACAATCTTAGCCGCCATGATCTC ATG GCT CTT   59
                                                                       M   A   L     3

  60 GCT CCA TCC AAA GTT TCC ACC TTT TCT GGT TTT TCT CCC AAA CCC AGT GTT GGG GGT GCT  119
   4 A   P   S   K   V   S   T   F   S   G   F   S   P   K   P   S   V   G   G   A     23

 120 CAG AAA AAC CCA ACT TGC TCT GTT TCT CTG AGC TTT GCG AAT GTG AAC TTG GGA AGC AGA  179
  24 Q   K   N   P   T   C   S   V   S   L   S   F   A   N   V   N   L   G   S   R     43

 180 AAC CTT AGG GTT TGT GCC TCA ACT GTG CCT CTC TCA GGG GTG ATA TTC GAA CCC TTC GAG  239
  44 N   L   R   V   C   A   S   T   V   P   L   S   G   V   I   F   E   P   F   E     63

 240 GAG GTT AAG AAG GGT GAA CTT GCT GTT CCA ACG GCT CCC CAA GTC TCG CTG GCT CGT CAG  299
  64 E   V   K   K   G   E   L   A   V   P   T   A   P   Q   V   S   L   A   R   Q     83

 300 AAC TAC GGT GAT GAG TGT GAA TCT GGC ATT AAC GAG CAG ATA AAT GTG GGA TAC AAT GGG  359
  84 N   Y   G   D   E   C   E   S   G   I   N   E   Q   I   N   V   G   Y   N   G    103

 360 TCC AAT GCG TAC TTT TGG TTG TTT GCG TAC TTT GCA AGG GGC AAC GGG GGG CTC AAG GGA  419
 104 S   N   A   Y   F   W   L   F   A   Y   F   A   R   G   N   G   G   L   K   G    123

 420 TTT TTC AGG TTC TTC AAG GAA TCT AGT GAG GAA GAA AGA GAG CAC GCT GAA AAG CTC ATG  479
 124 F   F   R   F   F   K   E   S   S   E   E   E   R   E   H   V   E   K   L   M    143

 480 AAA TAT CAG AAT ACT CGC GGT GGA AGG GTT GTC CTT CAC ACC ATC AAG AAT GCC CCC TCA  539
 144 K   Y   Q   N   T   R   G   G   R   V   V   L   H   P   N   K   N   A   P   S    163

 540 GAA TTT TCT CAT GTG GAA AAG GGG GAT GCA TTG TAT GCA ATG GAA TTA GCC TTG TCT TTG  599
 164 E   F   S   H   V   E   K   G   D   A   L   Y   A   M   E   L   A   L   S   L    183

 600 GAG AAA TTA GTG AAT GAG AAA CTT CTG AAT GTG CAC AGT GTG GCA GAT CGC AAC AAT GAC  659
 184 E   K   L   V   N   E   K   L   L   N   V   H   S   V   A   D   R   N   N   D    203

 660 CCT CAA TTG GCA GAC TTC ATT GAA AGC GAG TTT TTG TCT GAA CAG GTT GAA TCA ATT AAG  719
 204 P   Q   C   A   D   F   I   G   S   E   F   L   S   E   Q   V   E   S   I   K    223

 720 AAA ATT TCA GAG TAT GTG GCT CAG TTG AGA AGG GTT GGA AAG GGT CAC GGT GTT TTG CAC  779
 224 K   I   S   E   Y   V   A   Q   L   R   R   V   G   K   G   H   G   V   L   H    243

 780 TTT GAT CCA AGG CTT CTT GAT TAG GAAGATGCTGCATAATCTTGAATAGCCCTTTGAACAGCCTCTGCTTC  851
 242 F   D   P   R   L   L   D   *                                                    250

 952 CTAAATATGGCCTATGTGAAGTTATGTGTTGTCCTCTTGTAGGAAGTAGTGAATAAGTGTTTCTCTCCTAGGTGATAAA  930
 931 AATGTAGGAACTTTGTGTTGTATTATAGTTATTGTTGGTAGAATAGGTAAGTAGTAGTAGTATTATTATGTGCAATCCC 1009
1009 GTATGTTTGTAGCATGCAATAATTTTGTTAGAAGTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA           1076
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Fig. 1. Nucleotide sequence and drived amino acids of red pepper cDNA(Fp1). The region of the amino acid sequence unique to plants is underlined(transit peptide). A presumptive polyadenylation signal is underlined in the 3' -UTR.

the canonical eukaryotic translation initiation sequence, and contain a 5' untranslated region(UTR) of 51bp containing stop codons. An open reading frame of 250 amino acids was observed, followed by a 3' -UTR of 272bp, within which a consensus polyadenylation site was clearly defined(Fig.1). With the open reading frame, a stretch of 24 amino acids has a sequence identical to the N-terminus of the subunit of the soybean ferritin protein(overlined in Fig.1). Also, the precursor polypeptide of red pepper ferritin has a molecular mass of 28kDa and the mature ferritin subunit a value of 23.4kDa, as calculated from the amino acid sequence shown in Fig. 1. This sequence

(A)

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                          10                  20                  30                  40                  50
Fb -S   M A L A P S K V S P F S G F S L S D G V G A C - - R N P T C S V S L S F L N K K C G S R N L G C S A
Po IC   M A L S S S K F S S F S G F S L S P V S G N G V Q K P C F C D L R V G - - - E K W G S R K F R V S A
Pea S   M A L A P S K V S T F S G F S P K P S V S G A - Q K N P T C S V S L S F L N E K L G S R N S R V C A
PFe-1   M A L A P S K V S T F S G F S P K P S V G G A - Q K N P T C S V S L S F A N V N L G S R N L R V C A
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(B)

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       <---- Cleaved upon germination in plants ----->
        A B C D E F G H I J K L M N O P Q R S T U V W X                   10                  20                  30
HuL-H                                                   T T A S T S Q V R Q N Y H Q D S E A A I N R Q I N L E L Y A
HoS-L                                                           S S Q I R Q N Y S T E V E A A V N R L V N L Y L R A
Fb -S   S T V P L T G V I F E P F E E V K K E E L A V P T A G Q V S L A R Q Y Y A D E C E S A I N E Q I N V E Y N A
So IC   S T V P L T G V I F E P F E E V K K D E L A V P T A P Q V S L A R Q N Y A D E C E S A I N E Q I N V E Y N A
Pea S   T T A P L T G V I F E P F E E V K K D Y L A V P S V P L V S L A R Q N F A D E C E S V I N E Q I N V E Y N A
PFe-1   S T V P L S G V I F E P F E E V K K G E L A V P T A P Q V S L A R Q N Y G D E C E S G I N E Q I N V G Y N G

                        40                  50                  60                  70                  80
HuL-H   S Y V Y L S M S Y Y F D R D D V A L K N F A K Y F L H Q S H E E R E H A E X L M K L Q N Q R G G R I F L Q D
HoS-L   S Y T Y L S L G F Y F D R D D V A L E G V C H F F R E L A E E K R E G A E R L L K M Q N Q R G G R A L F Q D
Fb -S   S Y V Y H S L F A Y F D R D N V A L K G F A R F F K E S S E E E R E H A E K L M K Y Q N T R G G R V V L H P
So IC   S Y V Y H S L F A Y F D R D N V A L K G F A K F F K E S S E E E R E H A E K L M K Y Q N T R G G R V V L H P
Pea S   S Y V Y H S L F A Y F D R D N V A L K G F A K F F K E S S E E H R E H A E K L M K Y Q N T R G G R V V L H P
PFe-1   S N A Y F W L F A Y F A R G N G G L K G F F R F F K E S S E E E R E H V E K L M K Y Q N T R G G R V V L H P

                        90                  100                 110                 120                 130
              a   a                 a b
HuL-H   I K K - P - D C D D W E S G - - L N A M E C A L H L E K N V N Q S L L E L H K L A T D K N D P H L C D F I E
HoS-L   L Q K - P S Q - D E W - - G T T L D A M K A A I V L E K S L N Q A L L D L H A L G S A Q A D P H L C D F L E
Fb -S   I K N V P S E F E H V E K G D A L Y A M E L A L S L E K L V N E K L R S V H S V A D R N K D P Q L A D F I E
So IC   I K N V P S E F E H V E K G D A L Y A M E L A L S L E K L V N E K L L N V H S V A D R N N D P Q M A D F I E
Pea S   I K D V P S E F E H V E K G D A L Y A M E L A L S L E K L T N E K L L N V H S V A E R N N D L E M T H F I E
PFe-1   N K N A P S E F S H V E K G D A L Y A M E L A L S L E K L V N E K L L N V H S V A D R N N D P Q C A D F I G

                    90                  100
              a                 a b c
HuL-H   I K K - P D C D D W E S G L N - - - A M
HoS-L   L Q K - P S Q D E W G T T L D - - - A M
Fb -S   I K N V P S E F E H V E K G D A L Y A M
So IC   I K N V P S E F E H V E K G D A L Y A M
Pea S   I K D V P S E F E H V E K G D A L Y A M
PFe-1   N K N A P S E F S H V E K G D A L Y A M

                    140                 150                 160                 170                 180
HuL-H   T H Y L N E Q V K A I K E L G D H V T N L - R K M G A P E S G L A E Y L F D K H T L G D S D N E S
HoS-L   S H F L D E E V K L I K K M G D H L T N I Q R L V G S - Q A G L G E Y L F E R L T L K H D
Fb -S   S E F L S E Q V E A I K K I S E Y V A Q L - R M V G - - - K G H G V W H F D Q S L L H D G H A A
So IC   S E F L S E Q V E S I K K I S E Y V A Q L - R R V G - - - K G H G V W H F D Q R L L D
Pea S   G E Y L A E Q V E A I K K I S E Y V A Q L - R R V G - - - K G H G V W H F D Q R L L H G V H G A
PFe-1   S E F L S E Q V E S I K K I S E Y V A Q L - R R V G - - - K G H G V L H F D P R L L D
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Fig. 2. Alignment of the derived red pepper ferritin amino acid sequence with soybean(SoIC), pea(peaS), French bean(Fb-S), human river(HuL-H) and horse spleen(HoS-L) (A): Comparison of the sequence of various transit peptides from plant ferritins. (B): Comparison of primary structures of some plant and animal ferritins. Hyphens indicate gaps in the sequences to allow the best alignment. The additional amino acids in the plant sequences, owing to gaps in the animal sequences, are numbered using lower case letters.

of the mature ferritin subunit is preceded by a sequence of 91 amino acids showing the characteristics of a transit peptide for plastid targeting(Harrison et al., 1989). This is consistent with the finding that plant ferritins are synthesize as precursors(Proudhon et al., 1989; van der Mark et al., 1983) and transported to plastids(Seckbach, 1982; Lescure et al., 1991). The transit peptide of red pepper ferritin is respectively 62%, 42% and 66% identical to those of Freanch bean(48 residues), soybean(47 residues) and pea(49 residues) ferritins(Fig. 2a). Comparison of the remaining amino acid sequence of red pepper ferritin with those of French bean, pea, human liver H- and horse spleen L-ferritin is presented in Fig. 2b. Alignment between red pepper, French bean, pea and soybean ferritins show that amino acid sequences of plant ferritin are highly similar(90%-84% identity). Red pepper ferritin share 36% and 48% identity with horse spleen and human heavy-chain ferritin respectively. Four amino acids are classed as insertions in the plant sequences(position 87a and 88a and 96a and 96b)(Fig. 2b), and a gap of three amino acids was introduced at positions 160-162 to obtain the best alignment with animal ferritins.

It is of particular interest to note that 30 C-terminal amino acids of plant ferritins are highly conserved but they diverge entirely from the equivalent animal ferritin sequence.

Iron concentration in roots and leaves

Concentration of plant ferritins increases proportionally to iron loading. Therefore, the first step in this experiments to induce ferritin synthesis in red pepper plantlets in response to iron was to find conditions under which iron concentration in roots and leaves could be significantly increased. After 14days of iron starvation, followed by an addition of iron in the culture medium (500μM Fe-EDTA, 150μM trisodium citrate, 75μM $FeSO_4$), the iron concentration in roots and leaves of hydroponically grown seedlings increased up to 96h after treatment. In leaves, the cellular iron concentration had increased by 50% after 72 hours while in roots an 6-fold increase was observed after 24 hours, decreasing slightly until 96 hours(Fig.3 upper). It has been shown that in roots an important amount

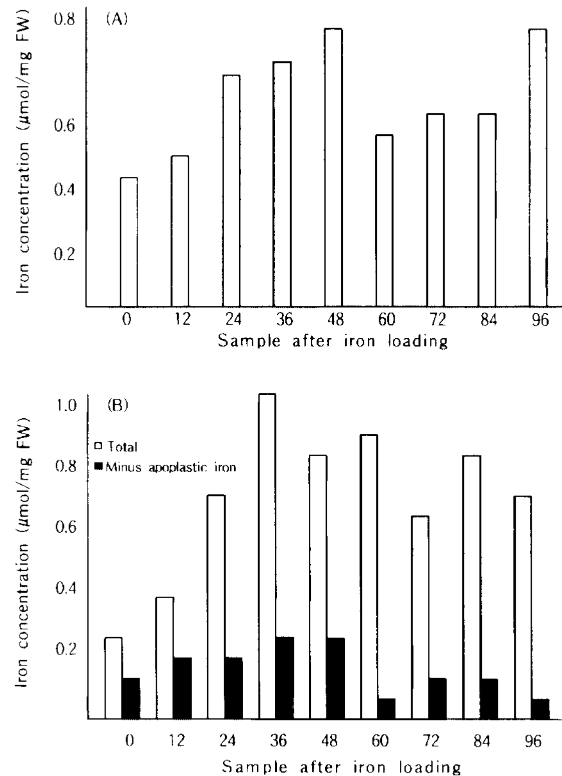

Fig. 3. Iron concentration in roots and leaves of red pepper at different times after iron loading. Results are the mean of 3 experiments in root and leaves.

of iron can be found in the apoplast and is therefore accessible to exogenous reducing agents(Longnecker et al., 1990). This property allows the removal of apoplastic iron from the roots and, therefore, to accurately determine the intercellular iron concentration in root tissue. As shown Fig. 3, after iron treatment of the plants, most of the root iron is apoplastic. Although the maxium amount found is lower in roots than in leaves, a 2-fold increase in the intracellular iron concentration was still observed after removal of apoplastic iron(Fig.3 lower).

Northern hybridization analysis

Northern hybridization were performed on leaf and root tissues to determine if the above treatment and development stage after germination was able to change ferritin mRNA content. Our observation raise the question of the fate of ferittin mRNA during germination. Fig. 4 shows

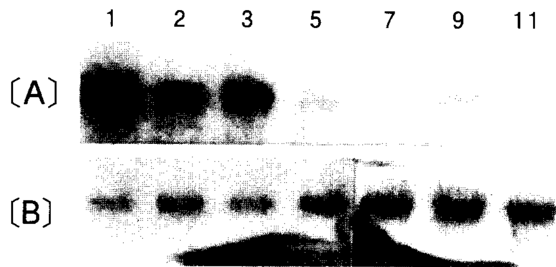

Fig. 4. Disappearance of mRNA in tissues of red pepper plantlet after germination. 40㎍ of total RNA extracts from tissues after germination. The nomenclature of samples is days after germination(A) and a 1.0kb red pepper actin CDNA(B). Hybridizations were performed in 6 × SSPE (0.1% SDS, 0.02% PVP, 0.02% Ficoll, 50㎍/ml salmon sperm DNA) with a partial pepper ferritin cDNA labelled with 32P-dCTP(Amersham 18.5Bq/mmol) and a random priming kit from Pharmacia, at 52℃.

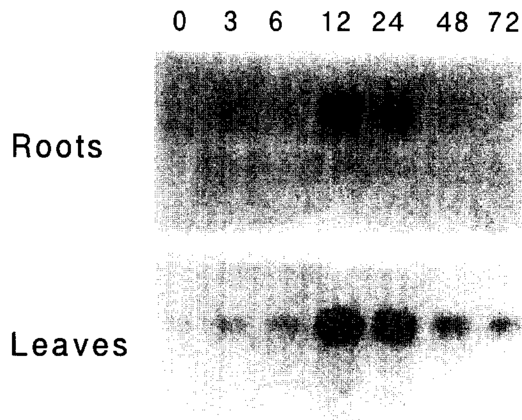

Fig. 5. Time course of mRNA accumulation in roots and leaves of red pepper plantlets in response to iron-stress. 20㎍ of total RNA extracts from roots and leaves prior to iron treatment(line 0) and 3, 6, 12, 24, 48, 72h after iron addition in the culture medium(line 3, 6, 12, 24, 48, 72). Hybridizations were performed in 6 × SSPE (0.1% SDS, 0.02% PVP, 0.02% Ficoll, 50㎍/ml salmon sperm DNA) with a partial pepper ferritin cDNA labelled with ^{32}P-dCTP(Amersham 18.5Bq/mmol) and a random priming kit from Pharmacia, at 52℃.

that ferritin mRNA was present in the same amount from 1day to 3days after germination. However, ferritin mRNA concentration decreases in leaf and root tissues during the first 5days of germination. These observations were true both for the leaf and root tissues. Ferritin degradation seems to be faster in leaves, since no ferritin was detactable in 7days old leaves although it was still detectable in a processed form in 7days old roots when 20μg of total RNAs was loaded on to the gel(not shown data). When 40μg of RNA was loaded on to the gel, traces of ferritin were visible in 11days old leaves. Interestingly, ferritin was not detectable in leaves after 11days old after iron addition(Fig. 5). Northern hybridization anlayses of total RNA extracted from leaves and roots, at different times after addition of iron, with a pepper ferritin probe internal to the coding region, shows that ferritin mRNA also expressed in response to iron. This ferritin mRNA, of about 1100nt, is particularly abundant in leaves where its transient accumlation peaks at 12h. The level of ferritin mRNA in roots is also affected, but to a lesser extent than in leaves.

The apparent discrepancy between mRNA levels and

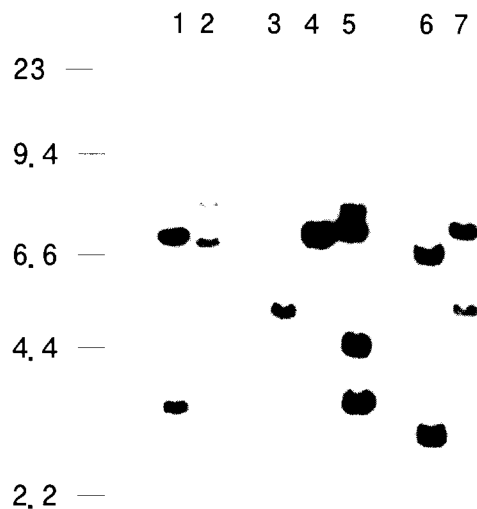

Fig. 6. Southern hybridization analysis of red pepper genomic DNA. 20㎍ of total DNA extracted from seedlings of Chungyang was digested with various restriction enzymes(*Xba*I(line 1), *EcoR*I(line 2), *EcoR*V(line 3), *Pst*I(Line 4), *BamH*I(line 5), *Hind*III(line 6) and *Xho*I(line 7), fractionated on a 0.8% agarose gel, and blotted onto a nylon membrane. fractionated on a 0.8% agarose gel, and blotted onto a nylon membrane, which was hybridization with with a partial pepper ferritin cDNA labelled with ^{32}P-dCTP(Amersham 18.5Bq/mmol) and a random priming kit from Pharmacia, at 52℃.

iron concentration can be explained that the number of iron atoms accommodated per ferritin molecule can vary, and increase, with time after iron induction(Harison et al., 1989; Theil et al., 1987).

Southern hybridization analysis

The number of pepper ferritin gene was indirectly estimated by Southern hybridization analysis. Total genomic DNA, extracted from seedling of the Bukang inbred line, was digested with various restriction enzymes, fractionated on a 0.8% agarose gel, and blotted onto a nylon membrane. which was hybridization with a red pepper ferritin probe internal to the coding region. With each restriction enzyme, one to two hybridized bands were observed(Fig. 6), indicating that ferritin related genes in red pepper might belong to single or two copy.

Indeed, in soybean cell cultures, ferritin iron accounts for only 5% of total cellular iron after iron treatment(Lescure et al., 1991). In the soybean system, ferritin mRNA accumulation was also transient and it was discussed that vacuoles could also play a role in iron detoxification in plants(Lescure et al., 1991). Therefore, Fp1 gene transcription can be controll by an integreted transduction pathway. Under normal conditions, Fp1 protein accumulates during seed formation, degrades during seed germination and was not detected in roots and leaves.

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