



Enhanced frataxin expression in *Arabidopsis thaliana* modulates iron metabolism and improves growth under iron stress

A. Terenzi, M.A. Pagani , M.V. Busi ^{*}, D.F. Gomez-Casati ^{**}

CEFOBI – CONICET. Centro de Estudios Fotosintéticos y Bioquímicos – Consejo Nacional de Investigaciones Científicas y Técnicas. Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, Rosario, Santa Fe, Argentina

ARTICLE INFO

Keywords:

Frataxin
Iron
Fe-S clusters
Heme

ABSTRACT

Frataxin plays a crucial role in iron homeostasis, Fe-S cluster, and heme biogenesis. This study investigated the impact of constitutive AtFH overexpression in *Arabidopsis thaliana*. The results demonstrated significant alterations in Fe-S protein activity, iron distribution, and the expression of genes associated with iron transport, Fe-S cluster assembly, and iron storage. Notably, AtFH-overexpressing plants displayed enhanced growth, with increased rosette area and improved root development, under both iron-deficient and iron-excess conditions. While these findings provide valuable insights into the regulatory potential of frataxin in optimizing plant iron homeostasis, they also highlight the need for tissue-specific and stress-inducible approaches to fully evaluate its application in crop resilience.

1. Introduction

Frataxin, a protein encoded in the nucleus but localized in mitochondria (and chloroplasts in photosynthetic eukaryotes), is fundamental to maintaining iron homeostasis (Lill and Mühlenhoff, 2008; Busi and Gomez-Casati, 2012; Gomez-Casati et al., 2018). This protein is postulated to facilitate the biogenesis of iron-sulfur (Fe-S) clusters, which are essential cofactors for numerous cellular processes, including energy production, DNA repair, gene expression, ribosomal function, and many metabolic pathways such as the citric acid cycle, amino acid biosynthesis, and the detoxification of reactive oxygen species. (Lill, 2009; Braymer and Lill, 2017; Rouault, 2019). In humans, frataxin deficiency causes a neurodegenerative disorder called Friedreich's ataxia, characterized by progressive mitochondrial iron accumulation. This iron overload disrupts Fe-S cluster assembly, leading to detrimental effects like increased oxidative stress, impaired cellular function, cardiomyopathy and neurodegeneration (Puccio et al., 2001; Gakh et al., 2006; Gentry et al., 2013; Bridwell-Rabb et al., 2014; Chen et al., 2016).

Within the mitochondria, Fe-S cluster biogenesis involves the coordinated action of the ISC (Iron-Sulfur-Cluster) multi-protein complex. Key components of this complex include frataxin, ISU (scaffold protein), NFS1 (cysteine desulfurase), ISD11 (a small chaperone which regulates

NFS1), and ACP (acyl carrier protein) (Boniecki et al., 2017; Fox et al., 2019). The recent elucidation of the ISC complex crystal structure has advanced our understanding of several key aspects of Fe-S cluster synthesis (Fox et al., 2019). However, many questions remain elusive, particularly the mechanism of Fe insertion into the clusters.

The first frataxin protein in a photosynthetic organism, *Arabidopsis thaliana* (AtFH), was identified in our laboratory in 2004. Its functionality was confirmed through complementation of a yeast frataxin-null mutant, suggesting its involvement in plant mitochondrial respiration and stress responses (Busi et al., 2004). *A. thaliana* is unique among some plants, since it possesses only one gene that codes for frataxin (AtFH; At4g032040.1); while other plants like *Zea mays* possess two isoforms with distinct properties, suggesting similar but non-identical roles in plant cell metabolism (Busi et al., 2006; Turowski et al., 2015; Buchensky et al., 2017; Sanchez et al., 2018). The dual targeting of plant frataxins to both mitochondria and chloroplasts, coupled with the observation that its deficiency negatively impacts chloroplast function and Fe-S protein levels, strongly suggests a modulatory role in both the ISC (mitochondrial) and SUF (chloroplastic) systems (Turowski et al., 2015).

AtFH-deficient plants exhibited retarded growth, increased levels of reactive oxygen species (ROS), and induction of oxidative stress

This article is part of a special issue entitled: SI Chile and Argentina published in Plant Physiology and Biochemistry.

^{**} Corresponding author.

^{*} Corresponding author.

E-mail addresses: busi@cefobi-conicet.gov.ar (M.V. Busi), gomezcasati@cefobi-conicet.gov.ar (D.F. Gomez-Casati).

<https://doi.org/10.1016/j.plaphy.2026.111687>

Received 6 April 2026; Received in revised form 13 August 2026; Accepted 24 August 2026

Available online 25 August 2026

0981-9428/© 2026 The Authors. Published by Elsevier Masson SAS. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

markers. Additionally, increased transcript levels of the mitochondrial Fe-S-containing proteins, aconitase and SDH2-1, were detected, but with reduced corresponding enzymatic activities. This suggests that AtFH is crucial for Fe-S cluster assembly or their subsequent insertion into ferrosulfofoproteins (Busi et al., 2004, 2006; Maliandi et al., 2007; Busi and Gomez-Casati, 2012; Gomez-Casati et al., 2018). In addition to its roles in iron homeostasis and Fe-S cluster assembly, AtFH also contributes to heme biosynthesis. Previous studies demonstrated that frataxin serves as a potential Fe donor to ferrochelatase for the final insertion of Fe into the protoporphyrin ring during heme synthesis (Yoon and Cowan, 2004). In *A. thaliana*, frataxin deficiency leads to decreased heme levels and impaired catalase activity, a defect that can be rescued by exogenous hemin (Maliandi et al., 2011). Furthermore, we have shown that AtFH directly catalyzes the *in vitro* formation of heme when incubated with iron and protoporphyrin IX. Although plant ferrochelatases are primarily chloroplastic, our results suggest that frataxin could function as the Fe donor for the final step of heme synthesis within plant mitochondria (Armas et al., 2019a,b).

All these results strongly indicate that frataxin plays a pivotal, regulatory role in Fe-S protein assembly, heme biosynthesis and iron homeostasis. These diverse functions potentially implicate sensing the cellular redox status or iron overload, thereby mitigating the accumulation of ROS within plant mitochondria. While numerous studies have explored the function of this protein in non-photosynthetic organisms, its comprehensive role in photosynthetic organisms remains to be fully elucidated. Therefore, to further clarify its function and explore its regulatory potential in plants, we investigated the effects of frataxin overexpression in *Arabidopsis thaliana*.

2. Materials and methods

2.1. Plant material and growth conditions

Arabidopsis thaliana (var. Columbia Col-0) was used as the wild-type (wt) line. To generate AtFH constitutive overexpressing lines (*s-AtFH1* and *s-AtFH9*), the *AtFH* gene coding sequence (At4g032040.1, 564 bp) was amplified by PCR using the primers *s-AtFHfw* and *s-AtFHrv* (Table S1) and cloned into the pCambia1302 vector (Hajdukiewicz et al., 1994) under the control of the cauliflower mosaic virus 35S (CaMV 35S) promoter. The resulting plasmid (pCambia-s-AtFH) was introduced into *Agrobacterium tumefaciens* strain GV3101 via the freeze-thaw method (Weigel and Glazebrook, 2002). *Arabidopsis* transformation was performed using the floral dip method (Clough and Bent, 1998). Transgenic plants were selected on MS medium supplemented with hygromycin, and homozygous lines were established after three generations. For general growth, surface-sterilized seeds were plated on MS medium. Following stratification, plants were grown in a greenhouse at 25°C under a 16-h-light/8-h-dark photoperiod (Grolux, Sylvania, and Cool White fluorescent lamps, Philips). Stage 1.05 plants (approximately 15 days old) were used for experiments unless stated otherwise (Boyes et al., 2001). Three distinct iron regimes were applied in Hoagland agar medium using Fe(III)-EDTA (EDTA Fe(III) sodium salt; Sigma-Aldrich, MO, USA) as the iron source: (I) Iron deficiency/limitation: 10 µM Fe-EDTA; (II) Iron sufficiency (control): 50 µM Fe-EDTA; and (III) Iron excess: 350 µM Fe-EDTA.

2.2. RNA Isolation and Quantitative Real-Time PCR (qPCR)

Total RNA was isolated from rosette leaves and roots of stage 1.05 plants using Trizol reagent (Invitrogen, Carlsbad, CA, USA). cDNA synthesis was performed using random hexamers and M-MLV reverse transcriptase (Promega, Madison, WI, USA) following the manufacturer's protocol. Quantitative real-time PCR was performed in a StepOne system (Applied Biosystems, Waltham, MA, USA) with SYBR Green I (Invitrogen, Carlsbad, CA, USA) as a fluorescent reporter and GoTaq polymerase (Promega, Madison, WI, USA). Primers were designed to

amplify products between 150 and 250 bp for each target gene (Supplementary Table 1). Three biological replicates (each pooling 15–20 plants) and three technical replicates were analyzed. Specificity was confirmed via melt curve analysis. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Pfaffl, 2001).

2.3. Phenotypic characterization

Rosette area, as well as the lengths of the main root and lateral roots, were evaluated in 15-day-old seedlings as previously described (Grisolia et al., 2017). Digital images of the plants were captured and subsequently analyzed using Image J software (Schneider et al., 2012).

2.4. Protein quantification and Western blot analysis

Total protein concentration was determined using the Bradford method, measuring absorbance at 595 nm with bovine serum albumin (BSA) as the standard (Bradford, 1976). Proteins were separated by SDS-PAGE on 12% (w/v) polyacrylamide gels using a Mini Protean II Cell (Bio-Rad, Hercules, CA, USA) as described (Laemmli, 1970). Separated proteins were then transferred to nitrocellulose membranes (Amersham Biosciences, Amersham, UK) using a Bio-Rad Mini35 Trans-Blot system (Bio-Rad, Hercules, CA, USA). Electro-blotted membranes were blocked and then incubated with anti-AtFH primary antibody. Detection was achieved using an HRP-conjugated anti-rabbit secondary antibody (Agrisera, Vännäs, Sweden), and the antigen-antibody complex was visualized using the BioLumina chemiluminescence kit (Kalium Technologies, Buenos Aires, Argentina).

2.5. Enzyme activity assays

Homogenates from *Arabidopsis* tissues (leaves and roots) were prepared from 3 independent biological pools, each consisting of 15–20 plants. For each pool, approximately 200 mg of tissue was frozen in liquid nitrogen and ground to a fine powder. The powder was then homogenized in 20 mM Tris-HCl extraction buffer supplemented with 1 mM PMSF (pH 7.4). The resulting mixture was centrifuged for 20 min at 10,000 rpm and 4°C to pellet debris. The supernatant containing the soluble protein fraction was collected and desalted using Sephadex-G25 resin (GE Healthcare, Sweden) to remove low-MW contaminants that could interfere with the assays.

Aconitase (ACO) activity was measured by monitoring the formation of *cis*-aconitate in a reaction mixture containing 1 mM D-isocitrate, 100 mM Tris-HCl (pH 8.0) and plant extract (Li et al., 1999). Succinate dehydrogenase (SDH) activity was determined by monitoring the reduction of 2,6-dichlorophenolindophenol (DCPIP) at 609 nm in a reaction mixture containing 30 mM succinate, 0.5 mM DCPIP, 40 mM sodium azide, 10 mM potassium phosphate (pH 7.4), and plant extract (Schirawski and Uden, 1998). Malate dehydrogenase (MDH) activity was assayed by monitoring the NADH decrease at 340 nm in a medium containing 50 mM imidazole-HCl (pH 6.9), 0.15 mM NADH, 1 mM oxaloacetate and plant extract (Tripodi and Podestá, 2003). All enzyme activities were assayed in a final volume of 200 µl using an Epoch2 microplate spectrophotometer (Agilent, Santa Clara, CA, USA).

Ferric-chelate reductase activity was determined *in vivo* using whole, intact root systems. Pooled roots from each group of 15–20 15-day-old seedlings were submerged in a solution containing 200 µM CaSO₄, 100 µM Fe(III)Na-EDTA, 1 mM phenanthroline and 5 mM MES (pH 5.5). After a 2 h incubation period, the medium's absorbance was measured spectrophotometrically at 510 nm. The concentration of Fe(II)-phenanthroline was calculated using a standard curve of Fe(NH₄)(SO₄)₂ (Yuan et al., 2008). All assays were performed using these three biological replicates, analyzed at least in triplicate (technical replicates), and results are presented as mean values ± SD.

2.6. Perl's staining and iron quantification

Perl's staining and total iron quantification were conducted on stage 1.05 plants grown under iron-deficient (10 μM Fe), iron-sufficient (50 μM Fe) or iron-excess (350 μM Fe) conditions. Perl's staining was performed on roots as previously described (Leaden et al., 2016). Briefly, roots were washed with distilled water, vacuum-infiltrated for 5 min with Perl's solution (4% (v/v) HCl, 4% (w/v) potassium-ferrocyanide, 1:1 (v/v)), and subsequently incubated for 30 min at room temperature. Stained roots were washed with distilled water and visualized using an SMZ-2T microscope with a C-0.45x TV lens (Nikon, Japan). This technique allows the detection of the most abundant non-heme Fe(III) species, which represent the main storage forms (vacuole and plastid ferritins) and xylem transport complexes (citrate-Fe(III)) of iron. Perl's staining does not detect heme Fe, which accounts for 0.5-1% of total iron in plants, or non-heme Fe (II), which is found in symplastic and phloem transport as chelates.

For total iron quantification, roots and shoots were separated and dried at 60°C until a constant weight was achieved. Dried tissues were completely digested at 100°C with 65% (v/v) HNO_3 until complete mineralization of the samples was achieved. The resulting digests were diluted to a final volume of 5 ml with milliQ water, supplemented with 10 ppb of ^{45}Sc from a PerkinElmer's Internal Standard Mix solution, and filtered through a 0.22 μm membrane. Iron content in these extracts was determined via Inductively Coupled Plasma Mass Spectrometry (ICP-

MS) using a PerkinElmer 350X instrument in standard reaction mode. The less abundant ^{57}Fe isotope was chosen to minimize spectral interferences of $^{40}\text{Ar}^{16}\text{O}^+$ polyatomic species, which interfere with ^{56}Fe detection. Quantification was based on interpolation against a calibration curve prepared from serial dilutions of a commercial monoelement iron standard (PerkinElmer, Waltham, MA, USA).

2.7. Statistical analyses

All data were obtained from a minimum of three independent biological replicates, each consisting of a pool of at least 15-20 plants, and analyzed in triplicate as technical replicates. Statistical significance among the different plant lines was assessed using one-way ANOVA. Statistically significant differences ($P < 0.05$) are denoted by asterisks.

3. Results and discussion

3.1. Frataxin overexpression enhances vegetative and reproductive development in *Arabidopsis thaliana*

Previous research in our lab has established a critical role for frataxin in plant mitochondrial iron metabolism, Fe-S cluster biogenesis (evidenced by decreased aconitase and SDH activity in deficient lines), and the mitigation of oxidative damage. Moreover, AtFH was found to be involved in heme biosynthesis, with AtFH-deficient lines exhibiting

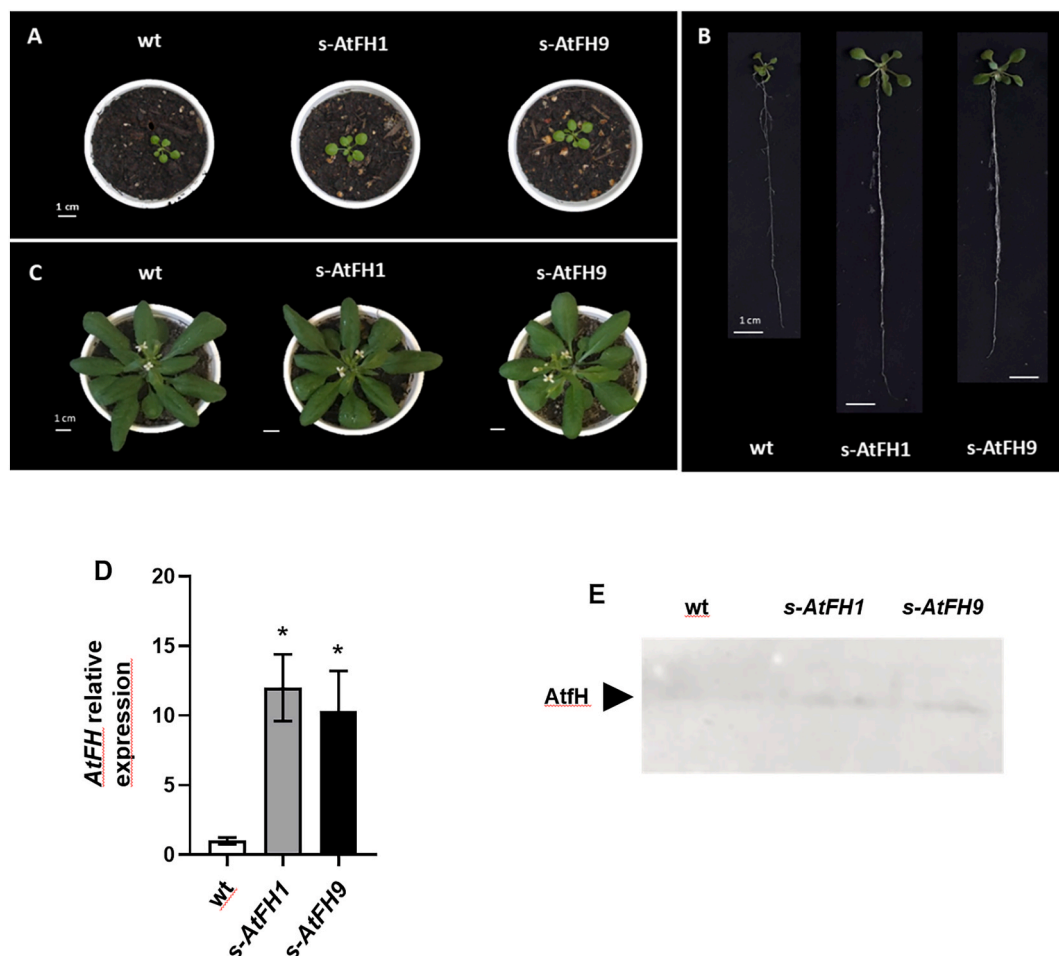


Fig. 1. Phenotypic characterization of overexpressing *AtFH* lines, *s-AtFH1* and *s-AtFH9*. A) Stage 1.05 plants grown on soil. B) Stage 1.05 plants grown on MS agar plates. C) Stage 5.10 plants grown on soil. White bars represent 1 cm. D) qPCR analysis of *AtFH* expression in leaves from wt, *s-AtFH1* and *s-AtFH9* lines. Asterisks indicate statistically different results from the control value ($P < 0.05$). Bars represent mean values (error $\pm$ standard deviation) of three independent experiments. Relative *AtFH* expression levels are shown as fold change values with respect to β -ACTIN (At1g01750) mRNA levels. E) Western-blot detection of AtFH protein in wt, *s-AtFH1* and *s-AtFH9* lines in leaves using serum anti-recombinant AtFH, HRP-conjugated secondary antibodies and developed using a chemiluminescent method.

altered heme-related transcripts, reduced heme content, and impaired catalase activity. Additionally, AtFH was found to be dually localized to both mitochondria and chloroplasts, influencing Fe-S cluster metabolism in both organelles (Busi et al., 2004, 2006; Maliandi et al., 2007, 2011; Busi and Gomez-Casati, 2012; Gomez-Casati et al., 2018; Armas et al., 2019a; Armas et al., 2019b; 2020).

Given AtFH's known involvement in coordinating respiratory and photosynthetic Fe processes, we hypothesized that its overexpression would enhance growth and development by optimizing iron homeostasis and Fe-cofactor synthesis leading to an increase in biomass accumulation. To investigate this, we generated transgenic *Arabidopsis thaliana* plants overexpressing AtFH under the control of the cauliflower mosaic virus 35S promoter (Fig. 1A–C). We selected two independent homozygous T3 overexpressing lines, *s-AtFH1* and *s-AtFH9*. Quantitative real-time PCR analysis confirmed successful transcriptional activation, showing AtFH mRNA levels increased 12-fold in *s-AtFH1* and 10-fold in *s-AtFH9* (Fig. 1D). Furthermore, AtFH protein levels, determined by Western blot analysis using specific antibodies, showed an approximately 3-fold increase in AtFH levels in both lines (Fig. 1E). The observed increase in both AtFH mRNA and protein levels confirms the successful generation of lines with stable AtFH overexpression, thereby validating their suitability for subsequent functional studies.

AtFH overexpression resulted in significant enhancement of early plant growth and development. In 15-day-old seedlings, both *s-AtFH1* and *s-AtFH9* lines exhibited enhanced vegetative biomass, reflected by a 30–40% increase in the fresh weight and dry weight (Fig. 2A). This advantage was evident in several organs: foliar area increased by 54% and 44% in *s-AtFH1* and *s-AtFH9*, respectively (Fig. 2B), and root architecture was significantly improved with primary root length increasing 1.6–1.9 fold and lateral root length increasing 57–78%

(Fig. 2C). While the foliar area difference was not maintained in adult plants (26-day-old plants, stage 5.10, not shown) (Boyes et al., 2001), the growth advantage translated into a higher reproductive capacity: both basal and distal siliques were larger (~30–47% increase) in the overexpressing lines (Fig. 2D), correlating with a ~20–30% increase in seed number per silique (Fig. 2E).

The transient increase in rosette area observed only in young AtFH-overexpressing lines (15-day-old seedlings, stage 1.05) reflects a classic ontogenetic shift in resource allocation (See also Supplementary Fig. 1). During early stages, iron uptake and enhanced total activities of Fe-S enzymes (such as ACO and SDH) optimize metabolic efficiency, accelerating exponential growth. However, by stage 5.10, limiting factors shift from metabolic capacity to physical constraints, including canopy self-shading and increased respiratory maintenance in non-photosynthetic tissues. While direct measurements of metabolic load, energy expenditure, or carbon partitioning were not performed in this study, this temporary vegetative advantage suggests a potential reallocation of early-accumulated biomass and carbon skeletons toward reproductive structures, culminating in increased silique length and seed number. However, we cannot rule out an underlying metabolic cost associated with constitutive overexpression under specific stresses or long-term growth conditions.

This transient vegetative benefit aligns with other iron-homeostasis mutants. For instance, overexpressing ferritin enhances biomass and accelerates development specifically during early stages without causing mature vegetative gigantism (Goto et al., 2000; Zhai et al., 2016). Similarly, upregulating iron uptake (e.g., IRT1 or FIT pathways) primarily promotes seedling establishment under fluctuating nutrient availability (Connolly et al., 2002; Vert et al., 2002). Once early metabolic constraints are optimized, adult plants reach an allometric plateau

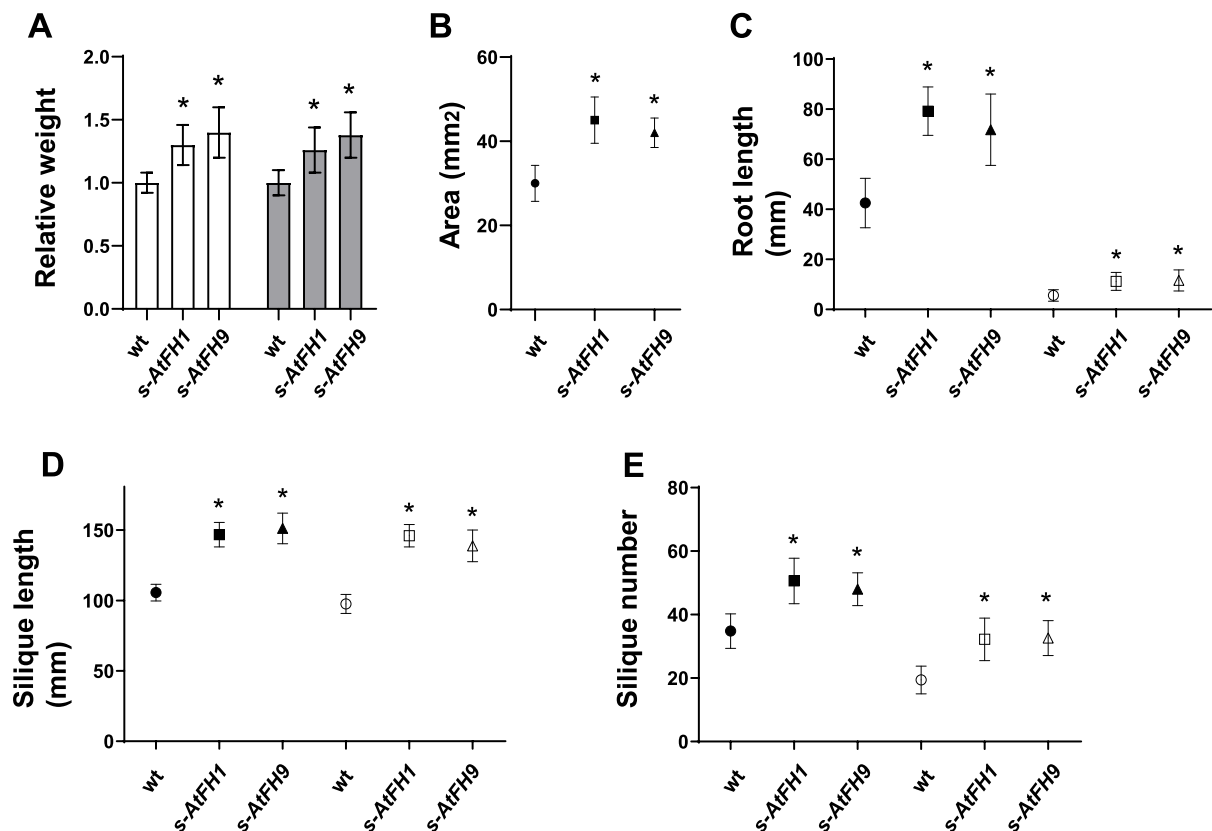


Fig. 2. Phenotypic characterization of AtFH overexpressing lines, *s-AtFH1* and *s-AtFH9*, grown on MS agar plates. A) Fresh weight (white bars) and dry weight (grey bars); B) Rosette area measurements; C) Primary root length (black symbols) and lateral root length (white symbols); D) Silique length (basal, black symbols and distal, white symbols); E) Silique number (basal, black symbols and distal, white symbols). Each analysis was performed on stage 1.05 plants grown on MS agar plates. Statistically significant differences are indicated by asterisks ($P < 0.05$).

that overrides individual metabolic advantages, explaining why rosette area differences disappear by stage 5.10 while securing greater final reproductive yield.

Interestingly, these phenotypic gains, which occur without affecting pigment content, silique morphology, or seed viability, strongly suggest that AtFH plays a key role in regulating early plant development. We propose that AtFH enhances organelle function and energy metabolism, which improves the metabolic capacity of the plant. AtFH would ensure optimal supply of Fe-cofactors, thereby accelerating energy-intensive processes like respiration and photosynthesis, resulting in higher biomass production and reproductive viability.

3.2. AtFH overexpression enhances Fe-S cluster biogenesis and enzyme activity

We hypothesized that overexpression of AtFH affects iron-sulfur cluster (ISC) biogenesis by modulating the expression of ISC pathway genes and mitochondrial Fe-S enzyme activities. We first analyzed transcripts of key ISC components in AtFH-overexpressing *Arabidopsis* lines (Fig. 3). Notably, *NFS1* (At5g65720), coding for the cysteine desulfurase which catalyzes the initial step of sulfur mobilization for Fe-S cluster assembly, was significantly upregulated in both, shoots (1.6-fold) and roots (4.1 to 5.6-fold). Similarly, *ISD11* (At5g61220) which codes for the chaperone that stabilizes NFS1, showed increased expression (ranging from −1.6 to 2.2-fold), while the transcript levels of *ISU1* (At4g22220), which codes for the scaffold protein, remained constant. We also evaluated the expression of chaperones involved in later stages of Fe-S cluster biogenesis: the heat shock protein *HSCA1* (At4g37910), which transfers mature Fe-S clusters to apoproteins, and the co-chaperone *HSCB* (At5g06410). Both chaperones showed elevated expression (approximately 2-fold) specifically in roots, with no significant changes observed in shoots for *HSCB*, whereas *HSCA1* showed a slight increase of approximately 10% (Fig. 3).

Consistent with our previous data demonstrating physical interaction and cooperative activity between AtFH, NFS1 and ISD11 proteins (Armas et al., 2020), AtFH overexpression influenced the expression of several key components of the ISC pathway. The observed induction of *NFS1*, *ISD11*, *HSCA1* and *HSCB* transcripts supports a model of coordinated regulation. This was further corroborated by prior findings in our laboratory, where *NFS1* overexpression in *A. thaliana* similarly increased AtFH and ISD11 transcript levels (Armas et al., 2019a; Armas et al., 2019b; 2020). This coordination ensures the efficient assembly of the multiprotein complex required for optimal NFS1 cysteine desulfurase

activity (Armas et al., 2019a; Armas et al., 2019b; 2020; Fox et al., 2019). This type of regulation may act as a compensatory mechanism to balance Fe-S cluster production, particularly under the altered iron homeostasis conditions caused by elevated frataxin. Our findings align with recent studies in humans, which show that these proteins interact to form a heterodecameric complex composed of five different components: frataxin, NFS1, ISD11, the scaffold protein ISCU and the acyl-carrier protein ACP (Fox et al., 2019).

To investigate the impact of AtFH levels on the activity of Fe-S cluster-containing enzymes, we measured the activities of ACO and SDH in total homogenates in wt and AtFH-overexpressing lines. We also measured the activity of MDH, a Krebs cycle enzyme that does not contain Fe-S groups.

Results showed that ACO activity in rosette leaves was approximately 1.5-fold higher in both AtFH-overexpressing lines compared to the wt line. In roots, ACO activity showed even more significant increases, ranging from 2.6-fold in *s-AtFH9* to 5-fold in *s-AtFH1*, compared to the wt line (Fig. 4A). Similarly, SDH activity was significantly increased in leaves (2.5- to 4.5-fold) and roots (approximately 3.5-fold) across both overexpressing lines (Fig. 4B). By contrast, MDH activity remained unchanged (Fig. 4C). Notably, while SDH is an exclusive marker of the inner mitochondrial membrane, our activity assays were performed on whole-tissue soluble fractions. Although sub-organellar fractionations were not conducted, the coordinated upregulation of core mitochondrial iron-sulfur cluster (ISC) components—such as NFS1, ISD11, HSCA1, and HSCB—alongside the overall increase in total Fe-S enzyme activities strongly points to a systemic impact of AtFH on Fe-S cluster maturation efficiency.

Consequently, these findings support the notion that AtFH overexpression primarily modulates Fe-S biogenesis, without necessarily altering general mitochondrial function or overall enzyme synthesis. The increased ACO and SDH activity suggests that AtFH overexpression functions in two possible ways: (i) it boosts the supply and loading of nascent Fe-S clusters onto their respective apo-enzymes, thereby converting an inactive pool of apo-proteins into active holo-enzymes; or (ii) it plays a key role in maintaining the redox state of existing clusters, which is vital for enzyme function. The latter possibility is consistent with previous findings in yeast demonstrating that ACO activity decreased when the [4Fe-4S] cluster is oxidized (Bulteau et al., 2004). Therefore, AtFH likely ensures a more stable and higher proportion of functional and active pool of Fe-S clusters, increasing total ACO and SDH enzyme activities.

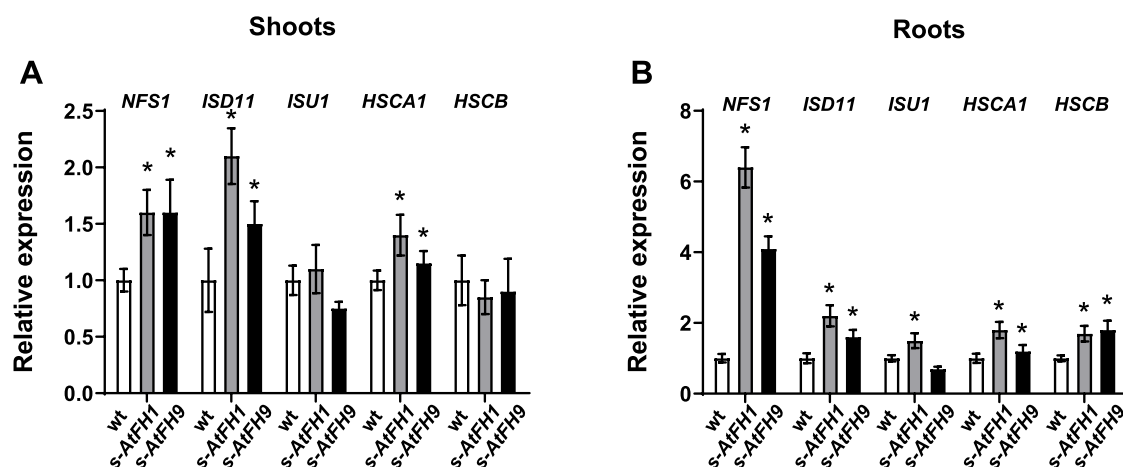


Fig. 3. Quantitative real-time PCR analysis of ISC pathway genes: cysteine desulfurase 1 (*NFS1*, At5g65720), *ISD11* (At5g61220), iron-sulfur cluster assembly protein 1 (*ISU1*, At4g22220), heat shock protein HSP70 (*HSCA1*, At4g37910, a chaperone in the transfer of the mature Fe-S cluster into apoproteins) and heat shock cognate protein B (*HSCB*, At5g06410, co-chaperone). RNA was extracted from A) shoots and B) roots of wt *s-AtFH1* and *s-AtFH9* plants. β -ACTIN (At1g01750) was used as a control gene. All experiments were performed using at least three independent biological replicates (pooling 15–20 plants). Statistically significant differences are indicated by asterisks ($P < 0.05$).

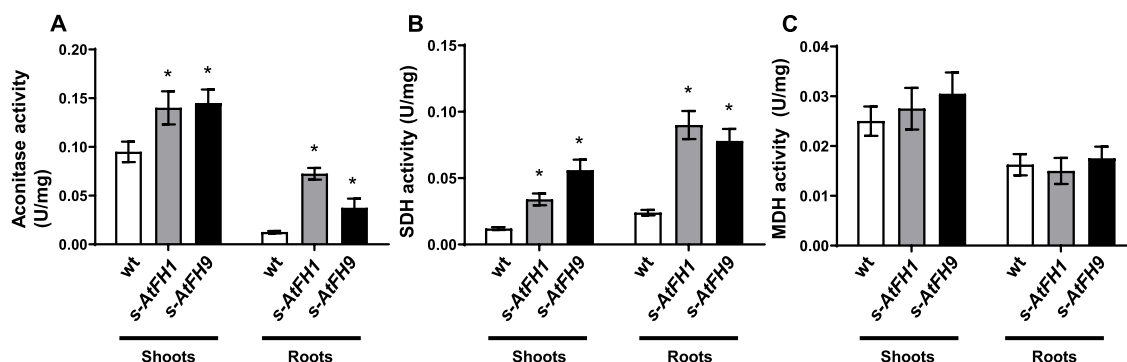


Fig. 4. Activity of Fe-S cluster enzymes under standard control conditions. Plants were grown on MS agar medium for 15 days and A) ACO B) SDH and C) MDH activities were measured in an extract made from pooled rosette leaves and roots from wt, *s-AtFH1* and *s-AtFH9* lines. All experiments were performed using at least three independent biological replicates (pooling 15-20 plants). MDH, an enzyme lacking Fe-S clusters, was used a negative control. Statistically significant differences are indicated by asterisks ($P < 0.05$).

3.3. *AtFH* overexpression enhances growth and development across varying iron conditions

Given that *AtFH*-overexpressing lines exhibited elevated expression of Fe-S cluster biosynthesis genes and enhanced activity of Fe-S cluster

enzymes, we hypothesized that *AtFH* overexpression increases the plant's capacity to tolerate varying external iron concentrations by promoting growth and robust root development. To test this, we compared the phenotypic responses of both *s-AtFH* and wt lines when cultivated under iron-sufficient, deficient, and excess conditions until

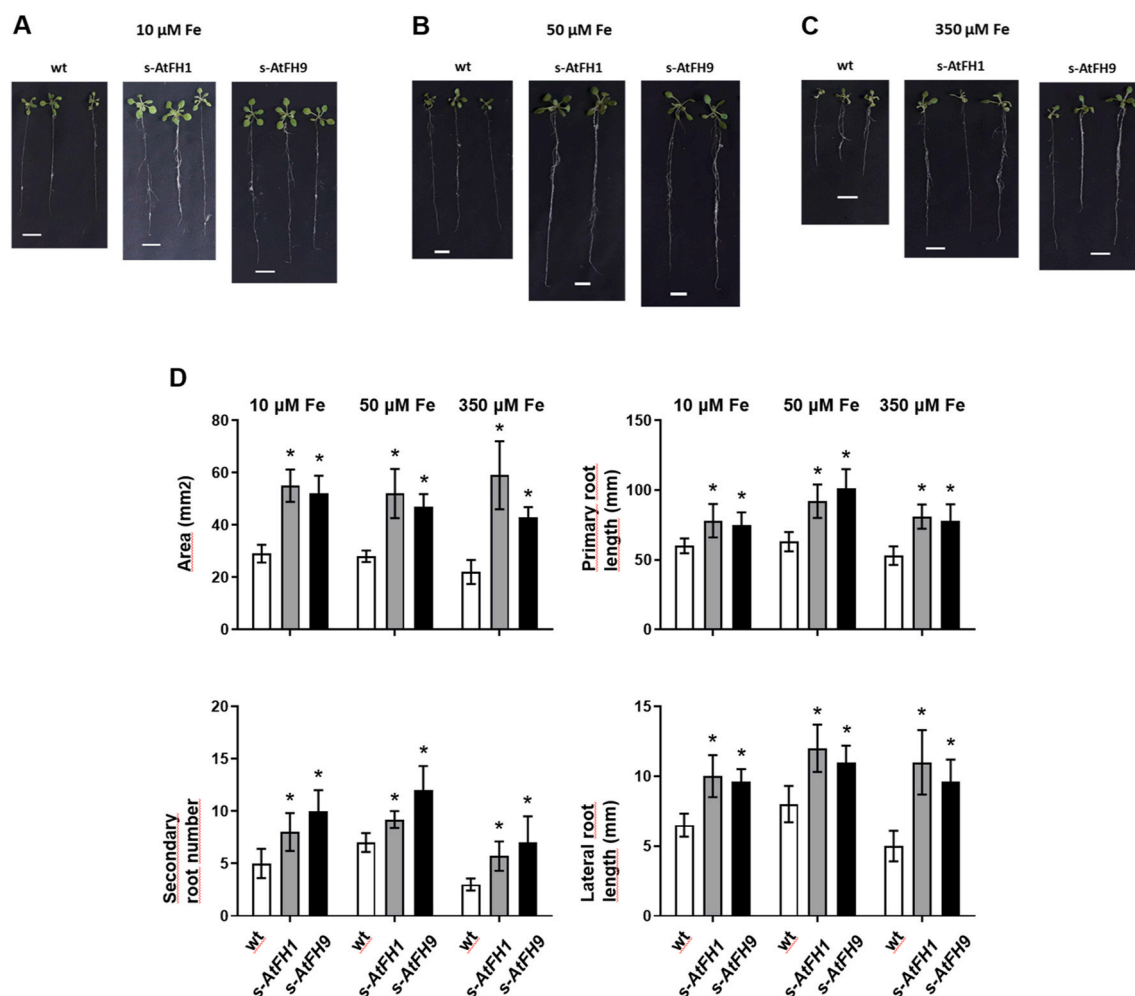


Fig. 5. Phenotypic characterization of *s-AtFH* and wt lines grown on A) iron deficiency conditions (Hoagland medium with 10 µM Fe) B) iron sufficiency conditions (Hoagland medium with 50 µM Fe) C) iron excess conditions (Hoagland medium with 350 µM Fe). Plants were grown up to stage 1.05 on Hoagland agar plates. White bars represent 1 cm. D) Rosette area measurements, primary and lateral root length and lateral root number quantification were performed on each condition. All experiments were performed using at least three independent biological replicates (pooling 15-20 plants). Statistically significant differences are indicated by asterisks ($P < 0.05$).

developmental stage 1.05.

Under iron-sufficient conditions (50 μM Fe), both *s-AtFH* lines exhibited a 1.6 to 2-fold increase in rosette area, and a 40–50% increase in primary root length. Furthermore, these lines showed approximately 40% longer secondary roots, with *s-AtFH1* specifically producing 20–30% more secondary roots than the wt (Fig. 5B and D).

Under iron-deficient conditions, the rosette area of *s-AtFH* lines was 1.8 to 1.9-fold larger than that of the wt. These lines also exhibited approximately 30% and 40% increases in primary and secondary root length, respectively. Moreover, both lines showed a significant increase in secondary root number, ranging from 50% to 90% (Fig. 5A and D).

Remarkably, the *s-AtFH* lines demonstrated striking resilience to iron-excess conditions. Both lines exhibited a substantial 2- to 2.7-fold increase in rosette area. This enhanced growth was accompanied by significant improvements in root architecture: primary root length increased by approximately 45%, secondary root length increased 1.9 to 2.2-fold, and the number of secondary roots increased by 90% to 110% compared to the wt (Fig. 5C and D).

Taken together, these data clearly show that AtFH overexpression confers a robust growth advantage, increasing shoot growth and particularly by enhancing root system development, across sufficient, deficient and excess iron conditions. This phenotype might be a secondary effect of their massive root architecture or changes in internal

iron utilization.

3.4. *AtFH* overexpression alters iron partitioning and enhances iron accumulation

Given the enhanced growth and activation of Fe-S cluster enzymes in both *s-AtFH*, we investigated whether these advantages are linked to altered iron accumulation and partitioning within the plant. To test this, we analyzed iron content in roots and shoots under varying iron conditions using two methods. Histochemical analysis using Perl's staining showed a significantly higher accumulation of ferric iron in *s-AtFH* roots compared to the wt, specifically under iron deficiency, suggesting enhanced storage and transport through xylem vessels. By contrast, no significant differences were observed under iron-sufficient (50 μM Fe) or iron-excess (350 μM Fe) conditions (Fig. 6A).

Quantification of total iron by ICP-MS corroborated these findings and provided further resolution across the different organs and treatments. Under iron deficiency, *s-AtFH* roots accumulated approximately 2-fold more iron than wt, while under iron sufficiency and excess, root iron content was similar in all lines (Fig. 6B). Notably, ICP-MS analysis of shoots revealed elevated iron content in *s-AtFH* plants under both sufficient and excess conditions (Fig. 6C), indicating a distinct partitioning pattern between both tissues.

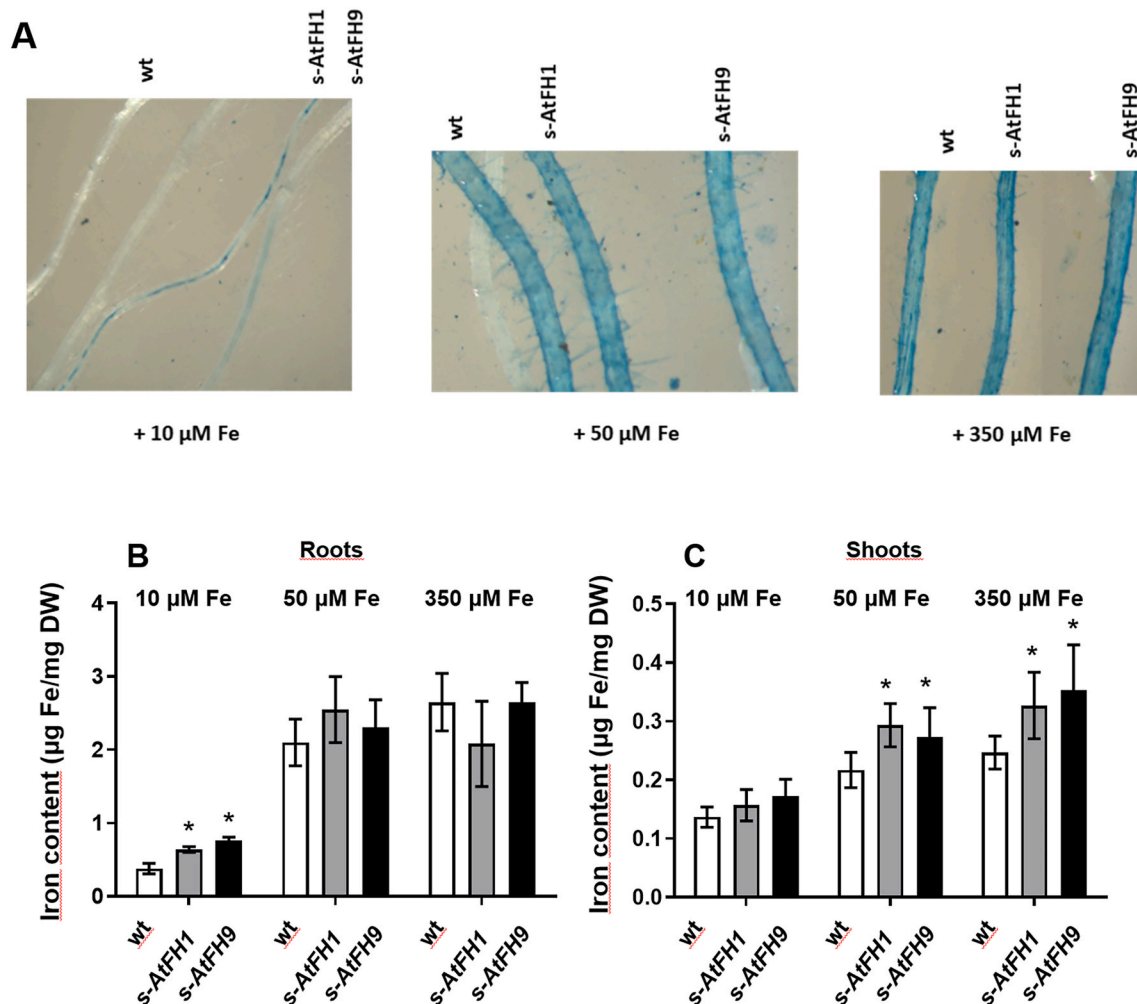


Fig. 6. Iron content determination. Perl's staining and iron quantification were performed on stage 1.05 plants grown under iron deficient (10 μM Fe), sufficient (50 μM Fe) and excess conditions (350 μM Fe). A) Histochemical Fe detection in roots by direct Perl's staining in 15-day-old wt and *s-AtFH* lines. B) ^{57}Fe quantification in roots and C) shoots. Dried tissues of wt and AtFH overexpressing lines were completely digested with nitric acid and ^{57}Fe content was measured with ICP-MS. All ICP-MS experiments were performed using at least three independent biological replicates (pooling 15–20 plants). Statistically significant differences are indicated by asterisks ($P < 0.05$).

Collectively, these data demonstrate a substantial alteration in iron partitioning mediated by frataxin overexpression. The enhanced iron accumulation in *s-AtFH* roots under deficiency likely contributes to the previously observed improved growth phenotypes—such as larger rosettes and enhanced secondary root development—by ensuring a greater iron supply for essential processes like Fe-S cluster biogenesis. Furthermore, the elevated iron content in *s-AtFH* shoots under both sufficient and excess conditions, suggests a role for overexpressed AtFH as a sequestering or storage protein, consistent with reports of the frataxin homolog in *Saccharomyces cerevisiae* (Park et al., 2003; Bulteau et al., 2004). Thus, we hypothesize that by binding and sequestering excess iron, AtFH prevents free iron toxicity via reactive oxygen species formation, thereby facilitating normal or enhanced development across a range of iron treatments (Fig. 6C).

These experiments were conducted in Hoagland medium supplemented with varying amounts of Fe-EDTA to maintain iron in solution. Because Fe-EDTA is a synthetic chelate with much higher bioavailability than naturally occurring soil iron forms—such as iron oxides, hydroxides, carbonates, phosphates, or complexes with humic and fulvic acids—future studies will be required to explore the behavior of *s-AtFH* *Arabidopsis* plants under more natural soil conditions and varying iron availability.

3.5. *s-AtFH* overexpression selectively enhances Fe-S enzyme activity under iron excess conditions

To investigate whether the overexpression of *Arabidopsis thaliana* frataxin enhances iron-sulfur (Fe-S) cluster biosynthesis, particularly under conditions of iron stress, we measured the activities of the Fe-S cluster-containing enzymes, ACO and SDH, in *s-AtFH* and wt seedlings grown under iron deficiency and iron excess.

Under iron-deficient conditions, no significant differences in ACO or SDH activities were observed (Fig. 7A and B), suggesting AtFH overexpression does not mitigate the effects of iron limitation. However, the results under iron excess were dramatic: *s-AtFH* lines showed a significant increase in enzyme activity. Specifically, ACO activity was increased by 3- to 4.5-fold in shoots and 6- to 12-fold in roots, and SDH activity was consistently increased by approximately 2- to 3.5-fold in shoots and 2- to 2.8-fold in roots compared to wt (Fig. 7A and B). These findings strongly support our hypothesis, demonstrating that when sufficient iron is available, elevated frataxin levels successfully enhance the assembly machinery for Fe-S clusters. This interpretation was confirmed by measuring the activity of MDH, where no significant differences among the *s-AtFH* and wt lines were detected under any iron conditions (Fig. 7C).

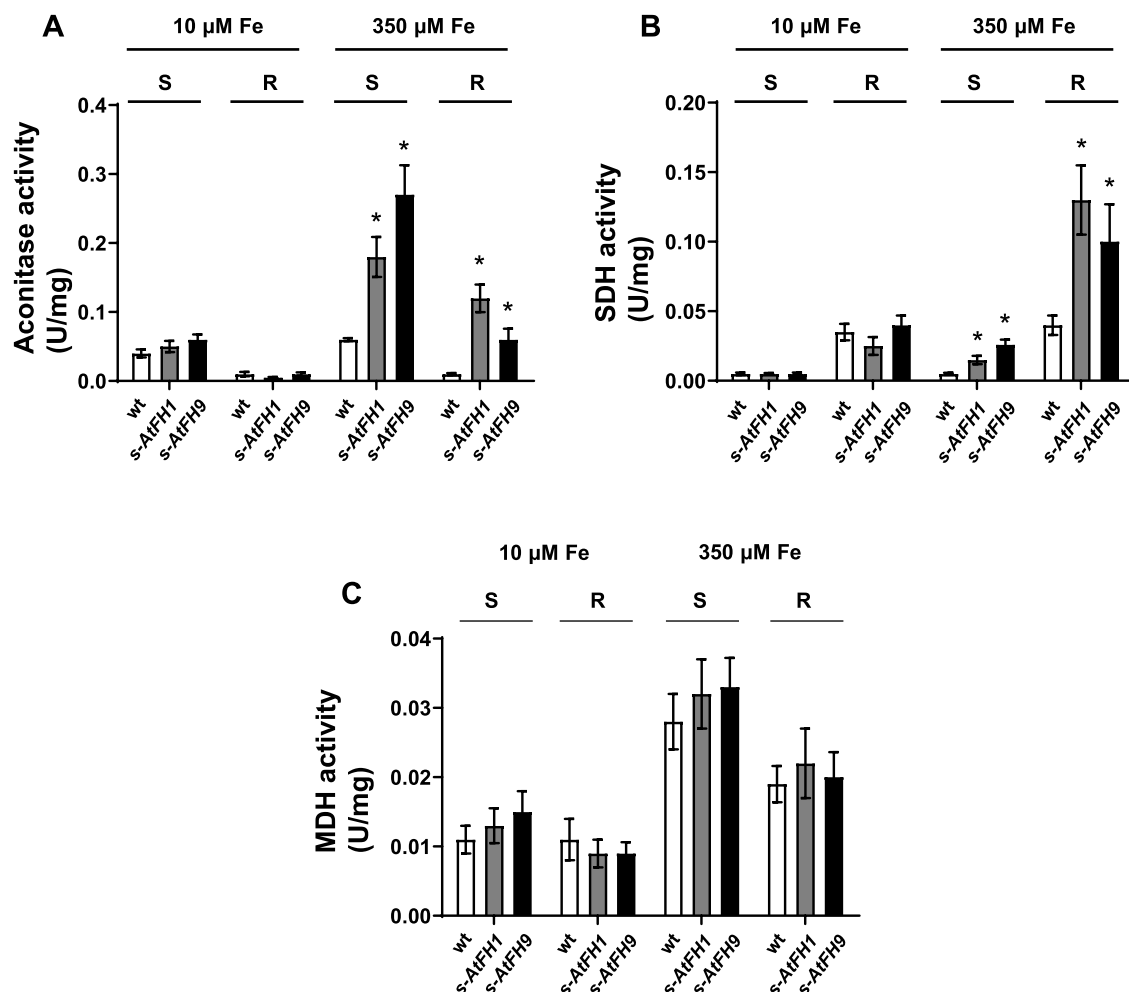


Fig. 7. Activity of Fe-S cluster enzymes under Fe-deficient and Fe-excess conditions. Plants were grown up to stage 1.05 on Hoagland agar medium with iron deficient (10 μM Fe) and excess conditions (350 μM Fe). A) ACO B) SDH and C) MDH activities were measured in shoots and roots extracts of wild-type, *s-AtFH1* and *s-AtFH9* lines. MDH, an enzyme lacking Fe-S clusters, was used as a negative control. All experiments were performed using at least three independent biological replicates (pooling 15–20 plants). Statistically significant differences are indicated by asterisks ($P < 0.05$).

3.6. Gene expression and induction of the iron uptake machinery

Based on the observed abnormal iron content and distribution, we hypothesized that overexpression of *AtFH* leads to an activation of the iron uptake machinery, independent of the external iron status. To test this hypothesis, we analyzed the expression of key genes involved in iron metabolism. These included *FIT1* (At2g28160), a transcriptional regulator of iron metabolism; *FRO2* (At1g01580), a low-iron-induced ferric-chelate reductase; *IRT1* (At4g19690), a divalent metal transporter responsible for iron uptake; and *FER1* (At5g01600) and *FER4* (At2g40300), two iron storage ferritins.

Our results showed increased expression of *FIT1* and *FRO2* in the *s-AtFH* overexpressing lines compared to wt (Fig. 8A). Consistent with these qPCR results, ferric-chelate reductase activity was also increased by 1.7- to 2.2-fold in *s-AtFH* lines compared to wt (Fig. 8B), suggesting a continuous need for iron uptake. *IRT1* mRNA levels further supported this upregulation, increasing by 1.7- and 2-fold in both *s-AtFH* lines (Fig. 8A). In addition, *FER1* transcript levels were significantly elevated in shoots, showing an increase of approximately 17- and 23-fold in *s-AtFH1* and *s-AtFH9* lines, respectively. In contrast, *FER4* transcript levels remained unchanged in all lines tested.

3.7. Limitations and experimental implications of CaMV 35S-driven overexpression

In this study, *AtFH* overexpression was driven by the constitutive cauliflower mosaic virus 35S (CaMV 35S) promoter. While the 35S promoter is a robust tool for functional characterization and initial proof-of-concept studies, its ubiquitous transcriptional activity introduces inherent experimental considerations. Constitutive expression bypasses native spatiotemporal and developmental regulatory mechanisms, driving ectopic gene expression across all plant tissues (e.g., roots, vascular bundles, and leaves) independently of environmental signals. This continuous, high-level expression may impose a metabolic burden or induce pleiotropic effects, particularly under severe stress scenarios or in non-target tissues.

Furthermore, because non-iron related abiotic stresses and precise metabolic energy costs were not directly assessed in this work, the observed phenotypes might differ if *AtFH* were expressed under its native promoter, or via tissue-specific (e.g., root- or leaf-specific) and stress-inducible systems. Employing inducible or cell-type-specific promoters in future studies will be essential to decouple the precise physiological functions of frataxin from potential artifacts of constitutive

ectopic expression, ensuring that optimized iron homeostasis can be achieved without incurring yield penalties in agricultural settings.

4. Conclusions

Collectively, these findings show that the iron uptake machinery in *s-AtFH* lines is activated, similar to the iron uptake response observed in wt plants under iron-deficient conditions, even when iron in this experiment is sufficient and externally available, as we explained before for the iron source Fe-EDTA. This leads to the continuous accumulation of iron in roots, which is subsequently translocated to and accumulated in the leaves. The differential expression pattern observed -increased *FER1* transcripts without concomitant changes in *FER4* levels-suggests that the resulting excess iron is primarily sequestered by the *FER1* protein. This sequestration mechanism likely prevents iron toxicity, thereby contributing to the enhanced biomass, larger rosette area, and improved root development observed in the *s-AtFH* lines. Similar phenotypic alterations have been reported by our group for *hscb* knockdown lines, which also displayed altered iron homeostasis and elevated leaf iron levels without significant changes in root iron content (Leaden et al., 2016). In that study, impaired *HSCB* expression was hypothesized to disrupt iron translocation from root to shoots. Moreover, *AtNFS1*-overexpressing and knockdown *Arabidopsis* mutant plants showed significant alterations in the expression of genes involved in Fe uptake and regulation, with the overexpressing lines accumulating higher amounts of Fe in both roots and shoots (Armas et al., 2019a,b). In summary, our present and previous findings support the hypothesis that the mitochondrial ISC biosynthetic pathway plays a crucial role in plant iron homeostasis and sensing. We propose that *AtFH* overexpression may interfere with normal upstream iron-sensing or regulatory mechanisms, resulting in a sustained upregulation of the iron uptake and subsequent non-toxic iron accumulation in foliar tissues.

However, the current results from the *s-AtFH* lines cannot distinguish between two potential mechanisms underlying the activation of iron uptake: (1) *AtFH* overexpression directly or indirectly activates iron uptake genes or proteins, decoupling this response from external iron availability; or (2) *AtFH* overexpression, by accelerating Fe-S cluster assembly and upregulating *FER1* ferritin expression, depletes the cellular labile iron pool. This depletion could functionally mimic an iron-deficient state, thereby triggering the feedback upregulation of iron uptake machinery through the canonical iron deficiency signaling pathway. Future work must be directed toward discriminating between these two scenarios.

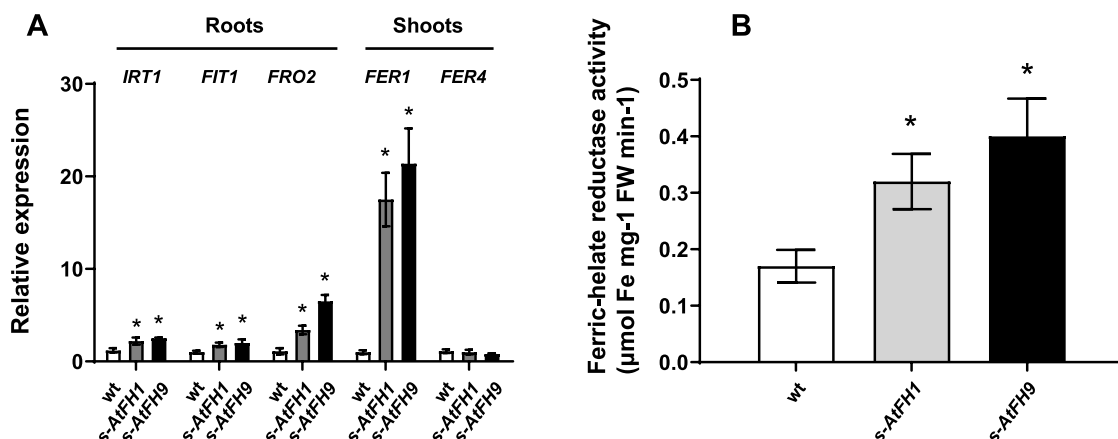


Fig. 8. Transcript levels of iron metabolism genes and FRO activity. A) qRT/PCR analysis of genes involved in iron metabolism: Fe²⁺ transport protein 1 (*IRT1*, At4g19690), Fe-deficiency induced transcription factor (*FIT1*, At2g28160), Ferric-chelate reductase (*FRO2*, At1g01580), ferritin 1 (*FER1*, At5g01600) and ferritin 4 (*FER4*, At2g40300). RNA was extracted from roots and shoots of wt, *s-AtFH1* and *s-AtFH9* plants. All experiments were performed using at least three independent biological replicates (from 15 to 20 pooled plants). B) Ferric-chelate reductase activity. At least 15-20 roots were pooled and subjected to enzyme activity analysis (n = 3). Statistically significant differences are indicated by asterisks ($P < 0.05$).

Although the exact identity of the iron sensor or the specific iron metabolite being sensed remains elusive, there is a consensus that this iron-sensing mechanism resides in the leaves and regulates root iron-deficiency responses via long-distance phloem-mobile signals (Enomoto et al., 2007). To test the integrity of this shoot-to-root regulatory mechanism in *s-AtFH* plants, classic foliar iron application experiments could be performed (Garcia et al., 2013). Painting the leaves of *s-AtFH* plants with iron—which enters via stomata—should deactivate the root iron-deficiency response. If no such deactivation occurs, it validates the first scenario. Conversely, to explore the second mechanism, it would be highly informative to specifically inhibit the iron-sulfur cluster (ISC) assembly pathway. A small molecule, “compound 53,” was recently reported to selectively inhibit human NFS1 and suppress ISC biogenesis in lung tumor cells (Zhu et al., 2025). Given the high evolutionary conservation of ISC machinery, compound 53 may be functional in plants as well. Treating *s-AtFH* plants with compound 53 could verify target engagement through decreased ACO and SDH activities, allowing researchers to evaluate downstream effects on iron homeostasis by monitoring changes in FRO2 activity and the expression of key regulatory genes like FIT, IRT1, and FRO2.

In summary, *AtFH* overexpression likely interferes with homeostatic iron-sensing or regulatory networks, causing a sustained upregulation of the iron acquisition machinery. This leads to enhanced iron uptake and subsequent non-toxic iron accumulation in leaves, ultimately yielding plants with improved growth under iron deficiency and greater resilience to iron excess. However, these phenotypic gains must be contextualized within the tested conditions (iron deficiency and excess supplied as Fe-EDTA in Hoagland agar media). Because these transgenic lines rely on the constitutive CaMV 35S promoter and were not evaluated under other abiotic stresses (e.g., drought, salinity, or heavy metal toxicity) or in natural soil, the broader agronomic applicability of this strategy remains to be established. Future studies using stress-inducible or tissue-specific promoters, alongside soil-based evaluations under multi-stress scenarios, will be essential to determine whether this engineered iron homeostasis mechanism maintains its benefits without incurring performance trade-offs in complex environments.

Author contributions

AT conceived, carried out the experiments, analyzed the data and wrote the first draft of the manuscript. MAP, MVB and DGC designed the experiments, analyzed the data, edited and prepared the final version of the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported in part by grants from ANPCyT (PICT 2018 01440 to MVB, PICT 2019 090 to MAP and PICT 2019 067 to DFGC). AT was a doctoral fellow from CONICET. MAP, MVB and DFGC are research scientists from the same institution.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plaphy.2026.111687>.

Data availability

Data will be made available on request.

References

- Armas, A.M., Balparda, M., Terenzi, A., Busi, M.V., Pagani, M.A., Gomez-Casati, D.F., 2019a. Ferroxidase activity of plant frataxin. *Biochimie* 156, 118–122.
- Armas, A.M., Balparda, M., Terenzi, A., Busi, M.V., Pagani, M.A., Gomez-Casati, D.F., 2020. Iron-Sulfur cluster complex assembly in the mitochondria of *Arabidopsis thaliana*. *Plants (Basel)* 9, 1171.
- Armas, A.M., Balparda, M., Turowski, V.R., Busi, M.V., Pagani, M.A., Gomez-Casati, D.F., 2019b. Altered levels of mitochondrial NFS1 affect cellular Fe and S contents in plants. *Plant Cell Rep.* 38, 981–990.
- Bonicki, M.T., Freibert, S.A., Muhlenhoff, U., Lill, R., Cygler, M., 2017. Structure and functional dynamics of the mitochondrial Fe/S cluster synthesis complex. *Nat. Commun.* 8, 1287.
- Boyes, D.C., Zayed, A.M., Ascenzi, R., McCaskill, A.J., Hoffman, N.E., Davis, K.R., Görlach, J., 2001. Growth stage-based phenotypic analysis of *Arabidopsis*: a model for high throughput functional genomics in plants. *Plant Cell* 13, 1499–1510.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Braymer, J.J., Lill, R., 2017. Iron-sulfur cluster biogenesis and trafficking in mitochondria. *J. Biol. Chem.* 292, 12754–12763.
- Bridwell-Rabb, J., Fox, N.G., Tsai, C.L., Winn, A.M., Barondeau, D.P., 2014. Human frataxin activates Fe-S cluster biosynthesis by facilitating sulfur transfer chemistry. *Biochemistry* 53, 4904–4913.
- Buchensky, C., Sanchez, M., Carrillo, M., Palacios, O., Capdevila, M., Dominguez-Vera, J. M., Busi, M.V., Atrian, S., Pagani, M.A., Gomez-Casati, D.F., 2017. Identification of two frataxin isoforms in *Zea mays*: structural and functional studies. *Biochimie* 140, 34–47.
- Bulteau, A.L., O'Neill, H.A., Kennedy, M.C., Ikeda-Saito, M., Isaya, G., Szewda, L.I., 2004. Frataxin acts as an iron chaperone protein to modulate mitochondrial aconitase activity. *Science* 305, 242–245.
- Busi, M.V., Gomez-Casati, D.F., 2012. Exploring frataxin function. *IUBMB Life* 64, 56–63.
- Busi, M.V., Maliandi, M.V., Valdez, H., Clemente, M., Zabaleta, E.J., Araya, A., Gomez-Casati, D.F., 2006. Deficiency of *Arabidopsis thaliana* frataxin alters activity of mitochondrial Fe-S proteins and induces oxidative stress. *Plant J.* 48, 873–882.
- Busi, M.V., Zabaleta, E.J., Araya, A., Gomez-Casati, D.F., 2004. Functional and molecular characterization of the frataxin homolog from *Arabidopsis thaliana*. *FEBS Lett.* 576, 141–144.
- Chen, K., Lin, G., Haelterman, N.A., Ho, T.S., Li, T., Li, Z., Duraine, L., Graham, B.H., Jaiswal, M., Yamamoto, S., Rasband, M.N., Bellen, H.J., 2016. Loss of Frataxin induces iron toxicity, sphingolipid synthesis, and Pdk1/Mef2 activation, leading to neurodegeneration. *eLife* 5, e16043.
- Clough, S.J., Bent, A.F., 1998. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* 16, 735–743.
- Connolly, E.L., Fett, J.P., Guerinot, M.L., 2002. Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. *Plant Cell* 14, 1347–1357.
- Enomoto, Y., Hodoshima, H., Shimada, H., Shoji, K., Yoshihara, T., Goto, F., 2007. Long-distance signals positively regulate the expression of iron uptake genes in tobacco roots. *Planta* 227, 81–89.
- Fox, N.G., Yu, X., Feng, X., Bailey, H.J., Martelli, A., Nabhan, J.F., Strain-Damerell, C., Bulawa, C., Yue, W.W., Han, S., 2019. Structure of the human frataxin-bound iron-sulfur cluster assembly complex provides insight into its activation mechanism. *Nat. Commun.* 10, 2210.
- Gakh, O., Park, S., Liu, G., Macomber, L., Imlay, J.A., Ferreira, G.C., Isaya, G., 2006. Mitochondrial iron detoxification is a primary function of frataxin that limits oxidative damage and preserves cell longevity. *Hum. Mol. Genet.* 15, 467–479.
- Garcia, M.J., Romera, F.J., Stacey, M.G., Stacey, G., Villar, E., Alcantara, E., Perez-Vicente, R., 2013. Shoot to root communication is necessary to control the expression of iron-acquisition genes in *Strategy I* plants. *Planta* 237, 65–75.
- Gentry, L.E., Thacker, M.A., Doughty, R., Timkovich, R., Busenlehner, L.S., 2013. His86 from the N-terminus of frataxin coordinates iron and is required for Fe-S cluster synthesis. *Biochemistry* 52, 6085–6096.
- Gomez-Casati, D.F., Busi, M.V., Pagani, M.A., 2018. Plant frataxin in metal metabolism. *Front. Plant Sci.* 9, 1706.
- Goto, F., Yoshihara, T., Saiki, H., 2000. Iron accumulation and enhanced growth in transgenic lettuce plants expressing the iron-binding protein ferritin. *Theor. Appl. Genet.* 100, 658–664.
- Grisolia, M.J., Peralta, D.A., Valdez, H.A., Barchiesi, J., Gomez-Casati, D.F., Busi, M.V., 2017. The targeting of starch binding domains from starch synthase III to the cell wall alters cell wall composition and properties. *Plant Mol. Biol.* 93, 121–135.
- Hajdukiewicz, P., Svab, Z., Maliga, P., 1994. The small, versatile pPZP family of *Agrobacterium* binary vectors for plant transformation. *Plant Mol. Biol.* 25, 989–994.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–685.
- Leadon, L., Pagani, M.A., Balparda, M., Busi, M.V., Gomez-Casati, D.F., 2016. Altered levels of ATHSCB disrupts iron translocation from roots to shoots. *Plant Mol. Biol.* 92, 613–628.
- Li, J., Kogan, M., Knight, S.A., Pain, D., Dancis, A., 1999. Yeast mitochondrial protein, Nfs1p, coordinately regulates iron-sulfur cluster proteins, cellular iron uptake, and iron distribution. *J. Biol. Chem.* 274, 33025–33034.
- Lill, R., 2009. Function and biogenesis of iron-sulphur proteins. *Nature* 460, 831.
- Lill, R., Muhlenhoff, U., 2008. Maturation of iron-sulfur proteins in eukaryotes: mechanisms, connected processes, and diseases. *Annu. Rev. Biochem.* 77, 669–700.

- Maliandi, M.V., Busi, M.V., Clemente, M., Zabaleta, E.J., Araya, A., Gomez-Casati, D.F., 2007. Expression and one-step purification of recombinant *Arabidopsis thaliana* frataxin homolog (AtFH). *Protein Expr. Purif.* 51, 157–161.
- Maliandi, M.V., Busi, M.V., Turowski, V.R., Leaden, L., Araya, A., Gomez-Casati, D.F., 2011. The mitochondrial protein frataxin is essential for heme biosynthesis in plants. *FEBS J.* 278, 470–481.
- Park, S., Gakh, O., O'Neill, H.A., Mangravita, A., Nichol, H., Ferreira, G.C., Isaya, G., 2003. Yeast frataxin sequentially chaperones and stores iron by coupling protein assembly with iron oxidation. *J. Biol. Chem.* 278, 31340–31351.
- Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 29, e45.
- Puccio, H., Simon, D., Cossée, M., Criqui-Filipe, P., Tiziano, F., Melki, J., Hindelang, C., Matyas, R., Rustin, P., Koenig, M., 2001. Mouse models for Friedreich ataxia exhibit cardiomyopathy, sensory nerve defect and Fe-S enzyme deficiency followed by intramitochondrial iron deposits. *Nat. Genet.* 27, 181–186.
- Rouault, T.A., 2019. The indispensable role of mammalian iron sulfur proteins in function and regulation of multiple diverse metabolic pathways. *Biometals* 32, 343–353.
- Sanchez, M., Palacios, O., Buchensky, C., Sabio, L., Gomez-Casati, D.F., Pagani, M.A., Capdevila, M., Atrian, S., Dominguez-Vera, J.M., 2018. Copper redox chemistry of plant frataxins. *J. Inorg. Biochem.* 180, 135–140.
- Schirawski, J., Unden, G., 1998. Menaquinone-dependent succinate dehydrogenase of bacteria catalyzes reversed electron transport driven by the proton potential. *Eur. J. Biochem.* 257, 210–215.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675.
- Tripodi, K., Podestá, F., 2003. Purification and characterization of an NAD-dependent malate dehydrogenase from leaves of the crassulacean acid metabolism plant *Aptenia cordifolia*. *Plant Physiol. Biochem.* 41, 97–105.
- Turowski, V.R., Akin, C., Maliandi, M.V., Buchensky, C., Leaden, L., Peralta, D.A., Busi, M.V., Araya, A., Gomez-Casati, D.F., 2015. Frataxin is localized to both the chloroplast and mitochondrion and is involved in chloroplast Fe-S protein function in *Arabidopsis*. *PLoS One* 10, e0141443.
- Vert, G., Grotz, N., Dedaldecamp, F., Gaymard, F., Guerinot, M.L., Briat, J.F., Curie, C., 2002. IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and for plant growth. *Plant Cell* 14, 1223–1233.
- Weigel, D., Glazebrook, J., 2002. *Arabidopsis. A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York, p. 448.
- Yoon, T., Cowan, J.A., 2004. Frataxin-mediated iron delivery to ferrochelatase in the final step of heme biosynthesis. *J. Biol. Chem.* 279, 25943–25946.
- Yuan, Y., Wu, H., Wang, N., Li, J., Zhao, W., Du, J., Wang, D., Ling, H.-Q., 2008. FIT interacts with AtbHLH38 and AtbHLH39 in regulating iron uptake gene expression for iron homeostasis in *Arabidopsis*. *Cell Res.* 18, 385–397.
- Zhai, L., Xiao, D., Sun, C., Wu, T., Han, Z., Zhang, X., Xu, X., Wang, Y., 2016. Nitric oxide signaling is involved in the response to iron deficiency in the woody plant *Malus xiaojinensis*. *Plant Physiol. Biochem.* 109, 515–524.
- Zhu, Z., Gan, H., Wang, Y., Jia, G., Li, H., Ma, Z., Wang, J., Shang, X., Niu, W., 2025. Identification of a selective inhibitor of Human NFS1, a cysteine desulfurase involved in Fe-S cluster assembly, via structure-based virtual screening. *Int. J. Mol. Sci.* 26, 2782.