

## Article

# Ionic and Transcriptomic Reprogramming Reveal Contrasting Iron Deficiency and Excess Responses in Potato (*Solanum tuberosum* L.)

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## Highlights

### What are the main findings?

- Contrasting potato genotypes showed tissue-specific ionic responses to Fe deficiency and excess.
- The 05P maintained higher leaf Fe under Fe deficiency, whereas CI5 exhibited stronger Fe accumulation under Fe excess.
- Fe excess caused pronounced Mn depletion in shoots and increased Fe/Mn ratios.

### What are the implications of the main findings?

- *FRO* and *IRT/ZIP* candidates provide priority targets for Fe uptake, divalent metal transport and biofortification studies.
- Tissue-resolved ionic and transcriptomic profiling can support potato breeding for improved Fe homeostasis.

## Abstract

Iron (Fe) is an essential micronutrient for plant growth, serving as a cofactor in chlorophyll synthesis, photosynthetic electron transport, and redox reactions. Both Fe deficiency and excess disrupt mineral nutrient homeostasis, but the ionic and transcriptomic mechanisms underlying genotype-specific responses in potato remain elusive. We profiled the ionome and transcriptome of two potato genotypes, 05P and CI5, grown under Fe-deficient, Fe-sufficient and Fe-excess conditions represented by 1, 40.4 and 120 mg L<sup>-1</sup> FeNaEDTA, respectively. Ionic analysis detected significant alterations in Fe, Mn, Zn, Ca, Mg, and Cu concentrations across roots, stems, and leaves, with the direction and magnitude of change varying by organ and genotype. Under Fe deficiency, CI5 showed steeper declines in root and stem Fe than 05P, while 05P retained higher leaf Fe, reflecting genotype-dependent patterns of shoot Fe distribution. Fe excess caused Fe accumulation in both genotypes, but coincided with Mn depletion in shoots, raising Fe/Mn ratios, consistent with potential antagonistic Fe–Mn interactions at the transport level. This pattern is consistent with potential antagonistic Fe–Mn interactions, although alternative mechanisms such as Mn uptake inhibition or dilution effects cannot be ruled out without direct experimental evidence. Transcriptome analysis showed organ-biased responses: Fe deficiency upregulated more genes in stems, whereas Fe excess triggered stronger transcriptional shifts in roots. No KEGG pathways remained significant after false discovery



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rate (FDR) correction, suggesting that genotype-dependent Fe-responsive divergence was not concentrated in a limited number of canonical KEGG pathways under the present analytical framework. Joint analysis of ionomic and transcriptomic data highlighted ferric-chelate reductase oxidase (*FRO*) and zinc-regulated transporter/iron-regulated transporter-like protein (*IRT*/*ZIP*) family members as genes associated with Fe reduction, divalent metal uptake, and Fe–Mn balance based on differential expression and orthology with functionally characterized *Arabidopsis* homologs. Comparative genomics showed that *FRO* and *ZIP* families have expanded in potato, with conserved domain architectures but divergent gene structures and promoter architectures, consistent with potential functional diversification related to metal transport. In conclusion, under Fe deficiency, genotype-dependent transcriptional divergence was most pronounced in stems, whereas under Fe excess it was more evident in roots, jointly maintaining systemic Fe–Mn homeostasis in potato.

**Keywords:** potato; iron stress; ionomics; transcriptome; iron homeostasis; biofortification

## 1. Introduction

Iron (Fe) is indispensable for plant growth, serving as a cofactor in chlorophyll biosynthesis, photosynthetic electron transport, respiration, and redox enzymes [1]. Despite its abundance in most soils, Fe bioavailability is limited by soil pH, oxygen levels, and chemical speciation, so plants often find themselves either deficient or exposed to excess [2]. Deficiency hampers chlorophyll formation and photosynthesis, leading to interveinal chlorosis and stunted growth; excess Fe, in turn, triggers reactive oxygen species (ROS) that damage membranes and disrupt mineral balance [3,4]. Hence, Fe homeostasis matters not only for crop yield but also for nutritional quality and biofortification efforts [5,6].

Potato (*Solanum tuberosum* L.) is the world's fourth most important food crop, supplying carbohydrates, vitamins, and minerals to human diets [7]. However, Fe nutrition in potato has drawn much less research attention than macronutrient studies [8,9]. Tuber Fe concentrations vary widely across germplasm, with heritability estimates around 0.81, indicating substantial genetic potential for biofortification [10]. This variation implies that genotypes differ in their capacities for Fe uptake, translocation, and storage. But how these differences are regulated at physiological and molecular levels remains poorly understood.

Potato, like other Strategy I plants, acquires Fe through rhizosphere acidification, Fe<sup>3+</sup> reduction, and Fe<sup>2+</sup> uptake [11]. In *Arabidopsis*, *AHA2*, *FRO2*, and *IRT1* coordinate these steps at the root surface [12–14]. *IRT1* is not Fe-specific; it also transports Zn<sup>2+</sup>, Mn<sup>2+</sup>, and Cu<sup>2+</sup>, and its activity is restrained by ubiquitin-mediated degradation triggered by intracellular metal sensing [15–17]. Transcriptional control of Fe deficiency responses involves the bHLH factors FIT and PYE [18–21], while systemic signaling includes IMA peptides and the BTS E3 ligase [22,23]. This regulatory hierarchy is well characterized in *Arabidopsis*, but whether potato deploys a comparable architecture—especially across genotypes with contrasting Fe accumulation—has barely been investigated.

Fe homeostasis intertwines with Mn, Zn, Cu, Ca, and Mg metabolism, making single-element analyses insufficient to capture Fe stress responses [24]. Ionomics allows simultaneous quantification of multiple elements, and transcriptomics identifies the regulatory pathways behind ionomic shifts [25,26]. Together, these approaches have advanced nutrient homeostasis research in several species [7,27], yet potato genotype-dependent Fe responses have rarely been examined in this way. Consequently, how potato genotypes

differentially allocate Fe and maintain multi-element balance under varying Fe supply is still largely unknown.

We therefore selected two potato genotypes at opposite extremes of tuber Fe concentration—05P (high Fe, top 0.3% of 404 accessions) and CI5 (low Fe, bottom 1.6%)—and grew them under Fe deficiency and excess. Choosing extreme phenotypes maximizes the contrast in Fe accumulation capacity, which should help reveal molecular differences that intermediate genotypes might obscure. We profiled ionomes across roots, stems, and leaves, paired with transcriptome analysis, qRT-PCR validation, and candidate gene characterization, to trace Fe redistribution under stress. Findings from these two genotypes will not capture the full diversity of potato germplasm, but they provide an initial framework for understanding genotype-dependent Fe homeostasis that wider genetic panels can later test.

## 2. Materials and Methods

### 2.1. Germplasm Screening and Genotype Selection

Fresh tubers of potato (*Solanum tuberosum* L.) germplasm accessions were harvested in mid- to late October of 2020 and 2021 from plants cultivated in the same experimental field at the Alpine Potato Experimental Station in Qinghai Province (Xiazhai Village, Sizhai Township, Huangyuan County, China; 36°42' N, 101°15' E; elevation 3000 m). Potato accessions were planted in May and harvested in October of the corresponding growing season. Tuber Fe concentrations were determined for 59 accessions harvested in 2020 and 345 accessions harvested in 2021, with 53 accessions measured in both years. Uniform and visually healthy tubers were selected for analysis.

Concentrated nitric acid (HNO<sub>3</sub>) and 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) of analytical grade were used for acid digestion. A certified iron standard solution (1000 µg mL<sup>-1</sup>) was purchased from the National Institute of Metrology, China. Instruments included a forced-air drying oven, analytical balance, pipettes, 50 mL volumetric flasks, assorted graduated cylinders, 100 mL conical flasks, a ceramic hot plate, and a novAA 400P atomic absorption spectrometer (Analytik Jena, Jena, Germany) equipped with a hollow cathode lamp (HCL).

Glassware was soaked in 20% nitric acid overnight, rinsed with tap water, washed five times with deionized water (18.2 MΩ·cm), and air-dried before use. Fresh tubers were washed with deionized water, peeled, and cut into small pieces. Samples were heat-inactivated at 105 °C for 30 min and then dried at 80 °C to constant weight. The dried material was ground into a fine powder (particle size < 0.25 mm) using a mortar and pestle.

Approximately 0.6 g (weighed to the nearest 0.0001 g) of powder was placed into a 100 mL conical flask. Fifteen mL of concentrated nitric acid and 10 mL of hydrogen peroxide were added, mixed thoroughly, and allowed to predigest overnight at room temperature. The flask was heated on a ceramic hot plate to 200 °C for approximately 30 min, then reduced to 150 °C, and acid digestion continued for approximately 1 h until the solution became clear and colorless, with a remaining volume of approximately 3 mL. After cooling to room temperature, the digest was transferred to a 50 mL volumetric flask and diluted to volume with 5% nitric acid.

Blank controls were prepared following the same procedure. The 5% nitric acid solution was prepared by diluting 73.5 mL of 68% concentrated nitric acid to 1 L with deionized water.

Working standard solutions of Fe (0.2, 0.5, 1.0, 2.0, and 4.0 mg L<sup>-1</sup>) were prepared by serial dilution of the stock solution with 5% nitric acid. Fe concentrations were determined by flame atomic absorption spectrometry (FAAS) at 248.3 nm. Instrumental parameters are provided in Table 1. The spectrometer was operated in single-beam mode with a

slit width of 0.2 nm and a lamp current of 2.0 mA. Calibration and quantification were performed using ASpect LS version 1.5.0.0 (Analytik Jena, Jena, Germany) according to the manufacturer's instructions.

**Table 1.** Instrumental conditions for flame atomic absorption spectrometry.

| Element | Analytical Line | Wavelength/nm | Slit Width/nm | Lamp Current/mA | Lamp Type | Optical Mode |
|---------|-----------------|---------------|---------------|-----------------|-----------|--------------|
| Fe      | Fe248           | 248.3         | 0.2           | 2.0             | HCL       | Single-beam  |

Each sample was analyzed in triplicate, with five readings per replicate, and the mean value was used for subsequent calculations.

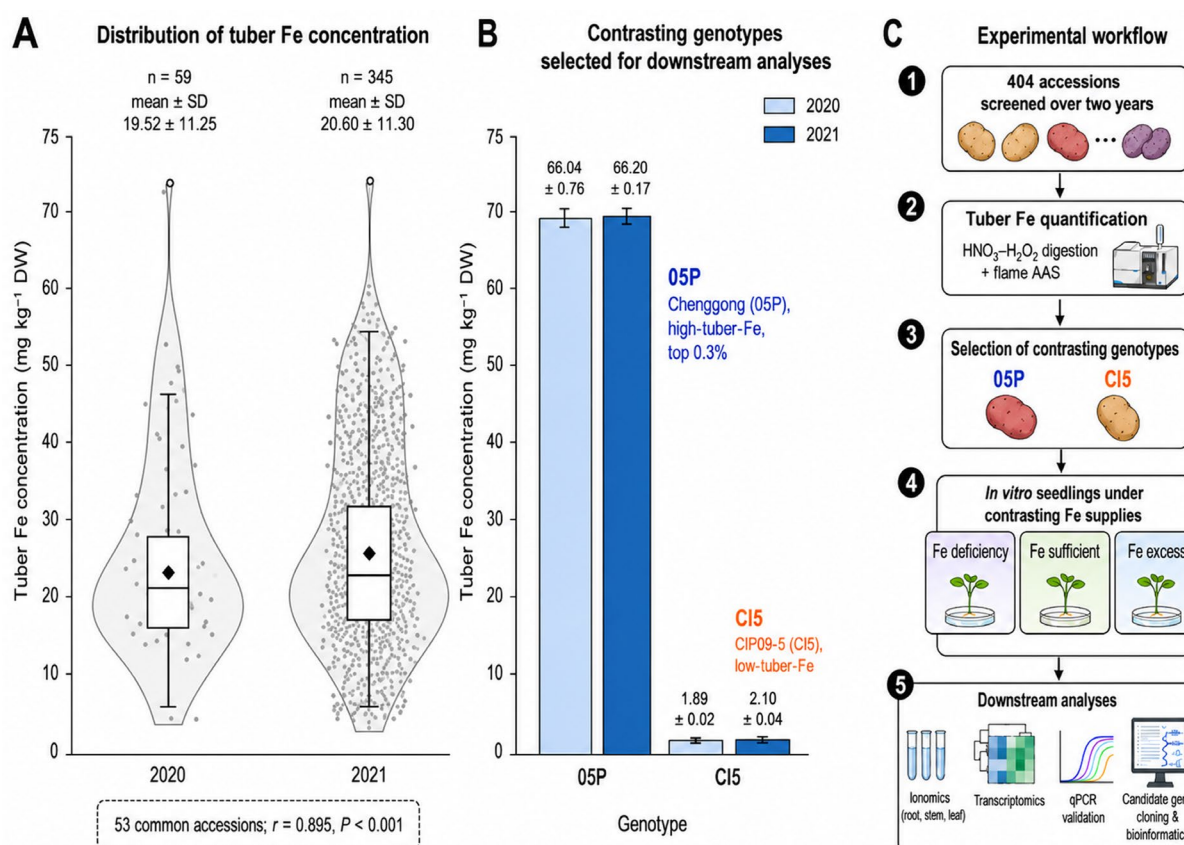
## 2.2. Iron Stress Treatments and Plantlet Culture

We selected two contrasting genotypes from the screening: 05P (high tuber Fe) and CI5 (low tuber Fe). In vitro virus-free plantlets were subcultured for three generations on 1/2 MS solid medium. Two-week-old seedlings were selected, and 1.5 cm stem segments containing 1–2 growth points (lateral meristems) were excised and inoculated onto 1/2 MS solid medium. After 10 days of culture (with 4–5 leaves), uniformly developed plantlets were selected and roots were rinsed three times with ultrapure water and transferred to iron stress media: Fe1 (1 mg L<sup>-1</sup> FeNaEDTA, deficiency), Fe40 (40.4 mg L<sup>-1</sup> FeNaEDTA, sufficiency), and Fe120 (120 mg L<sup>-1</sup> FeNaEDTA, excess) [28]. All media were supplemented with Na<sub>2</sub>EDTA to achieve a uniform EDTA concentration of 120 mg L<sup>-1</sup>, adjusted to pH 5.8 with 1 M NaOH, and contained 25 g L<sup>-1</sup> sucrose and 7 g L<sup>-1</sup> agar. Media and ultrapure water were autoclaved before use. Plantlets were maintained in a growth chamber at 20 °C under a 16 h light/8 h dark photoperiod with a light intensity of 100 µmol m<sup>-2</sup> s<sup>-1</sup> (LED, 08:00–24:00) and 85–95% relative humidity. After 7 days of Fe-deficient (Fe1), Fe-sufficient (Fe40), or Fe-excess (Fe120) treatment, roots, stems and leaves were harvested separately, immediately frozen in liquid nitrogen for RNA analyses or dried for ionic determination.

Figure 1 summarizes the germplasm screening and experimental design. Tuber Fe concentration varied widely across the collection (1.89–66.20 mg kg<sup>-1</sup> DW; Figure 1A). Mean values were similar between years (2020: 19.5 ± 11.3; 2021: 20.6 ± 11.3 mg kg<sup>-1</sup> DW), and the 53 accessions screened in both years showed strong rank consistency ( $r = 0.895$ ,  $p < 0.001$ ), indicating stable genotype performance across seasons. 05P ranked first for tuber Fe in both years, whereas CI5 ranked last in 2020 and remained in the low-Fe group in 2021 (Figure 1B). The Fe concentration difference between the two genotypes was ~35-fold in 2020 and ~32-fold in 2021. These contrasting phenotypes provided the rationale for comparing their ionic and transcriptomic responses to variable Fe supply (Figure 1C).

## 2.3. Ionic Determination and Analysis

After 7 days on stress media, intact plantlets of both genotypes were harvested, rinsed five times with deionized water, blotted dry, and separated into roots, stems, and leaves. Samples were dried at 80 °C to constant weight, ground to fine powder, and ~0.1 g of each tissue was digested using the same acid digestion procedure as for tuber Fe content determination. Digested solutions were transferred to 25 mL volumetric flasks and diluted to volume with 5% HNO<sub>3</sub>. Fe, Zn, Cu, Ca, Mg, and Mn concentrations were measured by flame atomic absorption spectrometry (novAA400P, Analytik, Jena, Germany) (mg kg<sup>-1</sup> DW). All reagents were analytical grade. Standard stock solutions were obtained from the National Center for Iron and Steel Materials Testing (NCS, Beijing, China). Each treatment combination included three biological replicates.



**Figure 1.** Screening of contrasting potato genotypes based on tuber Fe concentration. (A) Distribution of tuber Fe concentration in the potato germplasm collection screened in 2020 and 2021. (B) Tuber Fe concentrations of 05P and CI5 across two years. (C) Experimental workflow showing the selection of contrasting genotypes followed by Fe-deficiency, Fe-sufficient and Fe-excess treatments for ionomic, transcriptomic and candidate gene analyses.

Means, standard deviations, standard errors, relative percentage changes (vs. Fe40), and  $\log_2$  fold changes were calculated. Fe/Mn ratios were used to assess Fe–Mn homeostasis under excess Fe.

Genotypic differences were tested by Welch's *t*-test. For each tissue, two-way analysis of variance (ANOVA) was performed using OriginPro 2021 (OriginLab Corporation, Northampton, MA, USA), with genotype and Fe treatment included as fixed factors. When a significant genotype  $\times$  treatment interaction was detected, simple-effects analyses were conducted to evaluate treatment effects within each genotype and genotype effects within each treatment. Pairwise comparisons were performed using Tukey's honestly significant difference (HSD) test. Differences were considered statistically significant at  $p < 0.05$ .

#### 2.4. RNA Extraction and Transcriptome Sequencing

After 7 days of treatment, tissues were frozen in liquid nitrogen and sent for RNA-seq. For each genotype  $\times$  treatment  $\times$  tissue combination, three biological replicates were sequenced, yielding a total of 54 libraries (2 genotypes  $\times$  3 treatments  $\times$  3 tissues  $\times$  2 genotypes' shared design; see Supplementary Table S1 for the complete sample list).

Total RNA was extracted using the PureLink™ RNA Mini Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), DNase-treated, and purified with the RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA quality was assessed by NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA) ( $A_{260}/A_{280} = 1.8\text{--}2.0$ ,  $A_{260}/A_{230} \geq 2.0$ ) and RNA integrity number (RIN) was determined using an Agilent 2100 Bioanalyzer (all samples had  $\text{RIN} \geq 7.0$ ).

cDNA libraries were constructed using oligo(dT) enrichment, random hexamer priming, and PCR amplification. Paired-end sequencing (PE150) was performed on the Illumina HiSeq 2500/4000 platform (Illumina, San Diego, CA, USA). Each library yielded approximately 6.5–8.0 Gb of raw data, with 20–25 million raw reads per sample. After quality filtering (removing adapters, ambiguous bases >10% N, and low-quality bases >50% Q ≤ 20), clean reads ranged from 19.5 to 23.8 million per sample, with Q30 ≥ 93.5% (Supplementary Table S1).

Clean reads were mapped to the potato reference genome (PGSC\_DM\_v6.1) using STAR (v2.7.10a). The overall mapping efficiency ranged from 85.2% to 91.7% across all samples, with uniquely mapped reads accounting for 78.3–86.4% (Supplementary Table S1). Transcript assembly was performed with Cufflinks (v2.1.1) and alternative splicing analysis with rMATS, both with default parameters. Raw data are available in NCBI SRA (PR-JNA1312044). DEGs were used for GO and KEGG enrichment and candidate gene analysis.

## 2.5. GO and KEGG Enrichment Analysis

GO and KEGG enrichment was performed using DEGs from each comparison. GO terms with FDR < 0.05 were considered significant. Broad categories (e.g., “cellular process,” “binding,” “metabolic process”) were excluded from visualization.

KEGG pathways were ranked by nominal *p*-value; none reached significance after FDR correction, so results were interpreted descriptively rather than as mechanistic evidence.

## 2.6. Candidate Gene Selection and qRT-PCR Validation

Candidate genes were selected based on ionic variation, expression fold change, statistical significance, tissue specificity, and functional annotation. Priority was given to genes involved in Fe acquisition, divalent metal transport, Fe–Mn/Zn/Cu homeostasis, and stress signaling, including ferric-chelate reductases, ZIP transporters, copper transporters, heavy metal-associated proteins, and transcription factors (WRKY, bHLH families).

*FRO* (PGSC0003DMG400010125) and *IRT/ZIP* (PGSC0003DMG400010369) were selected for qRT-PCR validation based on their roles in Fe uptake and prior validation.

qRT-PCR was performed using the TB Green assay. Each 20 µL reaction contained 10 µL TB Green mix, 0.4 µL each primer, 2 µL cDNA, and 7.2 µL nuclease-free water. Cycling conditions: 95 °C for 30 s; 45 cycles of 95 °C for 5 s and 60 °C for 30 s. Expression was calculated by the  $2^{-\Delta\Delta C_t}$  method using *StActin* as the reference gene. *StActin* was selected as the housekeeping gene based on its stable expression across all samples in the RNA-seq dataset (coefficient of variation < 5%). Each reaction was run in three technical replicates. Primer efficiencies were validated by standard curve analysis ( $R^2 > 0.99$ , efficiency 90–110%). Primers are listed in Table 2.

**Table 2.** Primer information for qRT-PCR.

| Type          | Gene           | Gene ID              | Forward                              | Reverse                               |
|---------------|----------------|----------------------|--------------------------------------|---------------------------------------|
| Fe uptake     | <i>IRT1</i>    | PGSC0003DMG400010369 | 5'-GTTGCTGCACTTT<br>GCTTTCATC-3'     | 5'-CCTTAG<br>TCAATGTTATTAACAAGTTTG-3' |
| Fe reduction  | <i>FRO</i>     | PGSC0003DMG400010125 | 5'-<br>GCTCGTGATGGAATCTCTT<br>GGA-3' | 5'-TGTAACAAGATAAGGA<br>GAAAGATGTTC-3' |
| Fe reduction  | <i>FRO</i>     | PGSC0003DMG401018223 | 5'-GATGATTGAG<br>GAGGGCGAAGAAG-3'    | 5'-CTTGTGATCCACAC<br>TCACAAGG-3'      |
| transcription | <i>bHLH TF</i> | PGSC0003DMG400025976 | 5'-GGTGGA<br>GATGAGGTGGTTCCTT-3'     | 5'-GCTTGTGCTGGCAAGATA<br>GAGT-3'      |

Table 2. Cont.

| Type            | Gene              | Gene ID              | Forward                         | Reverse                          |
|-----------------|-------------------|----------------------|---------------------------------|----------------------------------|
| ion homeostasis | Ca-ATPase         | PGSC0003DMG400013022 | 5'-GGAGGAGCCTTTGTGGTGTG-3'      | 5'-CTGTTGTCGAAA GCCATGATCT-3'    |
| ion homeostasis | Na/K/Ca exchanger | PGSC0003DMG400027543 | 5'-GGAGAAGGTGAGGA GAATGGAG-3'   | 5'-CTCACAAGGAG TGTGATCTTAA-3'    |
| redox           | Ferredoxin-3      | PGSC0003DMG400026360 | 5'-GCTTCGAGTGAAGA ACAGGAAG-3'   | 5'-CTTGTCTTTTAA GAAATGGACTACC-3' |
| Reference Genes | StActin           |                      | 5'-AGATGCTTACGCTG GATGGAATGC-3' | 5'-TTCCGGTGTGGTTGGAT TCTGTTC-3'  |

Note: Primers were designed based on RNA-seq derived CDS sequences using Primer3. Amplicons were selected from unique coding regions avoiding low-complexity and repetitive sequences.

2.7. CDS Cloning of FRO and IRT Genes and Bioinformatic Analysis

Gene-specific primers were designed from the CDS sequences of *FRO* (PGSC0003DMG400010125) and *IRT/ZIP* (PGSC0003DMG400010369) in the PGSC database. PCR was performed on 05P root cDNA in 20 µL reactions containing 1 µL cDNA, 0.2 µL each primer, 10 µL 2× FastTaq Master Mix, and 8.6 µL RNase-free water. Cycling conditions: 95 °C for 3 min; 35 cycles of 95 °C for 20 s, 62 °C for 20 s, and 72 °C for 1 min; 72 °C for 5 min; and hold at 4 °C.

Products were purified, cloned into a T-vector, and transformed into *E. coli* DH5α. Positive clones were verified by colony PCR and Sanger sequencing. Primer sequences used for cloning are provided in Table 3.

Table 3. Primer information for cloning vector.

| Gene       | Forward Primer (5'→3')          | Reverse Primer (5'→3')         |
|------------|---------------------------------|--------------------------------|
| <i>FRO</i> | AAAAAGCAGGCTTCATGGAGGAAGTAACCAA | AGAAAGCTGGGTCTCACAACCTTGGGGCAG |
| <i>IRT</i> | AAAAAGCAGGCTTCATGGCCTCAGCATCTCT | AGAAAGCTGGGTCTTAGTAGACATAGCCTT |
| AttB       | GGGGACAAGTTTGTACAAAAAAGCAGGCT   | GGGGACCACTTTGTACAAGAAAGCTGGGT  |

Bioinformatic analysis included CDS assembly, ORF prediction, protein characterization, phylogeny, chromosomal localization, synteny, motif identification, gene structure, and promoter cis-element prediction. DNAMAN 9.0 (Lynnon Biosoft, Vaudreuil-Dorion, QC, Canada) was used for sequence assembly; SMART (<http://smart.embl-heidelberg.de/>, accessed on 10 June 2026) for domain analysis; MEGA 11 (Center for Evolutionary Medicine and Informatics, Tempe, AZ, USA) for phylogenetic reconstruction; TBtools v1.098 (South China Agricultural University, Guangzhou, China) for chromosomal distribution and synteny; MEME Suite version 5.4.1 (<http://meme-suite.org/>, accessed on 10 June 2026) for motif identification; and PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>, accessed on 10 June 2026) for cis-element prediction.

2.8. Statistical Analysis and Data Presentation

Ionome data are presented as mean ± SE. DEGs were summarized by number, regulation direction, and tissue distribution. GO terms with FDR < 0.05 were considered significant. KEGG pathways were ranked by nominal *p*-value; none reached significance after FDR correction, so results were interpreted descriptively. qRT-PCR data were analyzed by the 2<sup>−ΔΔCt</sup> method. Differences among treatments and genotypes were tested by two-way ANOVA. We used Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA) for data organization and initial processing of the germplasm screening and ionomic datasets, and Origin 2021 (OriginLab Corporation, Northampton, MA, USA) for statistical

analyses and graphing. Transcriptomic and qRT-PCR data were handled with Python version 3.9 (Python Software Foundation, Wilmington, DE, USA). The conceptual regulatory model was assembled in Microsoft PowerPoint, based on our experimental results and supported by evidence from the literature.

### 3. Results

To characterize genotype- and tissue-dependent responses to changes in Fe availability, the ionomes and transcriptomes of 05P and CI5 were examined under Fe-deficient (Fe1), Fe-sufficient (Fe40), and Fe-excess (Fe120) conditions. After 7 days of treatment, roots, stems, and leaves were collected separately for tissue-resolved analyses. Following a description of the germplasm screening that selected the two lines, we present their ionomic and transcriptomic responses to Fe deficiency and excess, together with functional enrichment, qRT-PCR validation, and candidate gene characterization, and conclude with a proposed regulatory model.

#### 3.1. *Ionome Reprogramming Reveals Genotype- and Tissue-Dependent Responses to Iron Availability*

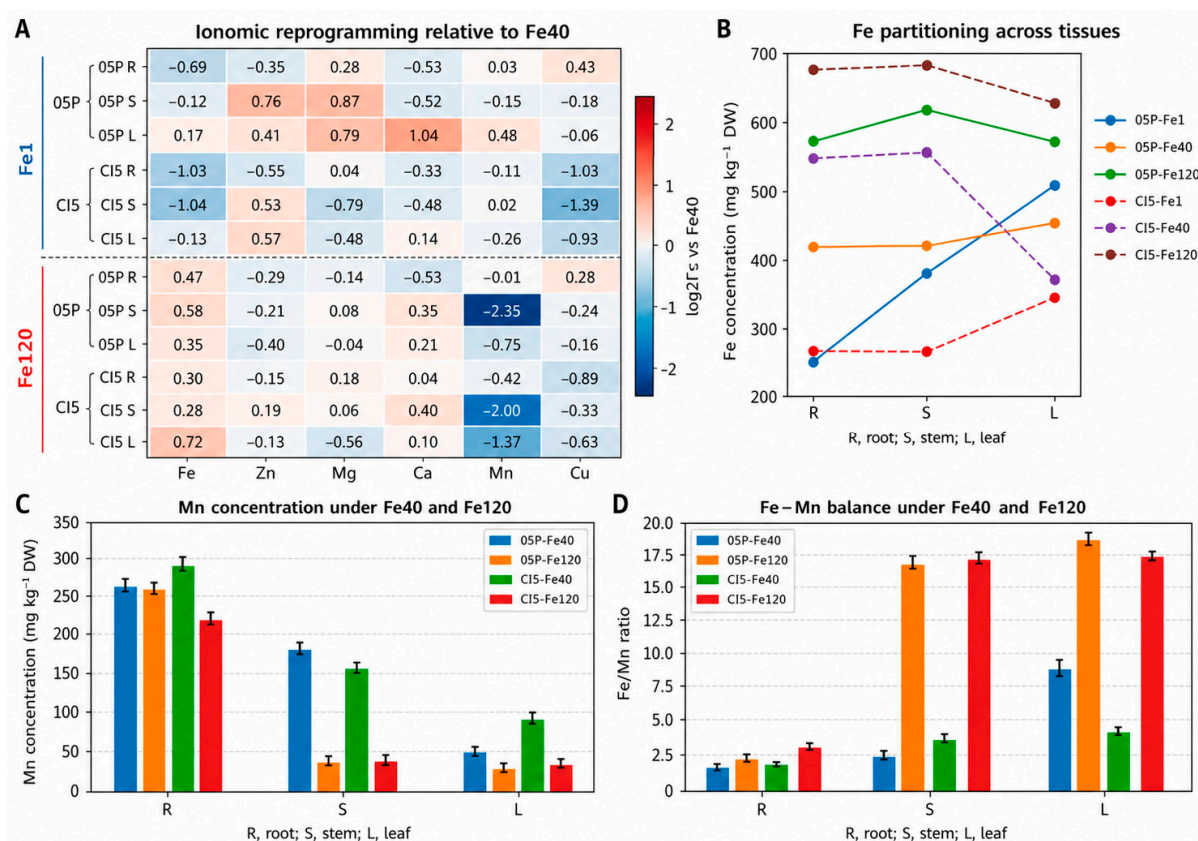
Having established the phenotypic extremes of 05P and CI5, we next examined how these genotypes respond to contrasting Fe supplies at the ionomic level. We quantified Fe, Zn, Mg, Ca, Mn, and Cu in roots, stems, and leaves of the two extreme-contrast genotypes 05P and CI5 under Fe1, Fe40, and Fe120 (Figure 2). These genotypes were selected to represent the phenotypic extremes of tuber Fe accumulation identified in the 404-accession screening (Figure 1A,B), thereby maximizing the potential to detect genotype-dependent molecular divergence. Iron availability reshaped ionomic profiles in both genotypes, with strong tissue specificity. Two-way ANOVA showed significant genotype  $\times$  treatment interactions for all elements across all tissues (Figure 2A), indicating that ionomic responses depend on both genotype and tissue context.

Under Fe1, Fe depletion in roots and stems was more severe in CI5 than in 05P, while 05P maintained leaf Fe. Root Fe fell by 37.9% in 05P and 50.9% in CI5 vs. Fe40; stem Fe fell by 7.8% in 05P but 51.4% in CI5. Leaf Fe rose by 12.7% in 05P but fell by 8.7% in CI5, giving higher leaf Fe in 05P (513.8 vs. 353.4 mg kg<sup>-1</sup> DW) (Figure 2B). Thus, 05P maintained relatively stable shoot Fe levels under deficiency, while CI5 showed more pronounced Fe declines in roots and stems.

Fe1 also triggered tissue-specific reallocation of other elements (Figure 2A). In 05P, leaves accumulated Zn, Mg, Ca, and Mn, with large increases in Ca (+106.1%), Mg (+72.8%), and Mn (+39.7%). CI5 showed reduced root Zn and Cu, with broader elemental disruption. Iron deficiency therefore induced coordinated multi-element redistribution, but the strategy differed between genotypes.

Under Fe120, both genotypes accumulated more Fe, though with different magnitudes and distributions (Figure 2A,B). Root, stem, and leaf Fe rose by 38.8%, 49.2%, and 27.4% in 05P, and by 22.8%, 21.2%, and 64.3% in CI5. CI5 showed higher absolute Fe concentrations (e.g., 681.4 vs. 578.5 mg kg<sup>-1</sup> DW in roots) (Figure 2B), reflecting distinct Fe accumulation patterns under excess supply.

Fe excess also disrupted Mn balance, especially in aerial tissues (Figure 2C). Root Mn fell moderately, but stem Mn dropped by 80.4% (05P) and 74.9% (CI5), and leaf Mn by 40.4% (05P) and 61.2% (CI5). Fe/Mn ratios in stems and leaves rose sharply (Figure 2D). While this pattern is consistent with Fe–Mn antagonism, other explanations—such as Mn uptake inhibition by high Fe at the root surface, reduced Mn mobility due to physiological changes, or dilution effects from increased biomass—cannot be excluded based on concentration data alone.

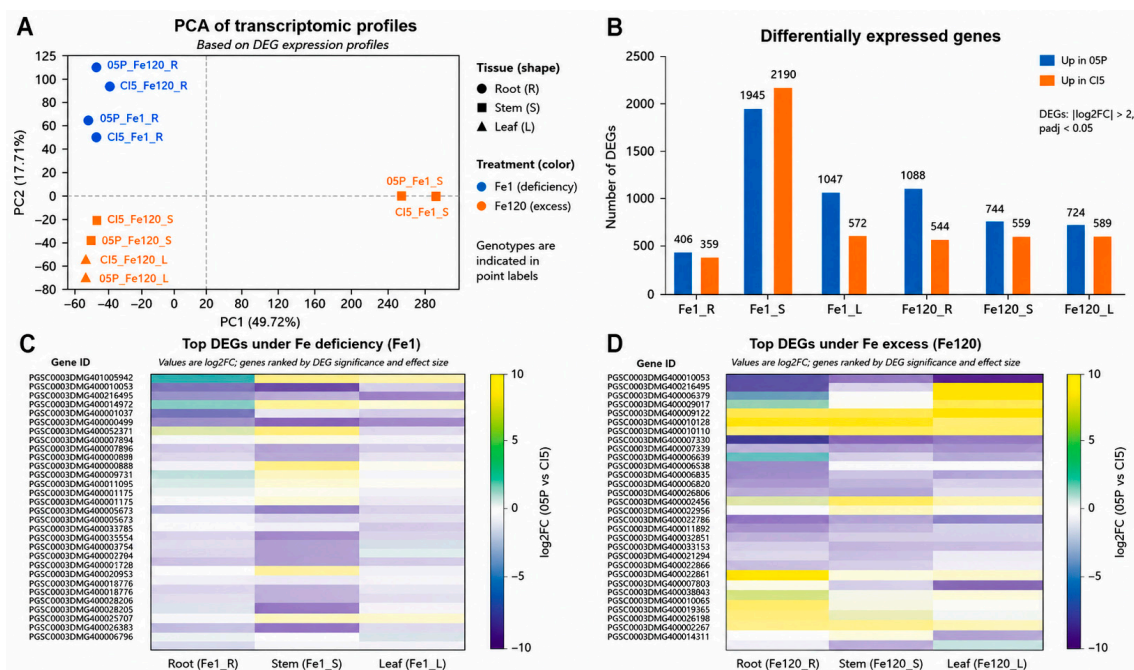


**Figure 2.** Ionomic reprogramming and Fe–Mn balance in 05P and CI5 under contrasting Fe supplies. (A) Heatmap showing log<sub>2</sub> fold changes in Fe, Zn, Mg, Ca, Mn, and Cu concentrations in roots (R), stems (S), and leaves (L) of the two potato genotypes relative to the Fe-sufficient control (Fe40). Values in each cell indicate log<sub>2</sub>FC relative to Fe40 within the same genotype and tissue. (B) Tissue-specific Fe partitioning in 05P and CI5 under Fe deficiency (Fe1), Fe sufficiency (Fe40), and Fe excess (Fe120). (C) Mn concentrations in roots, stems, and leaves of 05P and CI5 under Fe40 and Fe120 conditions, highlighting the strong decline in shoot Mn under excess Fe. (D) Fe/Mn ratios in roots, stems, and leaves of 05P and CI5 under Fe40 and Fe120. The increased ratios under Fe excess are consistent with potential Fe–Mn antagonism, though alternative explanations cannot be excluded based on concentration data alone. All values are shown as means  $\pm$  SE ( $n = 3$  biological replicates). In panel (A), red and blue indicate increases and decreases, respectively, relative to Fe40.

In summary, 05P and CI5 showed contrasting strategies: 05P buffered leaf Fe and coordinated multi-element uptake under deficiency, while CI5 accumulated more total Fe under excess but suffered stronger Fe–Mn imbalance in shoots. Genotypic differences thus reflect tissue-specific ionome reprogramming rather than simple Fe concentration changes. These ionomic differences prompted us to investigate the transcriptional changes that might underlie the contrasting Fe accumulation and redistribution patterns between the two genotypes.

### 3.2. Transcriptomic Responses Under Iron Deficiency and Iron Excess

We analyzed transcriptomes of roots, stems, and leaves from 05P and CI5 under Fe1 and Fe120. RNA-seq data quality was high across all samples. After filtering, 36 libraries yielded a mean of  $21.6 \pm 1.4$  million clean reads per sample, with Q30  $\geq 93.5\%$  and mapping rates  $\geq 85\%$  (Supplementary Table S1). Principal component analysis (PCA) of DEGs separated samples by tissue and treatment (Figure 3A), showing that Fe availability triggers substantial transcriptional reprogramming (Figure 3).



**Figure 3.** Transcriptomic responses of two potato genotypes under iron deficiency and iron excess conditions. **(A)** Principal component analysis (PCA) of transcriptomic profiles based on differentially expressed genes (DEGs) across root (R), stem (S), and leaf (L) tissues of two potato genotypes (05P and CI5) under iron deficiency (Fe1) and iron excess (Fe120) conditions. Samples cluster according to tissue type and treatment, indicating strong transcriptional reprogramming in response to iron availability. **(B)** Number of differentially expressed genes (DEGs) between 05P and CI5 under Fe1 and Fe120 conditions across different tissues. Upregulated genes in 05P and CI5 are shown separately. DEGs were identified using thresholds of  $|\log_2 \text{fold change}| \geq 2$  and adjusted  $p$  value  $< 0.05$ . **(C)** Heatmap showing the expression patterns ( $\log_2$  fold change, 05P vs. CI5) of the top 30 DEGs under iron deficiency (Fe1). Gene expression changes are displayed across root, stem, and leaf tissues, revealing tissue-specific and genotype-dependent transcriptional regulation. **(D)** Heatmap showing the expression patterns ( $\log_2$  fold change, 05P vs. CI5) of the top 30 DEGs under iron excess (Fe120). Distinct regulatory modules are observed compared with iron deficiency, indicating iron-level-dependent transcriptional reprogramming.

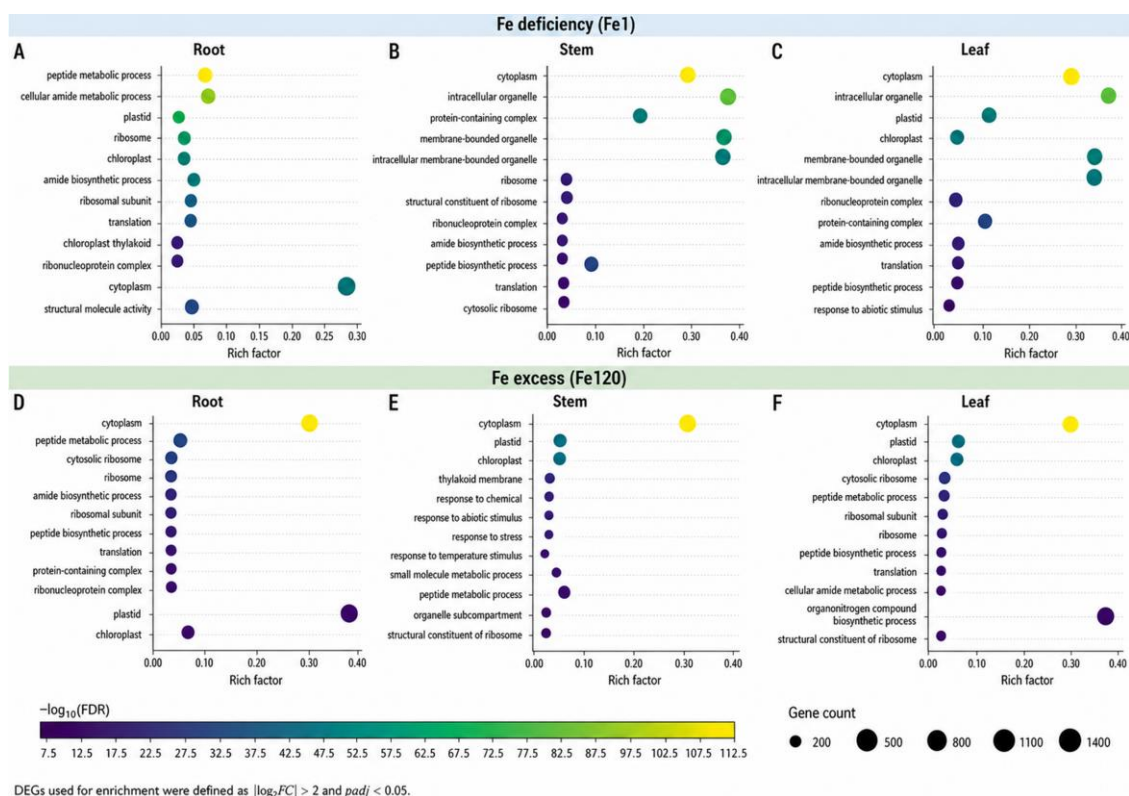
DEG numbers varied by tissue and treatment. Under Fe1, stems showed the highest transcriptional plasticity: 4135 DEGs in total, with 1945 up in 05P and 2190 up in CI5. Leaves had 1619 DEGs (1047 up in 05P, 572 in CI5), and roots had 765 (406 up in 05P, 359 in CI5). Only 323 DEGs were shared across all three tissues, while 3219 were stem-specific. Thus, Fe deficiency drove the strongest transcriptional divergence in stems.

Under Fe120, the pattern shifted to roots: 1632 DEGs (1088 up in 05P, 544 in CI5), compared with 1313 in leaves (724 up in 05P, 589 in CI5) and 1303 in stems (744 up in 05P, 559 in CI5). Root-specific DEGs reached 939, versus fewer tissue-specific DEGs elsewhere. Thus, Fe excess triggered the strongest transcriptional divergence in roots.

The top 30 DEGs from each condition showed tissue-specific patterns (Figure 3C,D). Under Fe1, these genes diverged sharply between genotypes, especially in stems. Under Fe120, a different set showed coordinated expression across tissues, pointing to distinct transcriptional strategies for deficiency versus excess. RNA-seq quality-related data are provided in Supplementary Table S1.

### 3.3. GO Enrichment Reveals Tissue-Specific Functional Reprogramming

GO enrichment was performed on DEGs from each tissue under Fe1 and Fe120 (Figure 4A–F).



**Figure 4.** GO enrichment analysis of differentially expressed genes in potato roots, stems, and leaves under iron deficiency and iron excess conditions. GO enrichment analysis of differentially expressed genes (DEGs) identified between the two potato genotypes (05P and CI5) under iron deficiency (Fe1) and iron excess (Fe120) across root (R), stem (S), and leaf (L) tissues. (A–C) GO enrichment results for DEGs under iron deficiency (Fe1) in root (A), stem (B), and leaf (C), respectively. (D–F) GO enrichment results for DEGs under iron excess (Fe120) in root (D), stem (E), and leaf (F), respectively. Each bubble represents an enriched GO term. The x-axis indicates the rich factor (gene ratio), while bubble size represents the number of DEGs assigned to each GO term. Bubble color indicates enrichment significance, expressed as  $-\log_{10}(\text{FDR})$ , with deeper colors representing higher statistical significance. DEGs were identified using thresholds of  $|\log_2FC| > 2$  and adjusted  $p$  value ( $padj$ )  $< 0.05$ . Only significantly enriched GO terms ( $\text{FDR} < 0.05$ ) are shown. R, root; S, stem; L, leaf. Rich factor = (number of DEGs in GO term)/(total DEGs in background set).

Under Fe1, roots showed enrichment in peptide metabolism, ribosome biogenesis, translation, and plastid functions (Figure 4A); stems in organelle organization and ribosomal structure (Figure 4B); and leaves in chloroplast components, ribonucleoprotein complexes, and abiotic stress responses (Figure 4C). Genotype-dependent DEGs under Fe1 were enriched in peptide metabolism, ribosome biogenesis, and translation-related categories, suggesting that translational processes contribute to the divergence between 05P and CI5 under Fe-deficient conditions. Under Fe120, roots again showed enrichment in ribosomal and peptide metabolic pathways (Figure 4D), stems in chemical and temperature stress responses (Figure 4E), and leaves in ribosomal structure and cytoplasmic organization (Figure 4F). Fe excess therefore disrupted translation across all tissues, but stems specifically activated stress signaling.

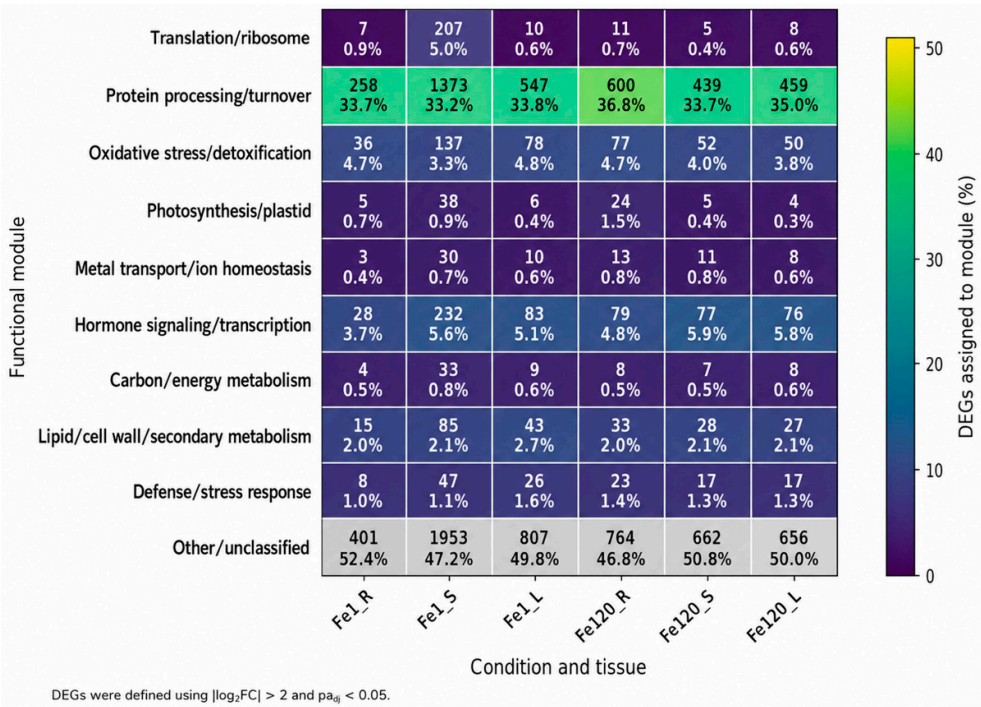
In summary, Fe1 and Fe120 induced partially overlapping but tissue-biased functional shifts: roots and leaves shared translational disruption, while stems showed the strongest stress-response activation under both conditions (Figure 4A–F). Detailed data of the GO enrichment analysis are provided in Supplementary Table S2.

3.4. KEGG Enrichment Analysis

KEGG enrichment was performed on DEGs from each tissue under Fe1 and Fe120. No pathways reached significance after FDR correction (adjusted  $p > 0.05$ ) in any genotype, treatment, or tissue (Supplementary Table S3). The lack of FDR-significant KEGG pathways indicates that the DEGs were not concentrated in a small number of annotated canonical pathways, although pathway-level effects may be underestimated due to incomplete annotation or limited statistical power.

3.5. Functional Classification of DEGs

DEGs from each tissue under Fe1 and Fe120 were grouped into nine functional categories based primarily on GO annotations and manual curation of gene functional descriptions (Figure 5): translation/ribosome biogenesis, protein processing/turnover, oxidative stress/detoxification, photosynthesis/plastid, metal transport/ion homeostasis, hormone signaling/transcription, carbon metabolism, lipid/secondary metabolism, and defense/stress response. It should be noted that these categories represent descriptive groupings of differentially expressed genes rather than statistically enriched KEGG pathways, as no KEGG pathways reached significance after FDR correction (Section 3.4).



**Figure 5.** Functional module-based classification of differentially expressed genes under iron deficiency and iron excess conditions in potato. Differentially expressed genes (DEGs) identified between 05P and CI5 under iron deficiency (Fe1) and iron excess (Fe120) conditions were classified into major functional modules based on KEGG and GO annotation. Modules include translation/ribosome, protein processing/turnover, oxidative stress/detoxification, photosynthesis/plastid, metal transport/ion homeostasis, hormone signaling/transcription, primary carbon metabolism, lipid/secondary metabolism, defense/stress response, and other/unclassified genes. Each cell represents the proportion of DEGs assigned to a given functional module within each tissue (root, stem, and leaf). Color intensity reflects the proportion of genes, and numbers indicate both gene counts and percentages. R, root; S, stem; L, leaf. Functional categories were assigned based on GO annotations and manual curation, not on KEGG pathway enrichment. No KEGG pathways reached significance after FDR correction (Section 3.4).

Under Fe1, genes associated with translation/ribosome biogenesis constituted the largest proportion of DEGs in roots and stems (Figure 5, Fe1), suggesting potential re-

pression of protein synthesis-related processes under Fe limitation. Leaves showed a higher proportion of DEGs annotated to photosynthesis/plastid functions, consistent with potential chloroplast-associated responses.

Under Fe120, the proportion of DEGs associated with protein processing/turnover and oxidative stress/detoxification increased (Figure 5, Fe120), suggesting potential activation of protein quality control and oxidative stress responses. Hormone signaling and metal transport-related genes were differentially expressed across all tissues and both stresses, suggesting their potential involvement in Fe stress responses.

In summary, Fe1 and Fe120 were associated with differential expression patterns across functional categories: Fe1 showed enrichment of translation-related DEGs, while Fe120 showed increased representation of protein processing/turnover and oxidative stress-related DEGs. Hormone signaling and metal transport-related genes were represented across both conditions, suggesting their potential relevance to Fe stress responses. These patterns are descriptive interpretations of gene category distributions and should be regarded as hypotheses pending functional validation.

### 3.6. Validation of RNA-Seq Results by qRT-PCR

To validate the RNA-seq dataset, representative Fe-responsive genes were selected for quantitative real-time PCR (qRT-PCR) analysis, including iron transporters (*IRT1*, *FRO1*, *FRO2*), transcription factors (bHLH family members), and ion homeostasis-related genes (*Ca-ATPase*, *NCX*, and *Fd3*). Expression patterns obtained from qRT-PCR were compared with RNA-seq-derived log<sub>2</sub> fold changes under iron deficiency (Fe1), iron sufficiency (Fe40), and iron excess (Fe120) conditions in root, stem, and leaf tissues (Figure 6).

Scatter plot analysis revealed a significant positive correlation between RNA-seq and qRT-PCR expression values across all selected genes (Figure 6A). Quantitative correlation analysis confirmed this relationship (Pearson  $r = 0.604$ ,  $p < 0.0001$ ; Spearman  $\rho = 0.520$ ,  $p = 0.0002$ ).

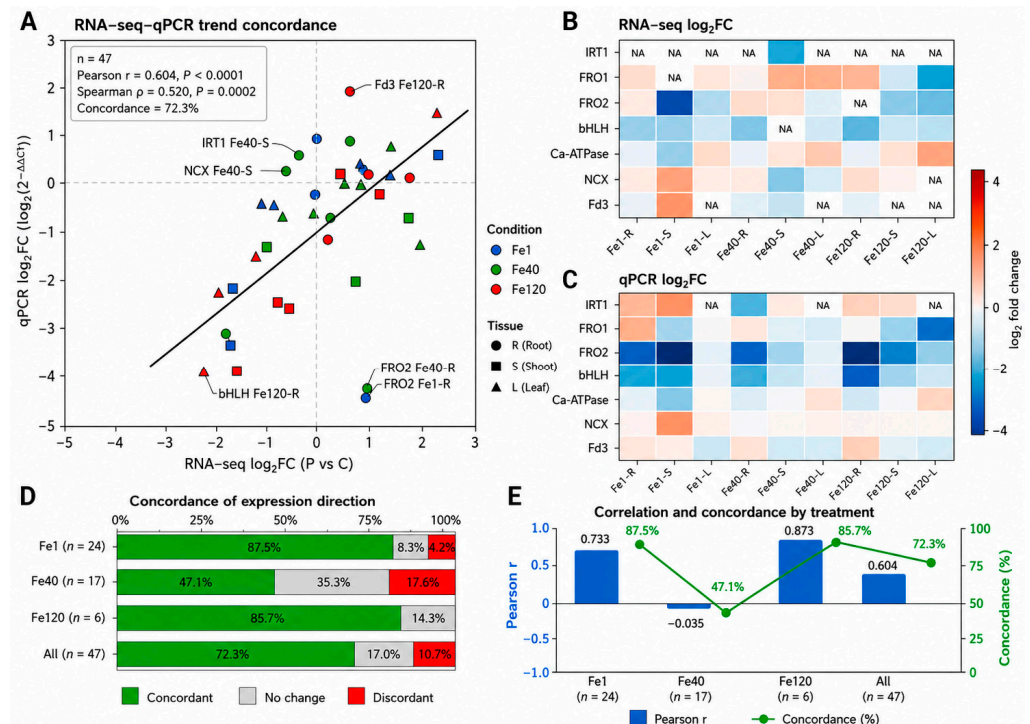
At the global level, 72.3% of genes exhibited concordant directional changes between RNA-seq and qRT-PCR across all treatments (Figure 6B), supporting the robustness of transcriptome-derived expression trends.

Treatment-specific concordance analysis showed that the highest consistency was observed under Fe1 (87.5%), followed by Fe120 (85.7%), whereas Fe40 displayed relatively lower concordance (47.1%) (Figure 6C). This suggests that transcriptional regulation under iron deficiency and excess is more pronounced and stable than under iron-sufficient conditions.

At the gene-level resolution, representative Fe uptake-related genes showed strong induction of *FRO2* and *IRT1* under iron deficiency across both platforms (Figure 6D). In contrast, *FRO1* and bHLH transcription factors exhibited tissue-dependent expression patterns consistent between RNA-seq and qRT-PCR.

Ion homeostasis-related genes, including *Ca-ATPase* and *NCX*, displayed moderate but consistent expression trends across treatments (Figure 6E), further supporting the reliability of the transcriptomic dataset.

qRT-PCR results generally supported the RNA-seq-derived expression trends, particularly under Fe1 and Fe120, although concordance was weaker under Fe-sufficient conditions.



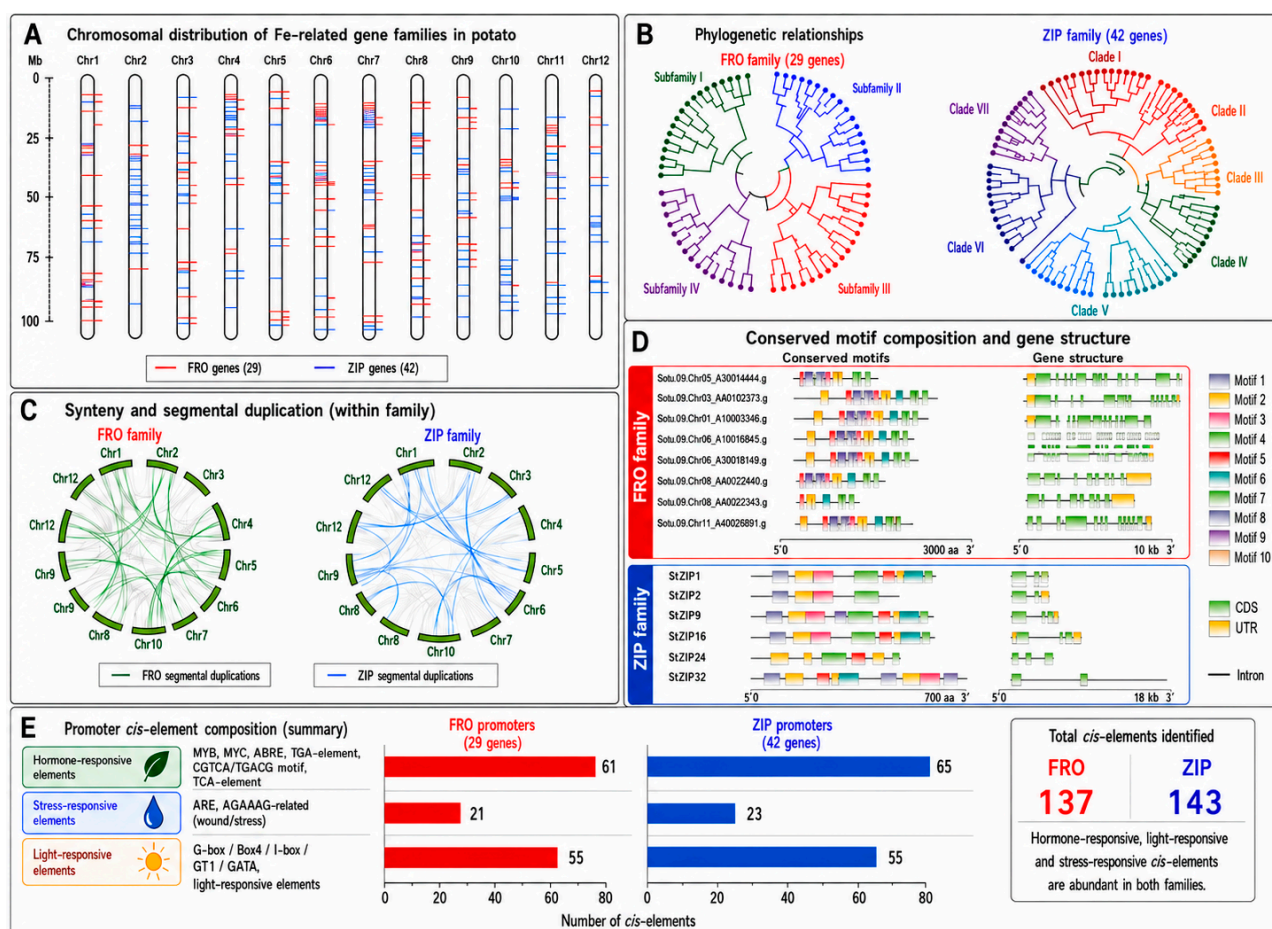
**Figure 6.** Validation of RNA-seq results by qPCR and assessment of cross-platform concordance for Fe-responsive genes. (A) Correlation analysis between RNA-seq-derived  $\log_2$  fold change (P vs. C) and qPCR-derived  $\log_2(2^{-\Delta\Delta Ct})$  values for selected Fe-responsive genes across root (R), stem (S), and leaf (L) tissues under Fe deficiency (Fe1), Fe sufficiency (Fe40), and Fe excess (Fe120) conditions. Pearson and Spearman correlation coefficients are indicated. Expression was normalized against *StActin* as described in Section 2.6 (see Table 2 for primer sequences). (B) Heatmap showing RNA-seq-derived  $\log_2$  fold changes in selected genes, including iron transporters (*IRT1*, *FRO1*, *FRO2*), transcription factors (*bHLH*), and ion homeostasis-related genes (*Ca-ATPase*, *NCX*, and *Fd3*) across different treatments and tissues. (C) Heatmap showing qPCR-derived  $\log_2$  fold changes for the same set of genes under corresponding conditions. (D) Concordance analysis of expression direction between RNA-seq and qPCR results. Genes were classified as concordant (same direction), non-changing, or discordant across Fe1, Fe40, and Fe120 treatments. Percentages represent the proportion of genes in each category. (E) Treatment-specific correlation coefficients (Pearson  $r$ ) and concordance rates (%) between RNA-seq and qPCR data for Fe1, Fe40, Fe120, and overall datasets. DEGs were defined using thresholds of  $|\log_2FC| > 2$  and adjusted  $p$  value  $< 0.05$  in RNA-seq analysis. Data represent comparisons between genotype 05P and CI5 across root, stem, and leaf tissues.

### 3.7. Characterization and Evolutionary Analysis of Fe-Related Candidate Genes in Potato

To characterize genes differentially expressed under Fe stress and potentially related to iron uptake and transport, selected candidates were cloned and systematically analyzed at genomic and evolutionary levels (Figure 7).

The full-length CDSs of *FRO* and *IRT* genes were successfully amplified from root cDNA of 05P. The *FRO* gene encodes a 728-amino-acid protein with a CDS length of 2184 bp, whereas the *IRT* gene contains an 846 bp CDS (Figure 7D). The successful amplification and sequencing of the CDSs confirmed the transcript sequences of the selected *FRO* and *IRT/ZIP* candidates in 05P roots, providing a basis for subsequent functional analyses.

At the genome-wide level, 29 *FRO* and 44 *ZIP* family members were identified in the potato genome (Figure 7A). Phylogenetic reconstruction classified *FRO* genes into four subfamilies and *ZIP* genes into seven distinct clades (Figure 7B), revealing substantial lineage-specific diversification within both transporter families.



**Figure 7.** Evolutionary and structural characterization of *FRO* and *ZIP* gene families in potato. (A) Chromosomal distribution of *FRO* and *ZIP* gene family members across the 12 potato chromosomes. Gene positions are mapped according to the physical coordinates of the *Solanum tuberosum* reference genome. (B) Phylogenetic relationships of *FRO* (29 members) and *ZIP* (44 members) gene families constructed using the maximum likelihood method with 1000 bootstrap replicates. Distinct clades are indicated by different colors. (C) Synteny and segmental duplication analysis of *FRO* and *ZIP* gene families in potato. Segmental duplication events are highlighted to illustrate evolutionary expansion patterns across chromosomes. (D) Gene structure and conserved motif composition of *FRO* and *ZIP* gene families. Exon–intron organization is shown together with 10 conserved motifs identified using MEME analysis. (E) Distribution of cis-regulatory elements in 2 kb promoter regions of *FRO* and *ZIP* genes. Elements are classified into hormone-, stress-, and light-responsive categories. Bootstrap support values (1000 replicates) values  $\geq 70\%$  are shown.

Synteny analysis identified 29 segmental duplication events within the *FRO* family (Figure 7C), indicating that gene duplication has been a major driver shaping the expansion and diversification of Fe transport systems in potato.

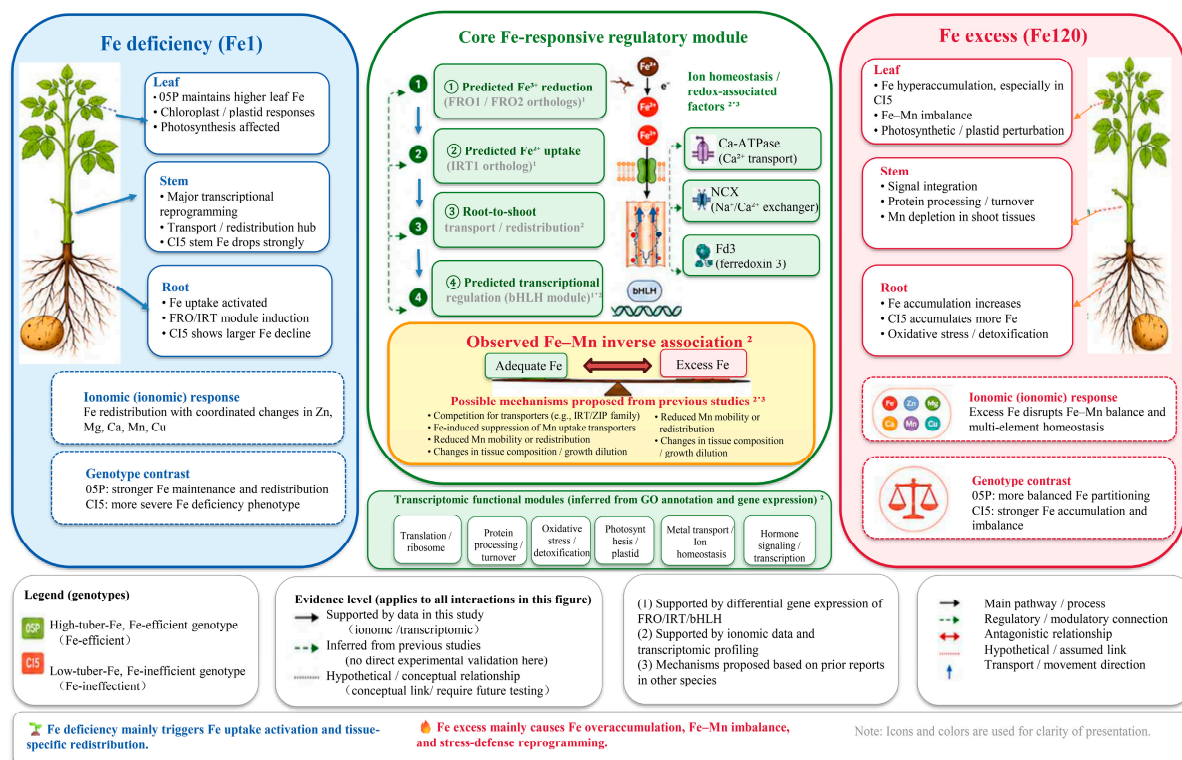
Structural analyses showed that members within the same phylogenetic clade exhibited highly conserved exon–intron organization and motif composition, whereas pronounced divergence was observed among different clades (Figure 7D). This pattern suggests a conserved structural backbone coupled with progressive functional divergence during evolution.

Promoter cis-element analysis revealed a rich repertoire of regulatory motifs, including MYB, MYC, ABRE, TCA-element, CGTCA-motif, TGACG-motif, G-box, and ARE elements (Figure 7E). The presence of these elements indicates that Fe-related genes are embedded within a multilayer regulatory network integrating phytohormone signaling, light responsiveness, and abiotic stress pathways.

These Fe-related gene families exhibit evolutionary expansion and structural diversification with conserved domain architectures and variable promoter elements. The integrated evidence from Figure 7A–E suggests their potential positioning within pathways associated with iron acquisition, transport, and environmental adaptation in potato, pending direct functional validation.

### 3.8. Integrated Model of Fe Supply-Dependent Ionic and Transcriptomic Regulation in Potato

Based on integrated ionic profiles, transcriptomic reprogramming, qRT-PCR validation, and candidate gene cloning and bioinformatic analyses, a systems-level model of iron supply-dependent regulation in potato was constructed (Figure 8). This model explains the contrasting Fe-use strategies observed between the high-Fe genotype 05P and the low-Fe genotype CI5.



**Figure 8.** A working model summarizing the differential responses of 05P and CI5 to Fe deficiency (Fe1) and Fe excess (Fe120). Solid arrows indicate relationships supported by the present ionic and transcriptomic data (e.g., tissue-specific Fe redistribution, Mn depletion under excess, differential expression of ribosomal/photosynthetic genes). Dashed arrows indicate predicted steps based on homology with Arabidopsis *FRO2/IRT1* and previous functional studies in other species. Dotted arrows indicate hypothesized regulatory connections that remain to be experimentally validated in potato. Under Fe deficiency, differential expression suggests activation of a predicted Fe acquisition module in roots, potentially involving *FRO1/FRO2* orthologs in  $\text{Fe}^{3+}$  reduction and *IRT1* in  $\text{Fe}^{2+}$  uptake based on Arabidopsis homology. Stem tissues showed the strongest transcriptional divergence between genotypes, while leaf tissues exhibited differential expression of plastid and chloroplast-associated genes. Under Fe excess, both genotypes accumulated more Fe, with CI5 showing higher Fe concentrations and more pronounced shoot Mn depletion. Oxidative stress responses and protein processing/turnover genes were differentially expressed. *Ca-ATPase*, *NCX*, and *Fd3* were validated by qPCR as differentially expressed under Fe stress; their functional roles in Fe homeostasis are inferred from expression patterns and orthology. This model integrates observations from the present study with predictions derived from comparative genomics and

should be regarded as a working hypothesis pending direct functional validation in potato. Superscript numbers indicate the evidence level: <sup>1</sup> supported by differential gene expression of *FRO/IRT/bHLH*; <sup>2</sup> supported by ionomic data and transcriptomic profiling; <sup>3</sup> mechanisms proposed based on prior reports in other species.

### 3.8.1. Iron Deficiency Module

Under iron deficiency, the regulatory network is characterized by activation of the canonical Fe acquisition pathway in roots, including predicted ferric chelate reduction involving *FRO1/FRO2* orthologs and predicted ferrous iron uptake involving *IRT1*, based on homology with Arabidopsis *FRO2* and *IRT1*. This root-localized activation is accompanied by pronounced transcriptional reprogramming in stems, suggesting that stem tissues function as an intermediate regulatory hub linking root acquisition and shoot redistribution of iron (Figure 8).

At the ionomic level, leaf tissues showed differential Fe concentrations between genotypes, with 05P maintaining higher Fe levels than CI5, consistent with distinct patterns of Fe retention and redistribution in aboveground tissues under limiting conditions. In parallel, plastid- and chloroplast-associated responses dominate in leaves, reflecting coordinated adjustment of photosynthetic and iron-dependent metabolic processes under Fe scarcity.

### 3.8.2. Iron Excess Module

Under iron excess, both genotypes exhibit enhanced Fe accumulation across tissues; however, CI5 shows a stronger tendency toward Fe overaccumulation in roots, stems, and leaves, indicating reduced control over Fe sequestration and distribution.

Fe excess also induces a pronounced disturbance of Fe–Mn homeostasis, particularly in stem and leaf tissues, as evidenced by reduced Mn levels and elevated Fe/Mn ratios. This elemental imbalance is accompanied by activation of oxidative stress responses, protein turnover pathways, and defense-related transcriptional programs, reflecting the cellular cost of iron toxicity (Figure 8).

Transcriptomic data indicate that iron excess triggers a shift from acquisition-centered regulation to stress-defense reprogramming, involving enhanced protein processing and redox-related pathways across tissues.

### 3.8.3. Genotype-Dependent Adaptive Strategies

Integration of multi-omics data supports two contrasting adaptive strategies between the genotypes (Figure 8). 05P is characterized by distinct ionomic patterns, including relatively stable Fe distribution across tissues and higher shoot Fe maintenance under deficiency, together with less pronounced multi-element disruption under excess conditions.

In contrast, CI5 showed more pronounced ionomic responses to Fe fluctuations, including steeper Fe declines under deficiency and higher Fe accumulation under excess supply, accompanied by more marked changes in elemental composition, particularly Fe–Mn imbalance.

### 3.8.4. System-Level Interpretation

These processes suggest a potential coordinated response framework in which iron availability is associated with integrated ionomic and transcriptional reprogramming across tissues, potentially contributing to genotype-dependent Fe stress responses in potato.

## 4. Discussion

The integrated model presented above raises several questions about the broader implications of our findings. Below, we discuss how these results relate to existing knowledge, acknowledge limitations, and identify future directions.

### 4.1. Natural Variation in Tuber Fe Content Provides a Genetic Foundation for Iron Efficiency Dissection

Initial screening of 404 potato accessions revealed extensive natural variation in tuber Fe concentration (1.89–66.20 mg kg<sup>−1</sup> DW), identifying 05P as a high-Fe extreme (top 0.3%) and CI5 as a low-Fe extreme (bottom 1.6%). This range exceeds and extends previously reported variation in cultivated potato germplasm (typically ~5–50 mg kg<sup>−1</sup> DW), underscoring substantial genetic potential for Fe accumulation and redistribution [10,29,30].

The use of extreme-phenotype genotypes follows the rationale that maximum phenotypic contrast enhances the statistical and biological power to detect molecular differences associated with Fe accumulation capacity. This approach has been successfully applied in multi-omics studies of mineral nutrition in crops, where extreme individuals from large populations are selected to identify candidate genes and pathways that may be diluted in comparisons of intermediate phenotypes. However, we acknowledge that 05P and CI5 represent the tails of the phenotypic distribution and may not fully capture the molecular diversity of intermediate Fe accumulators. The ionomic and transcriptomic patterns identified here therefore reflect the responses of extreme genotypes rather than the ‘average’ potato response.

To assess the representativeness of these findings, future studies should validate the identified candidate genes (e.g., *FRO* and *IRT/ZIP* family members) across a broader panel of genotypes spanning the full Fe concentration gradient. Such validation would establish whether the molecular mechanisms observed in 05P and CI5 are conserved across the germplasm or represent genotype-specific adaptations. Nevertheless, the pronounced phenotypic divergence between these genotypes provides a robust starting framework for identifying candidate molecular components associated with Fe stress responses, which can then be functionally validated and surveyed across diverse genetic backgrounds.

### 4.2. Iron Stress Induces Hierarchical Ionome Remodeling and Cellular State Reprogramming Rather than Canonical Pathway Activation

Integrated ionomic and transcriptomic analyses demonstrated that both Fe deficiency and excess trigger coordinated remodeling of multiple essential elements (Fe, Mn, Zn, Ca, Mg, and Cu), rather than isolated iron-specific responses. This coordinated behavior indicates that iron availability perturbs systemic elemental homeostasis at the network level.

GO enrichment analyses revealed differential expression of genes associated with translation and ribosome biogenesis under both stress conditions, whereas KEGG analysis did not identify significantly enriched pathways after multiple-testing correction. This pattern is consistent with the interpretation that iron stress may not induce coordinated activation of discrete metabolic pathways detectable by KEGG enrichment, but rather triggers broad transcriptional reprogramming across multiple functional categories.

These observations align with the hypothesis proposed in the recent literature that metal stress responses may involve translational control, proteostasis regulation, and cellular resource reallocation as complementary mechanisms to pathway-specific transcriptional modules [16,31,32]. At a systems level, iron perturbation may be associated with a shift in cellular equilibrium toward maintenance and stress buffering, although this interpretation remains speculative pending experimental validation of the functional consequences of the observed transcriptional changes [33,34].

#### 4.3. Systemic Iron Adaptation Involves Coordinated Gene Family Evolution and Shoot-Mediated Redistribution Rather than a Strict Root-Centric Model

Genome-wide analysis revealed significant expansion of *FRO* (29 members) and *ZIP* (44 members) gene families in potato, driven predominantly by segmental duplication events followed by structural diversification. This expansion pattern is consistent with potential evolutionary pressure for increased diversity in iron uptake and translocation systems, although functional divergence of individual paralogs remains to be experimentally validated [1,2,13].

Our results provide tissue-resolved evidence complementing the classical root-centric view of iron acquisition. Evidence from tissue-resolved transcriptomic patterns supports a systemic redistribution framework in which shoot tissues actively participate in iron homeostasis through long-distance transport and feedback regulation. Although roots remain the primary acquisition interface, the pronounced stem-associated transcriptional divergence under Fe deficiency suggests that stems may participate in coordinating long-distance Fe redistribution, although direct evidence from transport flux or tissue localization will be required to confirm this role.

In this context, the strong transcriptional responsiveness observed in stems likely reflects their role in coordinating inter-organ nutrient flux rather than serving as passive conduits. This interpretation aligns with increasing recognition of shoot–root communication networks in plant mineral homeostasis, particularly involving phloem-mediated signaling and systemic feedback regulation [1,2,35–37].

The concurrent rise in Fe and decline in Mn concentrations under Fe excess raises the possibility of Fe–Mn antagonism, although concentration changes alone cannot establish causality. Several mechanisms could explain the observed Mn depletion: (i) direct competition between  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  for uptake via shared divalent metal transporters such as *IRT1*, as reported in *Arabidopsis* and rice [38–41]; (ii) Fe-induced suppression of Mn uptake transporter expression or activity; (iii) reduced Mn mobility or redistribution due to physiological changes under Fe excess; or (iv) dilution effects if Fe accumulation increases tissue biomass disproportionately. Distinguishing among these mechanisms would require direct evidence, such as transport assays comparing  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  uptake kinetics in yeast or oocytes expressing potato *IRT1*/*FRO* candidates, or isotope tracing experiments to assess Mn flux under varying Fe supply. In the absence of such data, we interpret the elevated Fe/Mn ratios as consistent with potential Fe–Mn antagonism but do not claim to have demonstrated competitive transport in potato.

#### 4.4. Comparison with Previous Studies on Fe Homeostasis in Potato and Strategy I Species

Our findings extend earlier work on potato Fe homeostasis in several ways. Legay et al. [28] profiled Fe-related gene expression in potato plantlets under deficiency and overload, reporting that *StFRO1*, *StIRT1*, and *StNRAMP1* are upregulated under Fe limitation. That study convincingly demonstrated that potato uses the canonical Strategy I machinery, but it was largely confined to root-level expression under a limited set of conditions and did not explore tissue-specific ionomic changes or genotype-dependent variation. By profiling three tissues and two genotypes, we show that Fe stress responses are unevenly distributed across organs: stems show the most dramatic transcriptional shifts under deficiency, roots under excess—patterns a root-only approach would miss.

A second point of comparison is with the *Arabidopsis* regulatory framework. In *Arabidopsis*, FIT and PYE are known to coordinate *FRO2* and *IRT1* expression in roots during Fe deficiency [18,19]. Our finding that several potato bHLH genes are differentially expressed in stems hints at a possible shoot-localized signaling component that the *Arabidopsis*-centric model does not capture. Whether these bHLH paralogs are functional

equivalents of FIT or PYE is an open question. More broadly, the potato *FRO* family includes 29 members and the *ZIP* family 44 members—substantially larger than the corresponding *Arabidopsis* families (8 and 15, respectively). This expansion may reflect neofunctionalization linked to potato's tuber-based storage and vegetative propagation, but direct evidence for this is still lacking.

That said, our study leaves several questions unanswered. Transcriptomic data from whole tissues inevitably average over cell types; localized Fe distribution and cell-type-specific responses could be critical for understanding Fe trafficking but are not resolvable here. We also cannot claim that the observed expression changes in *FRO* and *IRT1* orthologs equate to functional activity—ferric chelate reductase assays or yeast complementation experiments will be needed to confirm catalytic or transport function. Our ionomic measurements report steady-state concentrations, not fluxes, so we cannot distinguish whether the Mn depletion seen under Fe excess reflects reduced uptake, increased efflux, or internal remobilization. Finally, the two genotypes we selected represent extremes of the Fe-response spectrum; whether the same mechanisms operate in intermediate genotypes remains to be tested.

#### 4.5. Limitations and Perspectives

A number of limitations should be kept in mind when interpreting these results. We focused on molecular and elemental responses, without measuring physiological endpoints such as biomass, chlorophyll content, photosynthetic performance, or oxidative stress markers. For this reason, the genotype-specific differences we observed are best understood as distinct transcriptional and ionomic response patterns, not as direct indicators of superior Fe tolerance or Fe-use efficiency.

Additionally, our transcriptomic data come from whole tissues, which averages out cell-type-specific expression and conceals spatial heterogeneity. The same tissue-level averaging applies to our ionomic measurements—they report total element concentrations, not subcellular Fe distribution or compartmentalization.

A third caveat concerns functional inference. While the expression patterns of *FRO* and *IRT* orthologs are consistent with their predicted roles in Fe homeostasis, those roles remain inferred from sequence homology and expression profiling rather than experimentally verified in potato. Direct assays of Fe transport or enzyme activity will be required to confirm function. Going forward, a combination of physiological phenotyping, genetic manipulation (e.g., CRISPR/Cas9), transport activity measurements, and high-resolution Fe imaging should help clarify the actual roles of these candidates and the cellular mechanisms that govern Fe uptake and distribution under contrasting Fe regimes. For now, the regulatory framework we propose is best viewed as a working model—one that integrates our data with existing knowledge and points toward specific hypotheses for future testing.

## 5. Conclusions

By integrating ionomic profiles with tissue-resolved transcriptomes, this study reveals how two potato genotypes with contrasting tuber Fe accumulation respond to Fe deficiency and excess.

Two key patterns stand out. Under low Fe, genotype 05P maintains higher leaf Fe levels than CI5. Under high Fe, CI5 accumulates more Fe overall and develops a stronger shoot Fe–Mn imbalance. These genotype-specific ionomic differences are accompanied by distinct transcriptional programs that vary sharply by tissue: stems show the largest genotypic divergence under deficiency, roots under excess. Several *FRO* and *IRT*/*ZIP* orthologs are differentially expressed in patterns consistent with predicted roles in Fe reduction and metal transport, making them plausible candidates for functional follow-up.

At a broader level, our findings highlight that Fe homeostasis in potato involves coordinated adjustments in tissue Fe distribution, cross-metal interactions (notably Fe–Mn), and organ-specific gene expression. The regulatory framework we propose integrates these observations, but it remains a working model. The functional assignments of *FRO* and *IRT/ZIP* candidates rest on expression evidence and sequence homology—not on direct assay in potato. Verifying their roles will require targeted genetic manipulation, transport activity measurements, and spatial mapping of Fe distribution. Such work will also be needed to assess whether these genes can be leveraged for Fe biofortification in potato breeding programs.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae12080945/s1>.

**Author Contributions:** J.W.: Conceptualization, Funding acquisition, Project administration, Supervision, Writing—review and editing. G.Y.: Conceptualization, Funding acquisition, Project administration, Supervision, Writing—review and editing. X.M.: Conceptualization (experimental design), Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing—original draft. Y.M.: Resources, Investigation. W.S.: Resources, Investigation. S.Y.: Writing—review and editing. M.H.: Writing—review and editing. G.P.: Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The original contributions presented in the study are included in the article/Supplementary Material. Ionomic data supporting the findings of this study have been deposited in Mendeley Data [42]. The plant materials (potato varieties CI5 and 05P) are available from the Potato Germplasm Bank of the Biotechnology Institute at Qinghai University, subject to material transfer agreement. Germplasm screening data for 404 accessions (tuber Fe content, 2020–2021) are provided in Supplementary Table S4.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

|       |                                      |
|-------|--------------------------------------|
| AHA2  | Arabidopsis H <sup>+</sup> -ATPase 2 |
| ANOVA | analysis of variance                 |
| ARE   | anaerobic-responsive element         |
| BTS   | BRUTUS                               |
| CDS   | coding sequence                      |
| DEGs  | differentially expressed genes       |
| DW    | dry weight                           |

|                      |                                                             |
|----------------------|-------------------------------------------------------------|
| EDTA                 | ethylenediaminetetraacetic acid                             |
| FAAS                 | flame atomic absorption spectrometry                        |
| FIT                  | FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR       |
| FRO                  | ferric-chelate reductase oxidase                            |
| FRO1                 | ferric-chelate reductase oxidase 1                          |
| FRO2                 | ferric-chelate reductase oxidase 2                          |
| IMA                  | IRON MAN                                                    |
| KEGG                 | Kyoto Encyclopedia of Genes and Genomes                     |
| LED                  | light-emitting diode                                        |
| log <sub>2</sub> FC  | log <sub>2</sub> -transformed fold change                   |
| MS                   | Murashige and Skoog medium                                  |
| Na <sub>2</sub> EDTA | disodium ethylenediaminetetraacetate                        |
| NCBI                 | National Center for Biotechnology Information               |
| NCS                  | National Center for Iron and Steel Materials Testing        |
| NCX                  | Na <sup>+</sup> /K <sup>+</sup> /Ca <sup>2+</sup> exchanger |
| ORF                  | open reading frame                                          |
| PCA                  | principal component analysis                                |
| PE150                | paired-end 150 bp sequencing                                |
| PGSC                 | Potato Genome Sequencing Consortium                         |
| PYE                  | POPEYE                                                      |
| ROS                  | reactive oxygen species                                     |
| SRA                  | Sequence Read Archive                                       |
| TF                   | transcription factor                                        |
| ZIP                  | ZRT/IRT-like protein                                        |

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