

苹果铁结合蛋白基因(**Apf1*)的克隆和序列分析

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铁结合蛋白是一种广泛存在于动物、植物和微生物中的铁储藏蛋白,是动、植物生长发育使用的储存铁的唯一共同来源。该蛋白在植物体中的主要功能是在种子形成、叶片衰老或环境中铁过量时积累,在种子萌发或质体绿化过程中释放铁,从而调节植物对铁的吸收和释放^[1,2]。同时,该蛋白也是解决全球动物和人类饮食缺铁有效的方法^[3]。转基因研究证明大豆铁结合蛋白基因能提高水稻种子中铁结合蛋白的含量^[4]。同时在转基因烟草植株后代中由于叶片中积累了铁结合蛋白而对病毒引起的坏死和真菌的感染表现出耐性,对因各种环境胁迫而导致的细胞氧化性损伤有保护作用^[5]。铁结合蛋白基因是植物体内唯一依赖于铁而进行表达的基因^[6]。目前国外仅报道了大豆植物铁结合蛋白基因的完整 DNA 序列以及豌豆、豇豆、苜蓿、玉米、油菜、拟南芥、马铃薯等的部分序列^[7]。小金海棠是苹果属植物中具有抗盐等各种逆境特性的优良遗传资源。本研究的目的是分离和克隆国内外尚未见报道的小金海棠铁结合蛋白基因,并进一步探索铁结合蛋白基因与苹果的抗盐等逆境特性的关系和基因的进化。

1 材料与方法

1.1 材料

取苹果属植物小金海棠 (*Malus xiaojinensis* Cheng et Jiang) 春季正在扩展的幼嫩叶用于 DNA 的提取。

1.2 方法

DNA 的提取、纯化及浓度和纯度的检验方法按文献 [8] 进行。PCR 扩增条件是:模板 DNA 2 μ L (40ng), 1 μ L (50ng/ μ L) 引物 1, 1 μ L (50ng/ μ L); 引物 2, 2.5 μ L 10 \times PCR 缓冲液, 2.0 μ L MgCl₂ (25mmol/L), 0.5 μ L dNTP (5mmol/L), 0.2 μ L Taq DNA 聚合酶 (5u/ μ L) 和 16 μ L 重蒸灭菌水, 总 25 μ L。PCR 程序为: 94 $^{\circ}$ C 5min 变性; 94 $^{\circ}$ C 1min, 52 $^{\circ}$ C 1min, 72 $^{\circ}$ C 2min, 35 循环; 72 $^{\circ}$ C 8min 延伸。PCR 产物的克隆用上海生工生物工程公司的

PUCm-T 载体,按《分子克隆实验指南》(II 版,金冬雁等译)上的方法进行。片段的测序由大连宝生物工程有限公司完成。序列的同源性比较在 <http://www.ncbi.nlm.nih.gov/blast/> 网站上进行。基因的结构分析用 Clustalx 软件进行序列对齐,用 DNASTar 软件的 Ediseq 分析开放阅读框并翻译成蛋白质。

2 结果与分析

2.1 PCR 扩增与克隆结果

利用设计的特异引物(序列见图 2)和不同方法提取的小金海棠基因组 DNA 进行了不同退火温度(37~52 $^{\circ}$ C)等不同条件下的 PCR 扩增,结果均稳定地获得一个大小为 771bp 的片段。将 PCR 产物连接到 pUCm-T 载体,转化 XL-blue 感受态细胞进行克隆并酶切检查克隆结果。PCR 产物和质粒 DNA 酶切电泳结果见图 1。

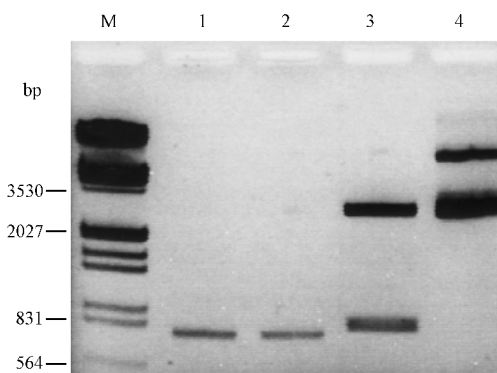


图 1 PCR 产物和重组质粒 DNA 酶切电泳结果

Fig.1 The electrophoresis result of the PCR products and digestion of recombinant plasmid DNA by *Pst* I restriction enzymes. In the figure lane M is λ DNA/*Eco*RI + *Hind*III DNA marker, Lanes 1~2 are PCR products at different annealing temperatures of 37 $^{\circ}$ C and 52 $^{\circ}$ C, Lanes 3~4 are recombinant plasmid DNA digested by *Pst* I enzyme and not digested

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** 基因库编号为 AF315505。

Soybean -----GGG-GCT-CTT-GCT-CCA-TCC-AAA-GTT-TCC-ACC-TTT-TCT
Xiaojin -----TTC-GCC-CAT-TGG-GLE-GTG-GTA-TTC-AAA-GTT-TCC-ACC-TTT-TCT
M A L A P S K V S T F S

Soybean GGT-TTT-TCT-CCC-AAA-CCC-AGT-GTT-GGG-GGT-GCT-CAG-AAA-ACC-CCA-CT-TGC-TCT-GTT-TCT
Xiaojin G F S P K P S V G G A O K N P S C S V S V S
GGT-TTT-TCT-CCC-AAA-CCC-AGT-GTT-GGG-GGT-GCT-CAG-AAA-ACC-CCA-CT-TGC-TCT-GTT-TCT
Xiaojin G F S P K P S V G G A O K N P S C S V S V S

Soybean CTG-AGC-TTT-TTG-AAT-GAG-AAA-CTT-GGA-AGC-AGA-AAC-CIT-GAG-TTT-TGT-GCC-TCA-ACG-GTG
Xiaojin C T G A G C T T T T G A A T G A G A A A C T T G G A A G C A G A A A C C I T G A G T T T T G T G C C T C A A C G G T G
P T A P Q V S L E A R Q N Y A D E C E S A

Soybean CCT-CTC-ACT-GGG-GTG-ATT-TTT-GAA-CCG-TTT-GAG-GAG-GTT-AGG-AGC-GAG-CTT-GCT-GTT
Xiaojin C C T C T C A C T G G G G T G A T T T T T G A A C C G T T T G A G G A G G T T A G G A A G A G C G A G C T T G C T G T T
P L T G T G R Q V I F E P P F E G E V K K S E L A V

Soybean CCA-ACT-GCT-CCC-CAA-GTC-TGC-TTG-GCT-CGT-CAG-AAC-TAC-GCT-GAT-GAG-TGT-GAA-TCT-CCG
Xiaojin C C A A C T G C T C C C C A A G T C T G C T T G C T C A G A A C T A C G C T G A T G A G T G T G A A T C T C C G
P T A P Q V S L E A R Q N Y A D E C E S A

Soybean ATT-AAC-GAG-CAG-ATA-AAT-GTG-GAA-TAC-AAT-GCT-TCC-TAC-GTG-TAC-CAC-TCC-TTG-TTT-GCA
Xiaojin A T T A A C G A G C A G A T A A A T G T G A A T A C A A T G C T T C C T A C G T G T A C C A C T C C T T G T T T G C A
N V A P Q V S L E A R Q N Y A D E C E S A

Soybean TAC-TTT-GAC-AGG-GAC-AAC-GTG-GCT-CTC-AAG-GGA-TTT-GCC-AGG-TTC-AAG-GAA-TCT-AGT
Xiaojin Y F D R D N G V A K G F A K F E R K E S S
TAC-TTT-GAC-AGG-GAC-AAC-GTG-GCT-CTC-AAG-GGA-TTT-GCC-AGG-TTC-AAG-GAA-TCT-AGT
Xiaojin Y F D R D N G V A K G F A K F E R K E S S

Soybean GAG-GAA-GAA-AGA-GAG-CAC-GCT-GAA-AGC-ATG-AAA-TAT-CAG-AAA-CTC-CGC-GGT-GGA-AGG
Xiaojin G A G G A A G A A G A G A G C A C G C T G A A A G C A T G A A A T A T C A G A A A C T C G C G G T G G A A G G
E F E R E H A E E K L M K Y Q N T R G G R

Soybean GTT-GT-TCT-CAC-CC-ATC-AAG-AAT-GTC-CCC-TCA-GAA-TTT-GAG-CAT-GTG-GAA-AGG-GGG-GAT
Xiaojin G T T G T T C T T C A C C C A T C A A G A A T G T C C C T C A G A A T T T G A G C A T G T G A A A G G G G G A T
E F H A M E L A R N V P S E F E H V E K G D

Soybean GCA-TTG-TAT-GCA-ATG-GAA-TTA-GCT-TTG-TCT-ITG-GAG-AGG-TTA-GTG-AAT-GAG-AAA-CIT-CTG
Xiaojin G C A T T G T A T G C A A T G G A A T T A G C T T T G T T G G A G A G G T T A G T G A A T G A G A A A C I T C T G
A L Y A M E L A L S L E K L V N E K L L

Soybean AAT-GTG-CAC-AGT-GTG-GCT-GAT-CGC-AAC-AAT-GAC-CCT-CAA-ATG-GCC-GAC-TTC-ATG-GAA-AGC
Xiaojin A A T G T G C A C A G T G T G G C T G A T C G C A A C A A T G A C C C T C A A A T G G C C G A C T T C A T G G A A A G C
N V H S V A D R N N D P Q C M A D F I E S

Soybean GAG-TTT-TTG-TCT-GAA-CAG-GTT-GAA-TTA-AGG-AAA-ATT-TCA-GAG-TAT-GTG-GCT-CAG-TTG
Xiaojin G A G T T T T T G T C T G A A C A G G T T G A A T T A A G G A A A A T T T C A G A G T A T G T G C T C A G T T G
E F L S E Q V E S I K K L S E Y V A O T

Soybean AGA-AGG-GTT-GGA-AGG-GGT-CAC-GGT-GTT-TGG-CAC-TTT-GAT-CAA-GTA-CTT-CIT-GAT-TAG-GAA
Xiaojin A G A A G G G T T G G A A G G G T C A C G G T G T T T G G C A C T T T G A T C A A G T A C T T C I T G A T T A G G A A
R R V G K G H G V W H F D R R L L D

Soybean GAT-GCT-GCT-TAA-TCT-TGA-ATA-GCC-TTA-TTA-TTA-GTC-TTC-ATT-TAC-ATT-TGG-TCT-TTT-CTG
Xiaojin G A T G C T G C T T A A T C T T G A A T A G C C T T A T T A T T A G T C T T C A T T T A C A T T T G G T C T T T T C T G
G A L C C T C R

图2 大豆铁结合蛋白基因与小金海棠铁结合蛋白基因的编码序列及氨基酸序列比较

Fig.2 The comparison of the coding sequence and their deduced amino acid sequence of ferritin gene of soybean and that of *Malus xiaojinensis*. In the figure the shaded nucleotides were the sequences of primers and the underlined letters were the mutation sites of nucleotides in the mature peptide region.

The initial and stop codes were boxed

表1 克隆片段同因特网上已报道的植物主要铁结合蛋白的氨基酸同源性比较

Table 1 The amino acid homology analysis of the cloned fragment to other plant ferritin reported in existing gene databases on internet

No.	Database No.	Gene names	Length	Identities	Positive	E-value
1	P19976	FRI soybean ferritin precursor	250	248/250(99%)	248/250(99%)	E-140
2	M72894	Glycine max ferritin L-chain	250	248/249(99%)	248/249(99%)	E-140
3	U31648	Glycine max ferritin	250	240/250(96%)	243/250(97%)	E-135
4	X58274	Phaseolus vulgaris ferritin	254	223/249(89%)	230/249(91%)	E-121
5	X73309	Pisum sativum ferritin	253	202/250(80%)	217/250(86%)	E-110
6	X97059	Medicago sativa ferritin	250	195/253(77%)	206/253(81%)	E-100
7	T08123	cowpea ferritin 3 precursor	256	193/254(75%)	208/254(80%)	E-100
8	X94248	Arabidopsis thaliana ferritin	255	168/257(65%)	199/257(77%)	E-90
9	U68217	Brassica napus ferritin	254	167/249(67%)	196/249(78%)	3E-89
10	AF133814	Solanum tuberosum	205	158/200(79%)	173/200(86%)	7E-89
11	X83076 173	Zay mays ferritin	253	95/130(73%)	110/130(84%)	4E-50

克隆片段包含了 Lescure *et al.*^[9]报道的全部编码序列,并具有完整的转录的起始和终止密码子。因此,初步推断本文报道

2.2 测序结果及小金海棠和大豆铁结合蛋白基因的结构比较

对克隆的不同单菌落的质粒 DNA 在大连生物工程有限公司通过 3 次测序结果完全一致,克隆片段大小为 771bp。用 DNASTar 软件的 Ediseq 程序分析共发现 11 个 ORFs,其中最大的 ORF 与 Lescure *et al.*^[9]报道的大豆铁结合蛋白基因编码的氨基酸序列除第 1 和第 157 位不同外,其余完全相同,二者氨基酸序列的同源性达 99%。克隆片段含完整的编码序列、转录起始和终止密码子。在基因的瞬时多肽编码区内 (TP 区,transit peptide)二者氨基酸序列完全一致,而在成熟多肽编码区内 (MP 区,nature peptide)二者间有 5 个碱基的差异,但其中 4 个是同义突变,详见图 2。

2.3 小金海棠铁结合蛋白基因与现有报道的主要植物铁结合蛋白基因的同源性比较

根据测序的结果,将所得序列同网站 <http://www.ncbi.nlm.nih.gov/blast/> 上的 nr database 进行比较,获得小金海棠铁结合蛋白基因与现有报道的主要植物铁结合蛋白基因的同源性比较,结果见表 1。由表 1 可以看出小金海棠铁结合蛋白基因与不同科的植物铁结合蛋白基因都有很高的同源性,尤其是与已报道的大豆铁结合蛋白基因的氨基酸序列的同源性高达 97%~99%。

3 讨论

Lescure *et al.*^[9]报道了大豆铁结合蛋白基因 cDNA 的部分序列(965bp),其中编码序列是 753bp。Goto *et al.*^[4]利用 Lescure *et al.*报道的序列设计特异引物,用 RT-PCR 的方法得到一个 0.84kb 的大豆铁结合蛋白基因的 cDNA,并将其与水稻种子储藏蛋白特异表达启动子连接,得到 3 倍于原种子铁结合蛋白含量的转基因水稻种子。本研究获得的 771bp 的

的是一个具有完整表达功能的苹果铁结合蛋白基因。有关小金海棠铁结合蛋白基因与大豆铁结合蛋白基因间高度的同

源性,由于缺乏苹果属近缘植物的铁结合蛋白基因的报道,对这种高度的同源性我们目前无法做进一步的分析。但该基因的获得已经为开发和利用我国特有的珍贵苹果遗传资源小金海棠以及研究它对各种逆境的适应提供了重要的条件。

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REFERENCES(参考文献)

- [1] Lahlere J P ,Briat J F. Iron release and uptake by plant ferritin :effects of pH reduction and chelation. *Biochem J* ,1993 **290**(Pt3) :693 ~ 696
- [2] Lahlere J P ,Barcelo F ,Fontecave M. Dynamic equilibria in iron uptake and release by ferritin. *Biometales* ,1996 **9**(3) :303 ~ 309
- [3] Beard J L ,Burton J W ,Theil E C. Purified ferritin and soybean meal can be sources of iron for treating iron deficiency in rats. *J Nutr* ,1996 **126**(1) :154 ~ 160
- [4] Goto F ,Yoshihara T ,Shigemoto N ,Toki S ,Takaiwa F. Iron fortifica-

tion of rice seed by the soybean ferritin gene. *Nature Biotechnology* ,1999 **17** :282 ~ 286

- [5] Deak M ,Horvath GV ,Davletova S *et al* . Plants ectopically expressing the iron-binding protein ,ferritin ,are tolerant to oxidative damage and pathogens. *Nat Biotechnol* ,1999 **17**(2) :192 ~ 196
- [6] Vansuyt G ,Lopez F ,Inze D ,Briat J F ,fourcroy P. Iron triggers a rapid induction of ascorbate peroxidase gene expression in Brassica napus. *FEBS Lett* ,1997 **410**(2 - 3) :195 ~ 200
- [7] Van Wuytswinkel O ,Briat J F. Conformational change and *in vitro* core-formation modifications induced by site-directed mutagenesis of the specific N-terminus of pea seed ferritin. *Biochem J* ,1995 **305**(Pt3) :959 ~ 965
- [8] ZHOU Z Q ,LI Y N. The RAPD evidence for the phylogenetic relationship of the closely related species of cultivated apple. *Genetic Resources and Crop Evolution* 2000 **47**(4) :353 ~ 357
- [9] Lescure A M ,Proudhon D ,Pesey H ,Ragland M ,Theil E C ,Briat J F. Ferritin gene transcription is regulated by iron in soybean cell cultures. *Proc Natl Acad Sci U S A* ,1991 **88**(18) :8222 ~ 8226

The Cloning of Apple Ferritin Gene (Apfl) from *Malus xiaojinensis* Cheng et Jiang and Its Structure Analysis

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Abstract The special primers were designed based on the sequence information of plant ferritin genes reported in the literature and used for polymerase chain reaction(PCR) with the genomic DNA of *Malus xiaojinensis* Cheng et Jiang in an attempt to clone apple ferritin gene. A single fragment of 771 bp was successfully obtained from the PCRs and cloned in this study. The sequence and homology analysis results of the fragment showed that the deduced amino acids of the fragment had a ninety-nine percent homology to that of the soybean ferritin gene reported by Lescure *et al* .(1991). A comparison in details of the nucleotide sequence of the clone and that of the soybean ferritin gene showed that they consisted of the same open reading frame(ORF ,753bp) and that the cloned fragment had complete initial and stop codes. Within the ORF no introns were found and there were no nucleotide difference in the transit peptide region(TP) of the gene ,but five nucleotide mutations were found in the mature peptide region(MP) ,among which four were synonymous mutations.

Key words ferritin gene , *Malus* Miller , *M. xiaojinensis*

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