IRON CONTENT AND FERRITIN GENE EXPRESSION IN COMMON BEAN (*Phaseolus vulgaris* L.)*

CONTENIDO DE HIERRO Y EXPRESIÓN DEL GEN FERRITINA EN FRIJOL COMÚN (*Phaseolus vulgaris* L.)

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ABSTRACT

An experimental analysis was conducted to determine iron content, ferritin gene activity and ferritin protein content, in different plant organs and growth stages of four common bean cultivars. Ferritin mRNA expression, determined by quantitative PCR, showed differential expression among cultivars and was not stable across growth stages and plant organs. Ferritin protein content was significantly higher in seeds (65.6-271.1 mg kg^{-1} fresh weight), whereas pods, roots, stems and leaves had significant lower levels (3.7-26.7 mg kg^{-1} fresh weight). Iron content in leaves was found in outstanding amounts (643 mg kg^{-1}), up to 12 times higher than in seeds (52 mg kg^{-1}); no relationship was found between ferritin transcriptional levels and ferritin protein contents with iron content. Results indicated that ferritin gene regulation is non-iron dependent in common bean.

Key words: gene expression, nutritional value, protein, quantitative PCR.

RESUMEN

Se condujo un análisis experimental para determinar el contenido de hierro, actividad del gen ferritina y contenido de la proteína codificada por el gen en diferentes órganos y etapas de crecimiento de cuatro cultivares de frijol común. La expresión de mRNA de ferritina por PCR cuantitativo mostró expresión diferencial entre cultivares y no fue estable a través de diferentes etapas de desarrollo y tipos de órganos. El contenido de la proteína ferritina fue significativamente superior en semillas (65.6-271.1 mg kg^{-1} peso fresco), mientras que en vainas, raíces, tallos y hojas los valores fueron significativamente menores (3.7-26.7 mg kg^{-1} peso fresco). El contenido de hierro en hojas tuvo niveles sobresalientes (643 mg kg^{-1}), representando niveles hasta 12 veces superiores a los encontrados en semilla (52 mg kg^{-1}). No se encontró relación entre los niveles transcripcionales de ferritina y los contenidos de proteína con el contenido de hierro. Los resultados indican que la regulación del gen ferritina en frijol común no depende del contenido de hierro.

Palabras clave: expresión de genes, PCR cuantitativo, proteína, valor nutricional.

INTRODUCTION

Ferritin is a class of iron storage and mineralization protein that plays a key role in the iron metabolism of

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most organisms (Theil, 1990; Andrews *et al*., 1992; Harrison *et al*., 1998; Chasteen and Harrison, 1999). Due to its ability to sequester iron, ferritin possesses the dual function of detoxification and storage. Previous studies have reported that ferritin contains a hollow spherical shell of 24 subunits and can accommodate 50-4000 iron atoms as an inorganic complex (Masuda *et al*., 2001). Ferritin genes have been documented from numerous animals, plants, and microbes (Theil, 1987; Harrison and Arosio, 1996; Proudhon *et al*., 1996; Andrews, 1998; Briat and Lobreaux, 1998; Carrondo, 2003; Pham *et al*., 2004).

In animals, ferritin protein is mainly cytoplasmic and is highly conserved (Theil, 1987), while in plants, ferritin is found in chloroplasts and other plastids but not in the cytoplasm (Ragland *et al*., 1990). Some authors reported a close correlation between ferritin messenger and ferritin protein content with varying iron levels (Van der Mark *et al*., 1983; Lescure *et al*., 1991). However, other authors indicated that ferritin protein and mRNA abundance did not show a relationship with iron content suggesting that iron might control plant ferritin protein accumulation at the post-transcriptional level (Fobis-Loisy *et al*., 1996).

Research focusing on the analysis of the iron demand by the plant is abundant. In legumes, iron is located mostly in ferritin (Ambe *et al*., 1987) and high levels of ferritin and iron depend on the large amounts of iron needed for nitrogen fixation by nodules (Bergersen *et al*., 1963; Ko *et al*., 1985; Kaiser *et al*., 2003).

However, from the nutritional point of view, the relationship between ferritin and iron content for breeding purposes has been less studied. Common bean, as one of the main staple foods in the world, is rich in proteins but less favored in iron content. A portion of a 70 g bean serving has 20% of nonheme Fe bioavailability yielding 2.4 mg or ~ 6 % of the 15 mg daily minimum recommended amount (DMR) (Anderson and Allen, 1994). Iron is an important microelement for living organisms and participates in hemoglobin production, a transport molecule that delivers oxygen into red cells. Iron deficiency in humans causes anemia, which affects nearly 30% of the worldwide population (Baynes and Bothwell,1990). Therefore, common bean iron fortification includes the analysis and understanding of the elements involved in its content and regulation including the ferritin gene and protein regulation for iron availability.

Our objective was to assess the relationship between ferritin and iron content in different organs of four common bean cultivars throughout its growth cycle to understand the regulation of ferritin synthesis and existing iron at different growth stages, as a preliminary analysis for nutritional breeding purpose. The utilized cultivars are representative of the commercial classes widely produced and consumed in Mexico.

MATERIALS AND METHODS

Plant material. One hundred seeds of each of four common bean cultivars: Azufrado Higuera (AH - Reg. Núm.747-FRI-001-220995) (Salinas *et al*., 1995), Flor de Junio Marcela (FJM - Reg. Núm. FRI-069-280597/C) (Castellanos *et al*., 2003), Negro Jamapa (NJ - FRI-012-000063) (SNICS, 2003) and Pinto Villa (PV - Reg. Núm. FRI-052-140890) (Acosta *et al*., 1995) were germinated in two gallon pots filled with Sunshine -3® (5 seeds/pot) and each pot was fertilized with 00-46-00 (10 g/pot) (Super Triple®) with no iron addition. Germination and growth conditions were 22-25 °C and light intensity of 170-285 mol m^2 seg^{-1}. Leaf, stem, pod, root and seed organs were collected at four phenological stages; stage I: 50% flowering I), stage II: beginning of seed filling II), stage III: filling pod III), and stage IV: physiological maturity IV). A pot (120 seeds for each cultivar were distributed in 20 pots, 5/pot) was considered as an experimental unit for each of the organ-stage samplings. Each sampling was randomly done in four pots/replicates. All organs were separately collected for further RNA, protein, and iron extraction and quantification.

Ferritin gene expression. RNA extraction was done using Trizol® (Reagent, Carisbald, CA, USA) following the manufacturer's instructions. RNA was quantified by fluorometry (TBS-380 Mini-Fluorometer) using the Ribo-Green kit (Molecular Probes, Eugene, Oregon, USA).

Primers and probe were designed from the 1 026 bp bean ferritin gene sequence (4331348, NCBI), using the software Primer Express (version 2.0, Applied Biosystems). Sense primer: 5´ AA GCA GGA ACC TTG GTG TTT CT 3´; antisense primer: 3´ CCT CCT CAA AGG GTT CAA AGA TCAC 5´ and probe: 5´ CCT CAA CTG TGC CTC TTA C 3´ labeled with fluorochrome FAM at 5´ end and a quencher at 3´end. Endogenous control ribosomal 18S (Applied Biosystem) was labeled with fluorochrome VIC.

Reactions were performed in multiplex and each reaction consisted of a total volume of 25 µL made of 1X TaqMan® Universal PCR Master Mix, AmpErase® UNG (2X), 1X MultiScribe™ and RNase Inhibitor Mix (40X), 1X Ferritin Assay Mix (20X), 1X Ribosomal Assay Mix 18S (20X) and 25 ng RNA. cDNA synthesis was performed by RT-PCR in one step (One-Step RT-PCR Master Mix, AppliedBiosystems), followed by amplification in ABI PRISM® Sequence Detection System (Applied Biosystems 7 000). The conditions for the amplification consisted of 48 °C, 30 min (Reverse Transcription), 95 °C, 10 min (denaturalization), 45 cycles (PCR amplification) at 95 °C, 15 sec and 60 °C, 1 min.

Assessment of relative expression. The relative expression ratio was calculated by normalizing the target gene Ct (ferritin) with reference housekeeping gene Ct (18S) to obtain only the efficiency of the ferritin gene (ΔCt= Ct fe-Ct18Sr). Each ΔCt was compared to a calibrator at each growth stage and plant organ within replicates (ΔΔCt = ΔCt (sample) - ΔCt (calibrator). Finally, the relative expression was shown as $2^{-\Delta\Delta Ct}$, unit of measurement of the expression of the gene (Livak and Schmittgen, 2001).

Ferritin protein content. Ferritin extraction was done following the method of Lane and Skopp (1986). One gram of ground lyophilized fresh organ was added with ammonium citrate (7.5 mL, 0.1 M pH 6.5), acetamide iodide (Sigma) (3 µg) and sodium thio carbamate (J. T. Baker) (8.5 µg), and filtered through fast filtration paper. The filtered solution was centrifuged for 15 min at 7 000 rpm (Damon-IEC Division) and the supernatant (7.5 mL) was then transferred to a 50 mL copolymer propylene tube added with 1.5 mL sodium dodecyl sulphate (SDS) (10%) and 2.5 mL sucrose (20%), and vortexed (Genie® 2 Daigger) for 1 min. The sample was centrifuged for 3 h at 13 000 rpm (Heraeus Biofuge Stratos rotor 3335) and the supernatant discarded. The pellet was dried and dissolved with 200 µL 1/2X ammonium citrate (0.1 M, pH 6.5) and centrifuged for 10 minutes at 7000 rpm. The supernatant was discarded and the pellet was resuspended in 100 µL 1/2X ammonium citrate (0.1 M, pH 6.5). For the detection of the protein use 3 mL Bradford dye (Sigma) (Bradford, 1976) was added and vortexed at 400 rpm and incubated at RT for 15 min. Ferritin was quantified by spectrophotometer (Jenway 6405) at a visible light wavelength of 595 nm based on the following formula:

µg Ferritin/µg fresh weight= (((Sample Abs/µL extract) x µL total Bradford dye)/µg FW) (Bradford, 1976).

where:

Sample Abs/µL=average absorbance (OD_{595} nm) of two replicates of organ extracts divided by the volume added to the Bradford dye
µL total extract= total volume of the Bradford dye
µg FW= fresh weight of each organ sample.

Iron content. Iron was determined in all samples of bean organs after HClO4 : HNO3 digestion (Jones and Case, 1990), with an inductively coupled plasma atomic emission spectroscopy analyzer (Perkin Elmer 3000SC, Wellesley MA, USA). Certified standards (Perkin Elmer) were run with every determination (Watson and Robert, 1990; Jones *et al*., 1991).

Statistical analysis. Statistical analysis of variance to test for significant differences among the mean scores was performed as well as Tukey's pairwise comparison to determine differences in gene expression, protein and iron content (α= 0.05) (Ott, 1993).

RESULTS

Relative expression of ferritin gene. The analysis of ferritin gene expression showed that cultivar AH (0.69) had higher ferritin gene relative expression ($2^{-\Delta\Delta Ct}$) than the other three cultivars, FJM (ΔΔCt= 0.45), PV (ΔΔCt= 0.42) and NJ (ΔΔCt= 0.38) ($p \leq 0.05$). Whereas the analysis of plant organs across cultivars showed consistent low expression in pods (ΔΔCt = 0.19-0.38) ($p \leq 0.05$), followed by leaf (ΔΔCt= 0.40-0.66), seed (ΔΔCt= 0.22-0.75) and stem (ΔΔCt= 0.34-0.78). Higher values in all plant organs always corresponded to AH (Table 1). Non expression of the ferritin gene was detected in roots because not profit an efficient extraction of RNA. It was noteworthy that the seed of AH and FJM expressed three times more than PV and NJ; and in overall NJ had the lowest ferritin gene expression in all plant organs (Table 1). No differences were observed among growth stages and plant organs in any of the evaluated cultivars and organs (Table 2). In other words, expression was stable across plant stages and plant organs.

Table 1. Relative ferritin gene expression, protein ferritin and iron content, in different plant organs of four common bean cultivars.[†]

Cultivar	Leaves		Stems		Roots		Pods		Seeds	
Azufrado	0.66[‡]±0.06	a[#]	0.78±0.06	a	---[††]	-	0.38±0.08	a	0.75±0.03	a
	9.7[§]±1.4	a	5.0±0.8	a	25.3±4.7	a	7.9±2.4	a	271.1±24.0	a
Higuera	490[¶]±31	a	39±5	a	155±18-	a	57±7	a	52±3	a
Flor de	0.40±0.10	b	0.51±0.08	b	---	-	0.29±0.13	ab	0.63±0.18	a
Junio	16.0±1.4	b	9.0±0.8	b	185±10.5	ab	5.3±2.0	a	230.9±22.1	a
Marcela	516±31	a	44±5	a	166±18 -	a	44±4	a	53±3	a
Pinto	0.48±0.09	b	0.52±0.10	b	---	-	0.19±0.04	b	0.22±0.06	b
	10.0±1.4	a	7.4±0.8	b	26.7±4.7	a	6.6±1.8	a	101.2±18.7	b
Villa	643±31	b	58±5	a	186±19	b	58±4	a	51±3	a
Negro	0.40±0.06	b	0.34±0.10	b	---	-	0.19±0.04	b	0.22±0.06	b
	13.7±1.4	b	3.7±0.8	a	14.0±4.7	b	8.6±2.3	a	65.6±24.6	b
Jamapa	439±31	c	47±5	a	156±18-	a	61±4	a	52±3	a

[†]Each value corresponds to the average of 4 replicates in 4 developmental stages; [‡]relative expression of ferritin gene $2^{-\Delta\Delta Ct}$; [§]protein ferritin content mg kg^{-1} Fresh Weight; [¶]iron content mg kg^{-1}; [#]different letters indicate significant differences within each variable at α= 0.05 by Tukey´s standardized range test; [††]not detected.

Table 2. Relative ferritin gene expression, protein ferritin and iron contents in different plant organs and growth stages average of the four common bean cultivars.[†]

Stage	Leaves		Stems		Roots		Pods		Seeds	
	0.47[‡]±0.07	a[#]	0.47±0.10	a	---[††]	-	---	-	N/A	
I	18.1[§]±1.4	a	4.8±0.8	a	20.3±6.1	a	6.2±2.0	a	N/A	
	89[¶]±31	a	44±5	a	124±19	a	68±5	a	N/A	
	0.50±0.08	a	0.60±0.10	a	---	-	0.22±0.05	a	---	
II	14.0±1.4	b	7.0±0.8	b	23.1±6.1	a	6.87±2.0	a	1 10.3±24.6	a
	516±31	b	49±5	a	154±18	ab	51±4	b	---	
	0.54±0.13	a	0.60±0.07	a	---	-	0.32±0.08	a	---	
III	9.0±1.4	c	5.5±0.8	bc	14.6±6.1	bc	8.3±1.6	a	149.0±18.2	a
	510±31	b	46±5	a	169±18	ab	46±4	b	---	
	0.53±0.11	a	0.53±0.14	a	---	-	N/A	-	0.52±0.08	
IV	8.4±1.4	c	7.7±0.88	c	26.6±4.7	c	N/A	-	242.2±16.2	b
	573±31	b	49±5	a	216±18	b	N/A	-	52±3	

[†]Each value corresponds to the average of 4 replicates in 4 cultivars (Azufrado Higuera, Flor de Junio Marcela, Pinto Villa and Negro Jamapa); [‡]relative expression of ferritin gene $2^{-\Delta\Delta Ct}$; [§]protein ferritin content mg kg^{-1} Fresh Weight; [¶]iron content mg kg^{-1}; [#]different letters indicate significant differences within each variable at α = 0.05 by Tukey´s standardized range test; [††]not detected; N/A material not available for determination.

Ferritin protein content. The analysis of protein content among cultivars, including all organs and growth stages, showed that FJM (50.9 mg kg^{-1} fresh weight (FW) and AH (47.5 mg kg^{-1} FW) had higher ferritin content ($p \leq 0.05$) as compared to PV (28.1 mg kg^{-1} FW) and NJ (21.0 mg kg^{-1} FW). As expected, seeds had up to 271.1 mg kg^{-1} FW, a high value as compared to the other plant organs (3.7-26.7 mg kg^{-1} g FW) (Table 1).

An analysis of plant organs by cultivar demonstrated that the seeds had the highest values in all cultivars ($p \leq 0.05$), and cultivars AH (271.1±24.0 mg kg^{-1} FW) and FJM (230.9±22.1 mg kg^{-1} g FW) ($p \leq 0.05$) were superior to cultivars PV (101.2±18.7 mg kg^{-1} FW) and NJ (65.6±24.6 mg kg^{-1} FW) (Table 1), similarly to ferritin gene expression. Ferritin content in pods was statistically similar through all growth stages in which this organ was present. However, seeds and leaves had important differences ($p \leq 0.05$). Seeds had the highest content starting at stage II (110.3±24.6 mg kg^{-1} FW) and doubling its content at stage IV ($p \leq 0.05$) (242.2±16.1 mg kg^{-1} FW). The other plant organs showed a modest increase in protein content across plant growth stages (Table 2).

Iron content. The analysis of iron content did not show differences among cultivars (AH (200± 26 mg kg^{-1}), FJM (214±34 mg kg^{-1}, PV (240± 33 mg kg^{-1}), NJ (177± 22 mg kg^{-1}) ($p \leq 0.443$). Regardless of cultivar and growth stage, iron content showed the highest concentration in leaves (643±31 mg kg^{-1}) and roots (186±19 mg kg^{-1}) followed by stems, pods and seeds (47-53 mg kg^{-1}) (Table 1). Iron leaf content showed differences ($p \leq 0.05$) among cultivars, PV (643±31 mg kg^{-1}) the cultivar with the highest iron content followed by FJM (516±31 mg kg^{-1}), AH (490±31 mg kg^{-1}) and NJ (439±31 mg kg^{-1}) (Table 1). Similar results were obtained when iron content was compared among plant organs and growth stages with a higher content in the leaf as the plant developed from stage I (489±31 mg kg^{-1}) to stage IV (573±31 mg kg^{-1}) (Table 2). The values of iron content in leaves were between 9 to 12-fold higher than stems, pods and seeds.

No relationship was observed between the expression of the ferritin gene and iron content. Similarly, correlation analysis (data not shown) showed an inconsistent pattern between protein content and iron in cultivars, plant organs, and growth stages.

DISCUSSION

There were significant differences among cultivars. AH had higher levels of ferritin gene expression than the other cultivars. Even though the levels of expression in leaf, stem, and seed were different among cultivars, the expression was stable across the growth cycle. Petit *et al*. (2001) reported that each of the four different ferritin genes found in *Arabidopsis* plants (*AtFer*1, *AtFer*2, *AtFer*3, *AtFer*4) showed differential expression in leaf, seed, stem and root at 17 to 42 days after germination (equivalent to stages II to IV in common bean). Moreover, these authors reported that *AtFer*2 gene expressed in seeds only whereas *AtFer*1, *AtFer*3 and *AtFer*4 expressed in vegetative organs. In our study we did not observe this since in the common bean, the ferritin gene expressed at similar levels in all plant organs and at all growth stages.

Wardrop *et al*. (1999) reported four different ferritin subunit cDNA´s in cowpea (*cp*1, *cp*2, *cp*3, *cp*5), which are present in leaf and root at different levels of expression. The *cp*5 sequence is identical to other legume ferritin sequences such as soybean (Ragland *et al*., 1990) and the common bean (Spence *et al*., 1991), whereas the other three sequences (*cp*1, *cp*2, *cp*3) are very similar among themselves but diverge from *cp*5. However, similarities in subunits *cp*1, *cp*2, *cp*3 sequences do not imply similar expression levels. In fact, mRNA´s for ferritin subunits *cp*2, *cp*3, *cp*5 were produced during leaf development in similar amounts from each gene. Subunits *cp*2 and *cp*5 were produced earlier than subunit *cp*3 in leaf expansion. However, subunit *cp*3 gene remained active in developed leaves, even one week after complete expansion when the amounts of subunits *cp*2 and *cp*5 were very low. Besides, *cp*1 expression did not vary during plant growth in leaves and roots. This is similar to what we found in the common bean, where no difference was observed in ferritin gene expression in any of the plant organs, neither among the different plant growth stages. Sequence similarities between *cp*5 and common bean ferritin did not represent a similar expression pattern. In fact, ferritin subunits may express differentially according to the species, metabolism, growth stage, or general iron homeostasis.

Contrary to the similar transcriptional levels of the ferritin gene, there were differences in ferritin protein contents in the plant organs, cultivars and growth stages probably due to the fact that in different tissues protease leads to different amounts of ferritin. These results were similar to those reported by Lobréaux and Briat (1991), who indicated an increasing content of ferritin protein in pea seed and pod as the plants developed.

Ferritin synthesis in animal cells is regulated at transductional level in the presence of iron (Theil *et al*., 1990; Masuda *et al*., 2001), whereas in plants, the control of ferritin synthesis occurs at transcriptional level as it was demonstrated in soybean (Lescure *et al*., 1991), and

corn (Savino *et al*., 1997) by inducing ferritin mRNA and protein with the addition of iron.

On the other hand, there are evidences of post-transcriptional regulation of ferritin gene in soybean nodules where mRNA level was constant during plant development, whereas protein content decreased 4 to 5-fold (Kimata and Theil, 1994). In this work mRNA levels remain constant, whereas protein content was variable along developmental stages and plant organs. Similarly to our observations, Ragland and Theil (1993) reported that developmental changes in ferritin protein concentrations were not coordinated with ferritin mRNA.

High iron content in leaves was found in all four cultivars at all growth stages, approximately at 9 to 12-fold higher than seed, pod, and stem. Differences in iron content among plant organs are due to the fact that much of the iron in leaves, for example, is associated with the ferrodoxins required for photosynthesis as part of the reverse tricarboxylic acid cycle (Fromme *et al*., 2001). This explains why approximately 80% of the iron in leaves is in the chloroplasts (Seckback, 1982; Theil and Briat, 2004) and the other 20% is in leaf vessels, so that it buffers the iron requirements in other parts of the plant (Van Der Mark *et al*., 1982; Van Der Mark and Van Den Briel, 1985).

Lobréaux and Briat (1991) reported that protein ferritin content increased in pea leaves as the plant developed, similarly to what we observed in the common bean, suggesting that protein synthesis is controlled by leaf development as a source of iron for metabolism of photosynthetic proteins (Theil and Briat, 2004). Moreover, constantly high iron contents in leaves indicates that iron reached the mesophyll cells and was stored in ferritin protein and, when released, is most likely used by the leaf cells themselves and not for export (Walter *et al*., 2003).

Drakaki *et al*. (2000) reported high iron concentrations in leaves of rice and wheat transformed with the soybean ferritin gene under the control of maize ubiquitin-1 promoter. Wheat leaves increased their iron levels from 104 mg kg^{-1} in non-transformed plants to 153 mg kg^{-1} in transformed plants and in rice leaves from 66.7 mg kg^{-1} in non-transformed plants to 132.5 mg kg^{-1} in transformed plants. In comparison, common bean leaves have outstanding concentrations of iron, reaching up to 6-fold (439-643 mg kg^{-1}) higher than rice and wheat transformed plants (Drakaki *et al*., 2000), providing evidence that common bean leaves have naturally high iron levels, representing an important alternative source of iron for nutritional purposes.

Van der Mark *et al*. (1982) reported iron concentrations of 139 mg kg^{-1} in common bean leaves from 25 day old-plantlets. High iron values were also observed in bean leaves from stage I (489 mg kg^{-1}). However, Lobréaux and Briat (1991) reported similar iron levels between pea stem (245 mg kg^{-1}) and seed (260 mg kg^{-1}). This contrast with our observations where common bean stems had 120 mg $kg^{-1}$and bean seeds barely reached 53 mg kg^{-1}. Lobréaux and Briat (1991) also reported that iron concentration in root organs were 10-fold higher than in seed and stem which, is a similar pattern as observed in the common bean, although the level of superiority was 3 to 4- fold higher than other plant organs.

There is also the possibility that the iron detected in bean organs is not only stored in the ferritin protein. Kim *et al*. (2006) reported that the *Arabidopsis* transporter gene *vit1* delivers iron to vacuoles in seed cells, expressing at low levels in root, stem, leaf, flower and floral organs. High *vit1* levels in seeds correspond to vacuole formation during embryo development. Moreover, these authors report that *vit1* over expression in yeast mutant *ccc1* increased vacuolar iron in seeds three fold over control cells and increased manganese but not zinc or cadmium. The increase in vacuolar iron content in seeds prove that *vit1* gene plays an important role in iron homeostasis and its expression is not affected by iron availability, as opposed to other proteins involved in iron metabolism.

Kim *et al*. (2006) also reported that *Arabidopsis* mutant seeds over expressing *vit1* changed iron distribution because iron was localized in provascular filaments of hypocotyl, radicule and cotyledons of wild type *Arabidopsis* seeds, whereas in mutant seeds, iron was localized in hypocotyl, radicule and cotyledonary epidermic cells; thus iron transport proteins to the vacuole such as *vit1* could help to increase iron levels in common bean seeds over expressing it together with the bean ferritin gene.

Some authors reported a close correlation between ferritin messenger and ferritin protein content with varying iron levels (Van der Mark *et al*., 1983; Lescure *et al*., 1991). However, other authors indicated that ferritin protein and mRNA abundance did not show a relationship with iron

content suggesting that iron might control plant ferritin protein accumulation at the post-transcriptional level (Ragland and Theil, 1993; Kimata and Theil, 1994; Fobis-Loisy *et al*., 1996). The same happens in this study, when ferritin gene expression, protein content and iron content analysis were compared, it is suggested a post-transcriptional regulation when ferritin protein content increased in seed while the level of expression was not different from that in the other organs. However no correlation was observed with iron content indicating an independent event between iron concentration and ferritin expression and protein content.

Finally, iron content was at similar concentrations in all cultivars tested at the whole plant level when we gather together all tested plant organs; however, leaf showed an exceptional high iron content (up to 12- fold more iron than bean seeds) showing even higher iron content than traditional plant species defined as excellent iron providers, such as spinach (46 mg kg^{-1}), lentils (61 mg kg^{-1}), chickpea (72 mg kg^{-1}) and faba bean (36 mg kg^{-1}) (FAO/WHO, 2002). Our results indicated that iron content at homeostatic level is not a regulatory component for ferritin expression suggesting that the post-transcriptional regulation is non-iron dependent in common bean.

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