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Table Grape Ferritin1 Is Implicated in Iron Accumulation, Iron Homeostasis, and Plant Tolerance to Iron Toxicity and H_2O_2 Induced Oxidative Stress

Zhenqiang Xie [1,2,†], Bin Peng [1,2,†], Matthew Shi [2,3], Guangrong Yang [1,2] and Zhizhong Song [2,4,*]

[1] School of Agriculture and Horticulture, Jiangsu Vocational College of Agriculture and Forestry, Zhenjiang 212499, China; xiezhenqiang@jsafc.edu.cn (Z.X.)
[2] School of Horticulture, Ludong University, Yantai 264025, China
[3] Faculty of Wolfson, University of Cambridge, Cambridge CB3 9BB, UK
[4] Department of Plant Science, University of Cambridge, Cambridge CB2 3EA, UK
* Correspondence: 3614@ldu.edu.cn
† These authors contributed equally to this work.

Abstract: In plants, Ferritin is the earliest discovered regulator of iron (Fe) metabolism and plays a critical role in maintaining Fe storage and sequestration, which contributes to cellular Fe homeostasis and tolerance to abiotic stresses. However, biological functions of Ferritin proteins in perennial fruit crops are largely rare. In this study, *VvFerritin1* was isolated from 'Irsay Oliver' table grape, and it was mainly expressed in roots and induced under Fe toxicity, H_2O_2 stress, and abscisic acid (ABA) treatment. Complementation of *VvFerritin2* in yeast mutant DEY1453 directly restored the mutant growth, and VvFerritin1 can transport Fe^{2+} in yeast. The heterologous expression of *VvFerritin1* in *fer1-2* mutant effectively rescued the dwarfed growth of *Arabidopsis fer1-2* mutant, under the control condition, Fe toxicity, or H_2O_2 stress, embodied in enhanced fresh weight (126%, 81%, or 48%), total root length (140%, 98%, or 64%), total root surface (70%, 84%, or 120%), and total leaf chlorophyll (56%, 51%, or 53%), respectively. In particular, tissue Fe concentration and activities of nitrite reductase (NiR), aconitase (ACO), and succinate dehydrogenase (SDH) were significantly enhanced in *fer1-2*/35S::*Ferritin1* lines, respectively, compared to that of *fer1-2* mutant. This work contributes to the study of molecular mechanisms of Fe storage and homeostasis in 'Irsay Oliver' table grape.

Keywords: table grape; Fe storage; ferritin; *fer1-2* mutant; Fe toxicity; H_2O_2 stress

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1. Introduction

Iron (Fe) is one of the most important mineral elements in plant cells, which was directly involved in photosynthesis, respiration, energy metabolism, DNA repair, and hormone synthesis [1–3]. In soils, Fe deficiency severely reduces fruit yield and fruit quality [4,5]. In plants, there are two types of Fe transport and absorption strategies, including Strategy I and Strategy II, especially under Fe deficiency conditions [5–9]. In dicotyledons and non-gramineous monocotyledons (Strategy I), Fe^{3+} is reduced to Fe^{2+} through ferric reduction oxide (FRO), and Fe^{2+} is absorbed by iron regulated transporters (IRTs). In gramineous plants (Strategy II), Fe^{3+} is absorbed through the Fe^{3+} chelator phytosideophore (PS) pathway, which depends on yellow stripe (YS) or yellow stripe-like (YSL) transporters [5,8,9].

Under Fe deficiency conditions, the mobilization of intracellular Fe^{2+} is indispensable for plant growth and development [5–10]. When Fe^{2+} is excessively stored in the cytoplasm, it will cause Fe^{2+} toxicity that hinders normal growth and development [5–12]. Previous studies showed that natural resistance associated macrophage proteins (NRAMPs), permease in chloroplast (PIC), and vacuolar iron transporter (VIT) are implicated in the transport and distribution of Fe^{2+} within plant cells [5–10]. Notably, intracellular Fe^{2+} is stored in vacuoles or might be chelated with Ferritins, which are being used in various metabolic pathways that depend on Fe^{2+} [9–12].

Ferritin plays a crucial role in maintaining cellular Fe balance and protecting plants against oxidative damage [9–13]. In *Arabidopsis*, four Ferritin family genes have been identified. Notably, *AtFerritin1* is enhanced by Fe toxicity and H_2O_2 treatment, *AtFerritin2* is increased by abscisic acid (ABA), and *AtFerritin3* is up-regulated by Fe toxicity [13–15]. AtFerritin proteins participate in the regulation of Fe^{2+} storage and sequestration, and contribute to plant tolerance to undesired abiotic stresses, including water loss [14], drought [16], and reactive oxygen species (ROS) [13,14,17,18]. The germination rate of *Arabidopsis fer2* mutant seeds was severely decreased under ROS stress [13,17,18]. The growth of *Arabidopsis fer1fer3fer4* mutant was severely impaired, and the intracellular Fe^{2+} concentration was significantly reduced. Moreover, Ferritin proteins are also implicated in regulating the root structure. The abrupt ROS production leads to the destruction of *fer1fer3fer4* roots [15]. Subsequently, *Ferritin* family genes have been identified in cut rose [14], cassava [16], and peach [17]. However, biological and molecular functions of Ferritin family proteins in perennial fruit crops are still unknown.

Table grape (*Vitis vinifera*) is a worldwide popular perennial fruit crop, and its genome has been published [18]. Fe as the highest amount of trace elements in vines correlates with grape quality and yield [4,5]. Grapes are highly sensitive to Fe deficiency, and when the soil Fe content is low, the young leaves of new shoots are the first to show chlorosis and yellowing. Due to nutrient deficiency in grapevines, they suffer from malnutrition, slow growth of new shoots, weakened tree vigor, and reduced fruit size, which greatly affects the quality and yield of grapes [4,5]. In this study, a Ferritin family gene *VvFerritin1* was isolated from table grape cultivar 'Irsay Oliver', and their expression profiles and putative biological function were further verified. This study helps to reveal the molecular mechanisms of Fe transport, storage, and utilization in fruit trees.

2. Materials and Methods

2.1. Plant Material and Growth Condition

The 'Irsay Oliver' seedlings grown in the National Grape Germplasm Repository (Yantai, China) were used throughout this study. One-month-old tissue-cultured 'Irsay Oliver' seedlings were cultivated on half-strength MS medium (pH 5.8) for 2 weeks, and then transferred to the half-strength MS liquid solution in plastic containers and cultured in the incubator under conditions of 25 °C day 16 h/20 °C night 8 h, with a relative humidity of 75%. For Fe depletion treatments, Fe was deleted from the MS solution [3,17,19]. For Fe toxicity treatments, 500 μmol·L^{-1} $FeCl_3$ was added in half-strength MS solution. For ABA treatments, 100 μol·L^{-1} ABA was supplied in half-strength MS medium. For H_2O_2 induced oxidative stress treatments, fresh H_2O_2 was added in half-strength MS solution to a final concentration of 10% (*v*/*v*) [14,19]. After being subjected to stress treatments for 48 h, samples of leaves, stems, and roots were collected, respectively, and quickly frozen in liquid nitrogen before further analyses.

The wild type *Arabidopsis* (Col-0), *fer1-2* knockout mutants, and *fer1-2*/35S::*Ferritin2* lines were germinated in half-strength MS medium and exposed to Fe depletion, Fe toxicity, or H_2O_2 stress for 14 days before physiological analysis. Biological repeats were carried out three times, each with 20 seedlings.

2.2. Physiological Analysis

The fresh weight of *Arabidopsis* seedlings was determined by the Analytical Balance (Thermo Electron, Waltham, MA, USA). Roots of *Arabidopsis* seedlings were scanned using the Epson Rhizo scanner (Epson, Long Beach, CA, USA), and the total root length and total surface area were calculated by the Epson WinRHIZO 2.0 software (Long Beach, CA, USA). Fe concentration was measured by ICP-AES systems (IRIS Advantage, Thermo Electron, Waltham, MA, USA). The activity of aconitase (ACO), nitrite reductase (NiR), and succinate dehydrogenase (SDH) was executed using commercial detection kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Total leaf chlorophyll was quantified by the BioRad SmartSpec 3000 spectrophotometer (BioRad, Wadsworth, IL, USA), as previously mentioned [17,19–21]. Biological repeats were carried out three times, each with 20 seedlings.

2.3. Isolation and Cloning of *VvFerritin1* from Table Grape

Both *Arabidopsis* Ferritin1 [1,22] and peach Ferritin1 [17] were taken as reference sequences, and one putative *Ferritin* family gene was screened throughout the Grape Genome Database [18]. The genomic DNA sequence, coding sequence (CDS), and amino acid sequence of putative *VvFerritin1* were downloaded, respectively. The amino acid sequence of VvFerritin1 protein was verified via InterProScan 4.8 and Pfam online servers. The independent prime pair of *VvFerritin1* was designed for CDS cloning. Total RNA of 1-month-old 'Irsay Oliver' seedling was extracted with the help of RNAprep Pure Plant Kit (TianGen, Beijing, China), and the first strand cDNA template was synthesized using the PrimeScript^{TM} RT reagent kit (Takara, Dalian, China). The CDS of *VvFerritin1* was amplified using Prime STAR^{TM} HS DNA polymerase (Takara, Dalian, China), and then sequenced in Shenggong Bioengineering Co., Ltd. (Shanghai, China). The tertiary structure of Ferritin homologous proteins was predicted utilizing the Phyre2 online server (https://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?%20Predicting%20using%20id=index) (accessed on 25 October 2024).

2.4. Phylogenetic Tree Construction

The alignment of amino acid sequences of Ferritin homologues from table grape (VvFerritin1), *A. thaliana* (AtFer1-4, Gene ID: 818622, 820276, 824775, 831720), *Arachis hypogaea* (AdFer1-4: 107485043, 107475384, 107469395, 107478374), *Camellia oleifera* (CoFer1-3: 106433816, 106452550, 106382764), *Brassica rapa* (BrFer1-3: 103855410, 103870409, 103830031), *Cicer arietinum* (CaFer1-3: 101503152, 101498435, 101510209), *Gossypium hirsutum* (GhFer1-3: 107943203, 107960065, 107904058), *Glycine max* (GmFer1-4: 547824, 547988, 547476, 547477), *Hevea brasiliensis* (HbFer2-4: 110640712, 110645561, 10638947), *M. domestica* (MdFer3-4: 103406424, 103450693), *Manihot esculenta* (MeFer1-4: 110619691, 110622202, 110624811, 110619936), *Nicotiana tabacum* (NtFer1-2: 107789800, 107832545), *Prunus persica* (PpFer1-2: 18787640, 18773611), *Ricinus communis* (RcFer2-3: 8263108, 8272083), *Solanum lycopersicum* (SlFer1-2: 102577492, 102581985), and *Fragaria vesca* (FvFer3-4: 101293015, 105353074) was conducted with the help of the ClusterX 2.0.13 software. The phylogenetic tree of these Ferritin homologues was well constructed using the maximum likelihood method in MEGA 13.0.

2.5. Quantitative Real Time PCR (qRT-PCR)

Specific primer pairs of *VvFerritin1* (forward: GATCCCCAGTTGACAGATTT, reverse: CCACCCTCTTCGAGGAGCAT) were designed via the NCBI/Primer-BLAST online server. A qRT-PCR analysis was executed on the 7500 Real Time PCR System (Applied Biosystems, New York, NY, USA), labelled with SYBR Premix Ex Taq (TaKaRa, Kyoto, Japan). The *Ubiquitin* of wine grape was used as the internal control as described in previous studies [19,23]. To calculate the concentration of the starting template and RT-qPCR efficiency for each cDNA sample, the linear regression of the log (fluorescence) per cycle number data was used by taking the logarithm on both sides of an equation as follows: log (*Nc*) = log (*No*) + log(*Eff*) × C, where *Nc* is fluorescence, *No* is the initial concentration of a template, *Eff* is efficiency, and C is the cycle number. The relative expression level of *VvFerritin1* was presented after normalization to the internal control from three independent biological repeats, each with four technical replicates.

2.6. Complementation of VvFerritin2 Gene in Yeast Mutant

The recombinant plasmid pYH23-*Ferritin1* was constructed by cloning the CDS region of *VvFerritin2* gene into the pYH23 vector [19,24], using the forward primer of 5′-GACGGATCCATGCTTGTGGGAGGTGTTTC- 3′ (*BamH* I was underlined) and reverse primer of 5′-GAGTCTAGA TCATGCTGCACCACCCTCTTC-3′ (*Xba* I was underlined). According to the description of Vert et al. [24] and Song et al. [19], the yeast *fet3fet4* double mutant strain DEY1453 was transformed with the empty plasmid pYH23 or the recombinant plasmid pYH23-*Ferritin1*. Yeast transformants were further cultured in liquid YPD (containing 10 μmol·L^{-1} $FeSO_4$) medium to OD_{600} of 1.0, and then diluted to 10^{-1}, 10^{-2}, and 10^{-3} concentrations. Yeast cell growth was determined in a synthetic-defined medium (containing 10 μmol·L^{-1} $FeSO_4$, pH 4.5) in the absence or presence of 30 μmol·L^{-1} bathophenanthroline disulfonic acid (BPDS), respectively. The plates were incubated at 30 °C for 60 h before colony observation.

2.7. Over-Expression of VvFerritin1 in Arabidopsis fer1-2 Mutant

The CDS region of *VvFerritin1* gene was cloned into the pBH vector [19,25] to obtain the recombinant plasmid pBH-*Ferritin1*, using the forward primer of 5′-GACGAGCTCATGCTTGTGGGAGGTGTTTC- 3′ (*Sac*I was underlined) and reverse primer of 5′- GAGTCTAGA TCATGCTGCACCACCCTCTTC-3′ (*Xba* I was underlined). The pBH vector or the recombinant plasmid pBH-*Ferritin1* was transformed into *Agrobacterium tumefaciens* EHA 105, and then further introduced into the *Arabidopsis fer1-2* knockout homozygote mutant [17,26]. Individual T1 generation of *fer1-2*/35S::*Ferritin1* lines were identified by screening hygromycin resistant transgenic *Arabidopsis* seedlings. The genomic DNA of T1 generation of *fer1-2*/35S::*Ferritin1* lines was extracted. Then, the existence of a 786-bp product of *VvIRT7* was further checked by reverse transcription PCR. Verified T3 generation seeds of #1, #2, and #6 *fer1-2*/35S::*Ferritin1* lines were harvested and cultivated on half-strength MS medium for 12 days before physiological analysis. Biological repeats were performed three times, each with 20 seedlings.

2.8. Statistical Analysis

Bar graphs were produced via the Origin 12.0 software. Significant differences were analyzed using IBM SPSS Statistics 23 (Armonk, New York, NY, USA) followed by Fisher's LSD test method at $p < 0.01$ level.

3. Results

3.1. Isolation of VvFerritin1 in Grape

One putative *Ferritin* family gene was isolated from grape genome, which was entitled as *VvFerritin1* with seven introns of different lengths (PQ862906, Figure 1A). Protein domain verification showed that VvFerritin1 contained the classical Ferritin domain (PF00210), implying that all of it belongs to Ferritin family transporters. The identity value of amino acid sequences among grape VvFerritin1, peach PpFerritin1, and *Arabidopsis* AtFerritin1 was 72.40% (Figure 1B). Meanwhile, the tertiary structure prediction analysis showed that four Ferritin homologous proteins (VvFerritin1, AtFerritin1, PpFerritin1, and MeFerritin1) exhibited a similar tertiary structure (Figure 2), implying that they may possess similar biological functions.

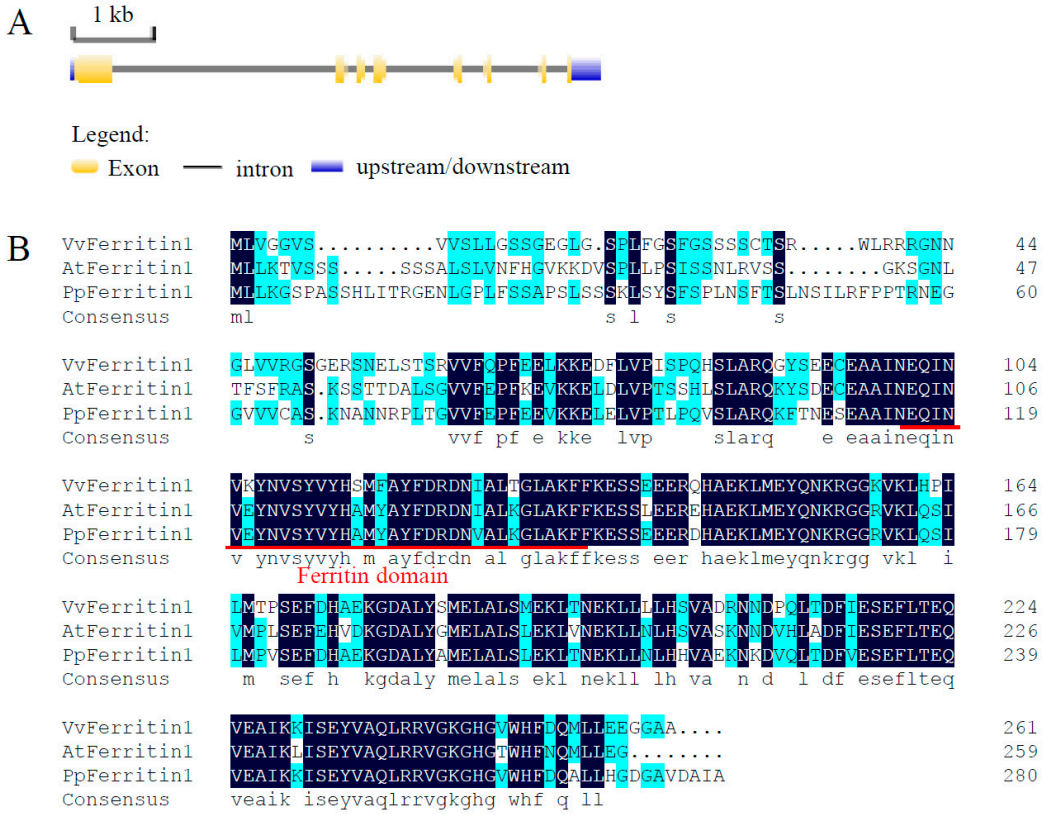

Figure 1. Analysis of gene structure and amino acid sequence alignment. (**A**) Intron and exon structure analysis of *VvFerritin1*. (**B**) Alignment of Ferritin proteins from *Arabidopsis*, peach, and grape.

A phylogenetic tree analysis indicated that Ferritin homologues belonging to the same genus, such as *Arabidopsis* and turnip (*Brassica rapa*) of *Cruciferae*, soybean (*Glycine max*), peanut (*Arachis hypogaea*), and chickpea (*Cicer arietinum*) of *Leguminosae*, rubber tree (*Hevea brasiliensis*), cassava (*Manihot esculenta*), and castor (*Ricinus communis*) of *Euphorbiaceae*, and peach (*Prunus persica*), apple (*Malus domestica*), and strawberry (*Fragaria vesca*) of *Rosaceae*, were prone to be closely clustered together and exhibited a closer genetic distance during evolution (Figure 2). Notably, VvFerritin1 was closely clustered with cassava MeFerritin1 and other *Euphorbiaceae* homologues (Figure 3).

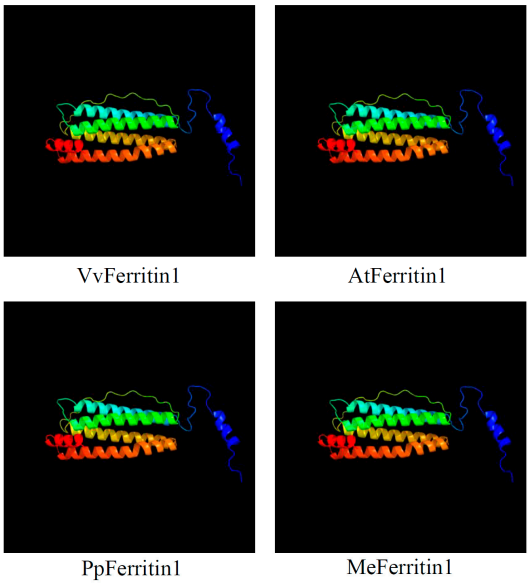

Figure 2. Tertiary structure prediction of Ferritin homologous proteins. The tertiary structure of VvFerritin1, AtFerritin1, PpFerritin1, and MeFerritin1 was predicted utilizing the Phyre2 online server (https://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?%20Predicting%20using%20id=index) (accessed on 25 October 2024).

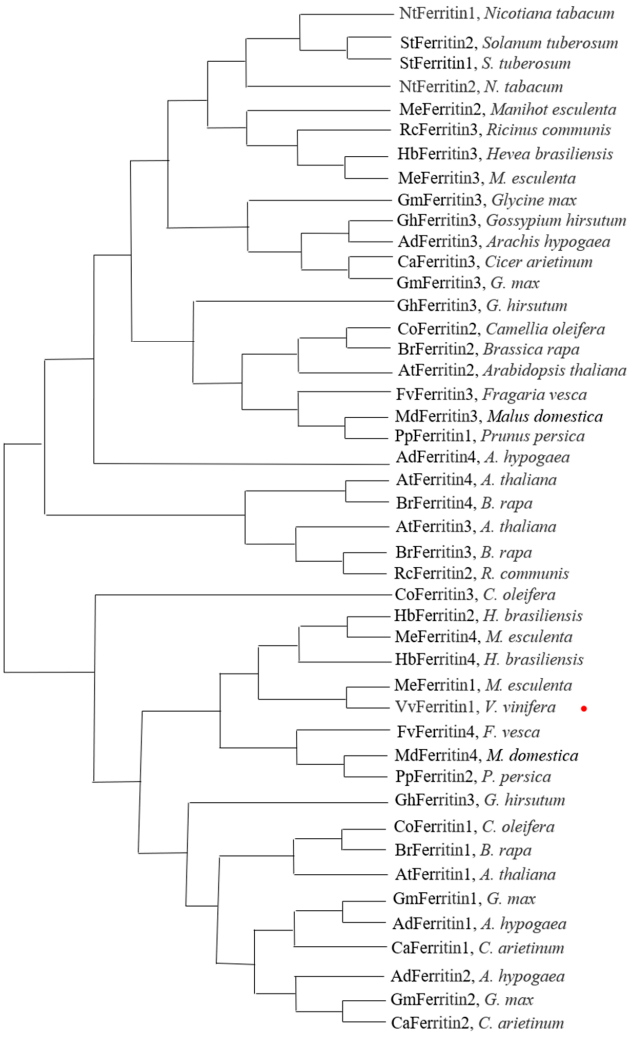

Figure 3. Phylogenetic tree analysis of plant Ferritin proteins. The alignment of Ferritin homologous proteins from grape (VvFerritin1), *Arabidopsis thaliana* (AtFer1-4, Gene ID: 818622, 820276, 824775, 831720),

Arachis hypogaea (AdFer1-4: 107485043, 107475384, 107469395, 107478374), *Camellia oleifera* (CoFer1-3: 106433816, 106452550, 106382764), *Brassica rapa* (BrFer1-3: 103855410, 103870409, 103830031), *Cicer arietinum* (CaFer1-3: 101503152, 101498435, 101510209), *Gossypium hirsutum* (GhFer1-3: 107943203, 107960065, 107904058), *Glycine max* (GmFer1-4: 547824, 547988, 547476, 547477), *Hevea brasiliensis* (HbFer2-4: 110640712, 110645561, 10638947), *M. domestica* (MdFer3-4: 103406424, 103450693), *Manihot esculenta* (MeFer1-4: 110619691, 110622202, 110624811, 110619936), *Nicotiana tabacum* (NtFer1-2: 107789800, 107832545), *Prunus persica* (PpFer3-4: 18787640, 18773611), *Ricinus communis* (RcFer2-3: 8263108, 8272083), *Solanum lycopersicum* (SlFer1-2: 102577492, 102581985), and *Fragaria vesca* (FvFer3-4: 101293015, 105353074) was conducted with the help of the Cluster X 2.0.13 software. A phylogenetic tree was constructed using the maximum likelihood method in MEGA 13.0. The grape VvFerritin1 protein is marked with a red dot.

3.2. Expression Profiles of *VvFerritin1*

Results showed that the expression levels of *VvFerritin1* were different among distinct tissues of tissue-cultured seedlings, and the maximum expression amount was observed in roots, followed by leaves and stems (Figure 4). In addition, *VvFerritin1* exhibited a different response to Fe depletion, Fe toxicity, H_2O_2 stress, and ABA stress, respectively. *VvFerritin1* was quite sensitive to Fe toxicity, whose expression levels were induced throughout the entire seedling. The expression of *VvFerritin1* was significantly increased in leaves and roots under H_2O_2 stress and enhanced in roots under ABA stress, while it slightly changed under Fe depletion (Figure 4).

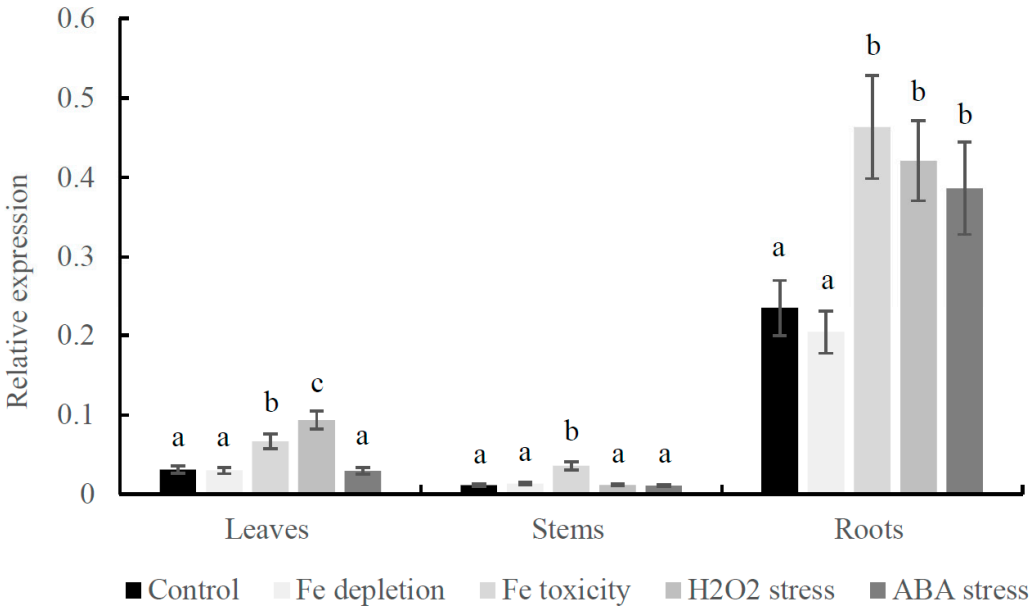

Figure 4. Expression profiles analysis of *VvFerritin1* in tissue-cultured seedling. One-month-old tissue-cultured seedlings were being exposed to Fe depletion, 500 μmol·L^{-1} $FeCl_3$ (Fe toxicity), 100 μol·L^{-1} abscisic acid (ABA), and 10% (*v*/*v*) H_2O_2 stress for 48 h before the q-RT-PCR analysis. Letters indicate differences among control condition, Fe depletion, Fe toxicity, ABA stress, and H_2O_2 stress.

3.3. *VvFerritin1* Restored the Growth of Yeast Mutant DEY1453

The DEY1453 mutant, which was deficient in Fe^{2+} uptake, cannot grow normally on YPD medium in the absence of Fe^2 [19,24]. In this study, DEY1453 cells harboring either the empty plasmid pYH23 or the recombinant plasmid pYH23-*Ferritin1* grew well on YPD medium (containing 10 μmol·L^{-1} Fe^{2+}) (Figure 5). When 30 μmol·L^{-1} BPDS was present in YPD medium, only DEY1453 cells harboring pYH23-*Ferritin1* thrived and DEY1453 cells harboring the empty plasmid pYH23 cannot grow normally, implying that VvFerritin1 is

directly implicated in Fe^{2+} transport or accumulation in yeast, thereby restoring the normal growth of the DEY1453 mutant.

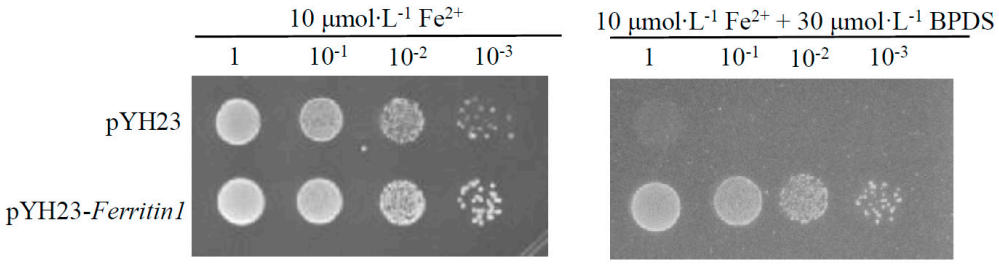

Figure 5. Functional complementation of *VvFerritin1* in yeast mutant DEY1453. The yeast mutant DEY1453 was cultured in liquid YPD medium until the OD_{600} value was reached at 1.0. The culture was diluted to the concentrations of 10^{-1}, 10^{-2}, and 10^{-3}, respectively. Yeast cell growth was determined in synthetic defined medium (containing 10 μmol·L^{-1} Fe_2SO_4, pH 4.5), supplied with 30 or 0 μmol·L^{-1} bathophenanthroline disulfonic acid (BPDS). Pictures were taken after 60 h of incubation at 30 °C.

3.4. *VvFerritin1* Recovered the Impaired Growth of Arabidopsis *fer1-2* Mutant

To determine whether *VvFerritin1* could restore the normal growth of *fer1-2* mutant, *VvFerritin1* was introduced into the expression vector pHB (Figure 6A). In this work, four positive (#1, #2, #6, and #9) T1 generation *fer1-2*/35S::*Ferritin1* lines were validated by PCR for the presence of a 786-bp amplification product of *VvFerritin1* (Figure 6B). Purified T3 generation of #2 and #6 *fer1-2*/35S:: *Ferritin1* lines were selected randomly for further physiological analysis. Given that #2 and #6 *fer1-2*/35S::*Ferritin1* lines exhibited a similar growth status, data of #2 *fer1-2*/35S::*Ferritin1* lines were shown in this present work.

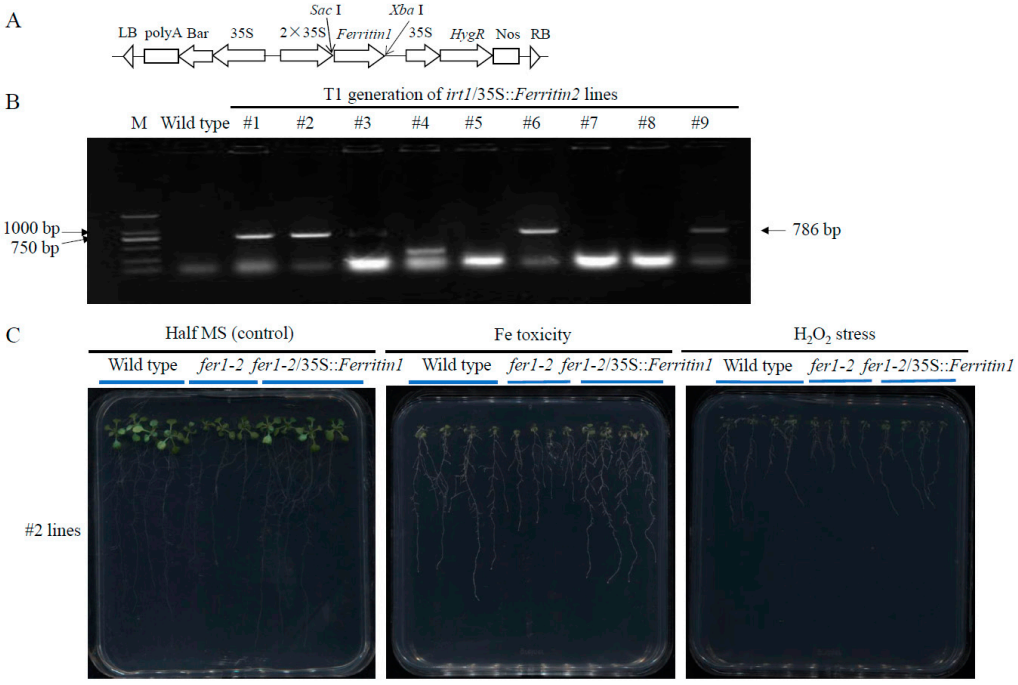

Figure 6. Generation and phenotype analysis of *VvFerritin1* over-expression transgenic *Arabidopsis* seedlings. (**A**) Scheme of the recombinant plasmid pBH-*Ferritin1*. (**B**) PCR verification of *VvFerritin1* in T1 generation *fer1-2*/35S::*Ferritin1* lines. Note: M, standard DL2000 DNA ladder (Takara, Dalian, China). (**C**) Phenotype of T3 generation *fer1-2*/35S::*Ferritin1* lines. Seedlings were germinated on half-strength MS solid medium, and subjected to 500 μmol·L^{-1} $FeCl_3$ (Fe toxicity) and 10% (*v*/*v*) H_2O_2 stress for 12 days before the phenotype analysis.

Compared to the wild type, the growth of *fer1-2* lines was seriously hindered, accompanied by decreased fresh weight, total root length, total root surface, and total leaf chlorophyll, respectively, under the control conditions, Fe toxicity, and H_2O_2 stress (Figures 6C and 7). In contrast, #2 *fer1-2*/35S::*Ferritin1* lines exhibited a better growth phenotype than that of the *fer1-2* mutant lines under all tested conditions. The fresh weight, dry weight, total root length, total root surface, and total leaf chlorophyll of #2 *fer1-2*/35S::*Ferritin1* lines were significantly increased, compared to the *fer1-2* mutant, similar to that of the wild type (Figures 6C and 7). All these findings imply that the complementation of *VvFerritin1* rescued the impaired growth of *fer1-2* mutant.

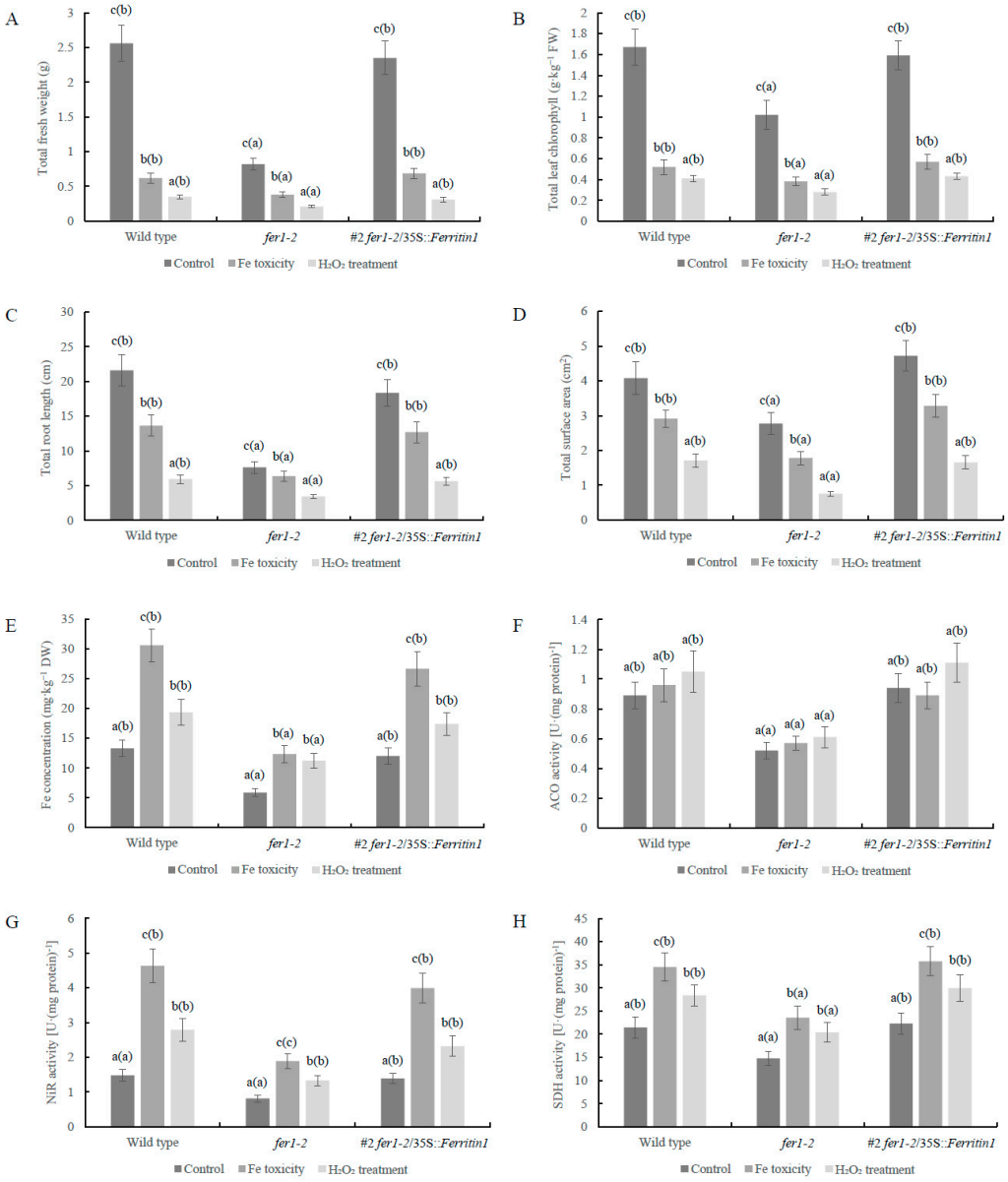

Figure 7. Physiological analysis of *VvFerritin1* over-expression transgenic *Arabidopsis* seedlings. (**A**) Total fresh weight. (**B**) Total leaf chlorophyll content. (**C**) Total root length. (**D**) Total root surface area. (**E**) Fe concentration. (**F**) ACO activity. (**G**) NiR activity. (**H**) SDH activity. Individual seedlings were germinated on half-strength MS medium, and subjected to 500 μmol·L^{-1} $FeCl_3$ (Fe toxicity) or 10% (*v*/*v*) H_2O_2 stress for 12 days before the phenotype analysis. Data are shown as means ± SE (n = 20). Letters outside the parentheses indicate differences among control condition, Fe depletion, and H_2O_2 stress and those inside the parentheses indicate differences among wild type, *fer1-2* mutant, and *fer1-2*/35S::*Ferritin1* lines, respectively.

In comparison to the wild type control, the Fe concentration, and ACO, NiR, and SDH activities of *fer1-2* mutant lines were significantly decreased under control conditions, Fe toxicity, and H_2O_2 stress, respectively (Figure 7). Compared to the *fer1-2* mutant, the Fe concentration, and ACO, NiR, and SDH activities of #2 *fer1-2*/35S::*Ferritin1* lines were significantly induced.

4. Discussion

Fe is the most indispensable mineral nutrient in fruit trees and it was closely associated with tree growth, flowering, fruit quality, and fruit yield [1,2,4,5]. However, molecular mechanisms of Fe uptake and transport in fruit trees are essentially unclear. In this study, VvFerritin1 transporter was isolated from grape, and amino acid sequences of VvFerritin1 and homologues from other 15 plants were highly conserved, with the identity of 64.67%. VvFerritin1 was tightly clustered with *Euphorbiaceae* homologues, implying that grape Ferritin1 has a close genetic distance to *Euphorbiaceae* plants. In particular, VvFerritin1 exhibited a similar tertiary structure with other plant homologous Ferritin proteins (AtFerritin1, PpFerritin1, and MeFerritin1), indicating that they may possess similar biological functions. Therefore, this study helps to reveal the biological function of Ferritin homologues from *Euphorbiaceae* and *Vitis* plants.

In this work, *VvFerritin1* was majorly expressed in roots of seedlings, which was consistent with *Arabidopsis* AtFer2 and tomato *SlFerrrine1*, but different from *AtFerritin1*, *AtFerritin3*, and *AtFerritin4* in *Arabidopsis* [22], *PbFerritin2* in pear [27], and *MeFerritin4* in cassava [16], which are highly expressed in leaves. Given that cut rose *RhFerrintin1* [14] and peach *PpFerritin1* (17) are highly expressed in flowers and peach *PpFerritin2* [17] is specifically expressed in young fruit, we speculate that *Ferritin* family genes have extensive expression profiles and *VvFerritin1* is prone to be functioned in grape roots. More convincingly, a functional verification in DEY1453 mutant cells demonstrated that VvFerritin1 could transport or utilize Fe^{2+}, indicating that it could be a functional Ferritin that directly contributes to Fe^{2+} accumulation and homeostasis in roots of table grape.

In pear, the expression of *PbFerritin2* was reduced by Fe deficiency [27]. However, *VvFerritin1* was not responsive to Fe depletion, similar to that of *PpFerritin* genes in peach seedlings. These findings indicate that different Ferritin family genes from perennial fruit crops are likely to possess different physiological roles due to the external Fe supply status, and *VvFerritin1* may be active in table grape under excessive Fe status, but not Fe depletion treatment. Moreover, *AtFerritin1* and *AtFerritin3* in *Arabidopsis* [22] and *PpFerritin1* and *PpFerritin3* in peach [17] were induced by Fe toxicity and H_2O_2 stress, and RhFer1 in cut rose [14] and *AtFerritin2* in *Arabidopsis* [22] were induced by the ABA treatment. Consistently, *VvFerritin1* was responsive to Fe toxicity, H_2O_2 stress, and ABA treatment, which was mainly induced, implying that VvFerritin1 may be implicated in the Fe uptake/transport in grape roots under adverse environmental stresses, thus maintaining Fe accumulation and homeostasis, so as to secure Fe-dependent basic metabolic activities. The abrupt increase in *VvFerritin1* expression may be one of the crucial indicators that vines respond to such environmental stresses.

In *Arabidopsis*, *AtFeritin1* regulates the cellular Fe accumulation and knockout of *AtFerritin1* accelerated plant senescence and impaired the normal growth [26]. The maximum expression of *VvFerritin1* was observed in roots and was up-regulated under both Fe toxicity and H_2O_2 stress. Remarkably, the heterologous expression of *VvFerritin1* in *fer1-2* mutant effectively recovered the retarded growth of *fer1-2* mutant. In particular, the Fe content and activity of Fe-dependent enzymes (ACO, NiR, and SDH) were significantly enhanced in *fer1-2*/35S::*Ferritin1* lines, which may partially account for the restored growth performance. Over-expression of *VvFerritin1* in *fer1-2* mutant may positively strengthen the Fe

transport and storage capacity in *fer1-2*/35S::*Ferritin1* lines, maintaining basic Fe-dependent metabolic processes, thereby preventing the transgenic seedlings from Fe toxicity or H_2O_2 stress. Meanwhile, the Fe content and total leaf chlorophyll were indeed induced in *fer1-2*/35S::*Ferritin1* lines. Furthermore, *MeFerritin4* was up-regulated by a low temperature, and the heterologous expression of *MeFerritin4* in cassava favorably enhanced plant resistance to cold stress [16]. These findings favor the proposition that Ferritin transporters are implicated in regulating Fe homeostasis and H_2O_2 induced stress in plants, especially under undesired abiotic stresses [14–17]. Nonetheless, this study provides a foundation for genetic improvement programs aimed at enhancing stress tolerance and nutrient use efficiency in table grape cultivars.

5. Conclusions

VvFerritin1 was isolated and determined from table grape. It was mainly expressed in roots and was enhanced under Fe toxicity, H_2O_2 stress, and ABA treatment. *VvFerritin1* can transport Fe^{2+} in yeast mutant DEY1453. Over-expression of *VvFerritin1* recovered the impaired growth of *fer1-2* knockout mutant, especially under Fe toxicity and H_2O_2 stress. *VvFerritin1* may be a crucial Ferritin transporter that is involved in Fe storage and homeostasis in table grape, especially under Fe toxicity or H_2O_2 stress. This study provides a foundation for genetic improvement programs aimed at enhancing stress tolerance and nutrient use efficiency in table grape cultivars.

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