Changes in the Transcriptomic Profiles of Allohexaploid Wheat in Response to Low Fe Stress

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Research Article

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Abstract

Background: Around the world, especially in aerobic soils, because the solubility of iron (Fe) is very low, plants are often stressed by low Fe stress, and the growth, yield, and quality of crops will be inhibited in the case of severe Fe deficiency. The metabolism of Fe in plants is controlled by a series of complex transport, storage, and regulatory mechanisms to maintain the homeostasis of Fe in cells. Allohexaploid wheat (*Triticum aestivum* L.) is an important food crop and is more sensitive to low Fe stress. Although some studies have been conducted on the low Fe stress response of different plant species, these mechanisms are still unclear in wheat.

Results: According to the results of transmission electron microscope, and paraffin section, under low Fe stress, leaf chlorosis was due to damage to the chloroplast structure, and the effect on root structure was mediated by reducing the rate of cell division in meristems and reducing cell elongation in the elongation zone. ICP-MS showed that low Fe stress significantly limited the absorption of essential elements, including N, Pi, K, Ca, Mg, Fe, Mn, Cu, Zn, and B nutrients. RNA sequencing revealed the transcriptomic changes of wheat under low Fe stress. The results showed that 378 and 2,619 differentially expressed genes (DEGs) were identified in the shoots and roots, respectively. These DEGs were mainly involved in the synthesis of Fe chelating agents, ion transport, photosynthesis, amino acid metabolism, protein synthesis, and other processes. Next, to find the core genes responding to low Fe stress, the gene co-expression network diagram was constructed. The results indicated that *TaIRT1b-4A*, *TaNAS2-6D*, *TaNAS1a-6A*, *TaNAS1-6B*, and *TaNAAT1b-1D* might play a key role in dealing with low-Fe stress.

Conclusions: These research results might help to fully understand the morphological and molecular responses of plants to low Fe stress, and provide excellent genetic resources for the genetic modification of Fe deficiency crops.

Background

Iron (Fe) plays an important role in maintaining the normal growth and development of higher plants, such as participating in chlorophyll synthesis, respiration, redox reactions, and electron transfer [1]. Although the Fe content in the soil is very high, it is often in the form of Fe$^{3+}$ which is hardly soluble in water due to the influence of soil pH and oxygen partial pressure. The total amount of soluble Fe is less than $10^{-10}$ mol·L$^{-1}$ and the Fe concentration required by plants to maintain growth is about $10^{-8}$ mol·L$^{-1}$, so it is difficult for plants to use [1, 2], especially in calcareous soils with higher pH, low Fe is more serious [3]. Studies have shown that the lack of Fe will not only affect the growth and development of plants but also affect the absorption of Fe by animals and humans [2]. According to investigations, more than 40% of the world's soils are severely Fe deficient [4]. If there is no active regulation mechanism to allow plants to obtain sufficient Fe, most plants will show Fe-deficiency symptoms. Therefore, low Fe and chlorosis in plants have become a universal concern around the world, and it is also been one of the hot spots in plant nutrition research since the beginning of this century.

Faced with different Fe nutritional statuses in the environment, higher plants have formed two different strategies in the long-term evolution process to adapt to the changes in Fe content in the external environment: reduction strategy (strategy I) and chelation strategy (strategy II) [5]. Monocotyledonous non-graminaceous plants and all dicotyledonous plants adopt strategy I to deal with a low Fe environment. When these plants are lacking Fe, their root epidermis adopts three processes of acidification, reduction, and absorption to absorb rhizosphere Fe [3]. First, a proton ATPase (H$^+$-ATPase) is expressed in large quantities, pumping a large number of protons (H$^+$) to the root environment to acidify the rhizosphere environment, thereby improving the availability of Fe in the rhizosphere environment. Second, up-regulate the expression of a gene encoding Fe reductase (FRO), and then reduce the Fe$^{3+}$ adsorbed on the root epidermis to Fe$^{2+}$. At the same time, the iron-regulated transporter (IRT) gene encoding specific uptake of Fe$^{2+}$ is also up-regulated to transport Fe$^{2+}$ into root cells [3]. Strategy II adopted by monocotyledonous gramineous plants consists of two parts [6]. First, by synthesizing a plant Fe carrier (phytosiderophore, PS) that can bind to Fe$^{3+}$ with high affinity, it is then secreted into the rhizosphere environment.
and combined with Fe\(^{3+}\) to form a Fe\(^{3+}\)-PS complex. At the same time, under the action of the YSL (YS1-like protein) family transporter, the Fe\(^{3+}\)-PS complex near the root epidermal cells is transported across the membrane to the inside of the plant, thereby completing the absorption of Fe in monocotyledonous grasses [7]. The proposal of strategy I and strategy II opened up a new field of research on the specific response of plant nutrient stress, enabled people to have a deeper understanding of the mechanism of plant low Fe stress, and also provides an important theoretical basis for solving the problem of low Fe in plants.

*Triticum aestivum* L. is the most important crop in the world and the second-largest crop in my country after rice. Its yield and quality directly affect the improvement of the human diet [8]. Fe, as an essential micronutrient element for *Triticum aestivum*, has important biological functions. Low Fe stress will cause distortion or destruction of the cell membrane system and intracellular organelles [9], and the phenotype will be chlorosis and chlorosis [10]. Low Fe stress can also induce the reduction of photosynthetic pigments in the shoots, weaken the capture and transmission of light energy, damage the photosystem II reaction center, and lead to the reduction of excitation energy capture efficiency, electron transfer efficiency, and photosynthetic efficiency. Thus inhibiting the growth and development of seedlings leads to a decrease in biomass [11, 12]. Under low Fe stress, the activity of Fe reductase in the rhizosphere is significantly increased, thereby increasing the ability of plants to reduce Fe and increasing the Fe use efficiency of *Triticum aestivum* roots [13]. Previous studies have shown that the *Triticum aestivum* genome contains two homologous genes *TaVIT1* and *TaVIT2* of vacuolar Fe transporter (VIT), which have different expression patterns, but the expression in the endosperm is lower. Overexpression of *TaVIT2* can enhance the transport of vacuolar Fe in the endosperm, thereby increasing the transport of Fe to seeds [14]. Although many scholars have studied *Triticum aestivum* roots with low Fe stress, most of them mainly focus on photosynthetic characteristics, root characteristics, seed germination, and plant physiological and biochemical effects. There is still a lack of in-depth research on the adaptability of *Triticum aestivum* to low Fe stress. Therefore, studying the resistance mechanism of *Triticum aestivum* under low Fe stress has very important theoretical and practical guiding significance for improving the quality of crops.

The nutritional quality of crops, especially the effect of microelements represented by Fe on human health, has attracted more and more attention. In recent years, important progress has been made in the molecular mechanisms of Fe absorption, transport, and metabolism in higher plants. Although some key genes, such as fer-like iron deficiency-induced transcription factor (*FIT*), have been successfully cloned, their relationship with other regulatory factors in the molecular regulatory network has become a hot spot for future research. In addition, the lack of Fe is also a major problem faced by the growing population. Increasing the Fe content in grains has become a hot issue for enhanced biological breeding in the future. Therefore, the research on the molecular mechanism of plants' efficient absorption and utilization of Fe has become an important direction for future plant nutrition research, and at the same time, it provides a basis for the cultivation of high-quality new varieties of Fe with high-efficiency utilization.

### Materials And Methods

#### Plant material and low Fe stress treatment

The bread wheat (*Triticum aestivum* L.) cultivar zhengmai 1860 cultivated by Henan Academy of Agricultural Sciences (Zhengzhou, Henan Province, China) was used in this research. We germinated the seeds of zhengmai 1860 on wet gauze for 7 days and then selected the seedlings with the same growing vigor for hydroponic cultivation in a black plastic bottle containing 10 L of Hoagland nutrient solution (pH 5.8). Half of the seedlings were cultivated in a nutrient solution containing sufficient Fe, and half were cultivated in a nutrient solution containing low Fe stress. Hydroponic solutions using basic nutrition solution contained 1.0 mM KH\(_2\)PO\(_4\), 5.0 mM KNO\(_3\), 5.0 mM Ca(NO\(_3\))\(_2\), 2.0 mM MgSO\(_4\), 6.7 mM HSO\(_4\), 2.0 mM MnSO\(_4\), 1.0 μM ZnSO\(_4\), 4.8 mM H\(_2\)O, 0.30 μM CuSO\(_4\), 5.43 mM H\(_2\)O, 0.10 μM Na\(_2\)MoO\(_4\), 2.0 mM MoO\(_4\)·2H\(_2\)O, and 46 μM H\(_3\)BO\(_3\) with different Fe(III)-EDTA concentrations (Fe-sufficient condition: 50 μM; low Fe stress condition: 2 μM). The rapeseed seedlings were
cultivated in an illuminated chamber with the following growth condition: the light intensity of 200 μmol m\(^{-2}\) s\(^{-1}\), the temperature of 25°C daytime/22°C night, the light period of 16 h photoperiod/8 h dark, the relative humidity of 70%. During hydroponic culture, the nutrient solutions were refreshed every 5 days. The pH of the nutrient solution was adjusted to 5.8 with NaOH.

**Determination of physiological indexes**

10 days after the treatment, selected 5 seedlings with uniform growth under different treatments, took samples from the shoots and roots, and measured their fresh weight. Then, dried at 65°C to constant weight, weighed the dry weight, and calculated the root-to-shoot ratio and relative water content of the shoots. According to the previous research [48], the shoots were cut into silk and mixed, and 0.2 g of the samples were weighed and placed in a test tube containing 5 ml of 80% acetone solution and soaked in a dark place at room temperature for 24 h. 80% acetone solution was used as the reference solution, and the DU®640 UV-Vis spectrophotometer (Beckman) was used to measure the absorbance at wavelengths of 470 nm, 645 nm, and 663 nm to determine the chlorophyll content.

We took fresh root samples of the whole plant in 5 mL 0.4 M mannitol at 25°C, 100 r/min shaker for 4 hours, and passed the conductivity meter (DDSJ-318, Shanghai, China) to measure the conductivity of the solution and record it as the initial leakage rate. Then put the samples in a water bath at 85°C for 20 minutes, measured the leakage rate of the solution, and recorded it as the total leakage rate. Ion leakage rate = initial leakage rate/total leakage rate [49].

The root scanner (Microtek Scan Maker I800, WinRHIZO Pro) was used to measure the total root length, primary root length, root surface area, root volume, number of root tips, lateral root length, and average root diameter of each plant, and 5 biological replicates were made for each treatment. The Suzhou Mengxi Biomedical Technology Kit was used to determine the root activity of wheat under different treatments by the tetrazolium (TTC) reduction method, and 5 biological replicates were performed for the different treatments.

The Keming biological kit was used to determine the content of proline (Pro), superoxide anion (OFR), hydrogen peroxide (H\(_2\)O\(_2\)), and malondialdehyde (MDA) in the shoots and roots of wheat under different treatments, and 5 biological replicates were made for each treatment.

**Microscopy analysis**

10 days after treatment of the wheat plants, the roots were taken and placed in a glass container filled with ddH\(_2\)O. The morphology of the root hairs was observed with a stereoscopic microscope (Leica M125C).

Paraffin sections were used to study the cell size in wheat root tips. First, put fresh wheat root tips of about 0.5 cm into a 5 ml reagent tube, added 2:3 ml FAA fixative (formaldehyde: glacial acetic acid: 70% ethanol=1:1:18), and performed aspiration treatment on the fixed material in time. It was then embedded after dehydration, transparency, and wax dipping. When using conventional paraffin section technology to slice the fixed material, the material needed to be stained by the safranine-fast green double staining method and then sliced with a microtome (Leica RM2235). The slice thickness was 8-10 μm, and a typical section was selected for microscopy, and finally took pictures under a stereoscopic microscope (Leica M125C) [50].

The shoots of wheat under different treatments were cleaned with distilled water, the excess water was absorbed by the absorbent paper, and they were fixed in fresh 4% glutaraldehyde fixative for 12 h. Then rinsed with 0.1 mol/L phosphate-buffered solution (PBS) of pH 6.8 for 4-5 times, and put the rinsed material into 1% osmium acid solution for fixation at 4 °C for 1 h, and then dehydrated with 100% ethanol. Infiltration, finally resin embedding and polymerization, the tissue block was taken out and stored in a desiccator. The sample was cut into ultra-thin sections of 50-70 nm, double-stained
with uranyl acetate-lead citrate, observed and photographed by transmission electron microscope (H-7650; Hitachi, Tokyo, Japan) [51, 52].

**Detection of ROS in wheat plants under different treatments**

ROS accumulation can be estimated based on the histochemical staining intensity of total H$_2$O$_2$ and O$_2$¯ in the roots. 3’3’-Diaminobenzidine (DAB) can react with H$_2$O$_2$ in the presence of peroxidase to form a dark brown polymer product, thereby detecting the H$_2$O$_2$ content in plant tissues [53]. Nitro blue tetrazole (NBT) can react with oxygen-free radical O$_2$¯ to produce dark blue insoluble formazan, which can be used to detect the O$_2$¯ content in plant tissues [54]. Finally, a stereoscopic microscope (Leica M125C) was used to take pictures.

**Ionoqmic analysis**

The *Triticum aestivum* seedlings after 7-day seed germination were hydroponically grown under Fe-sufficient and low-Fe solution for 10 days until sampling. Each sample contained 5 independent biological replicates for the ionomic analyses under low Fe stress. The over-dried shoot and root tissues were subsequently transferred to an HNO$_3$/HClO$_4$ mixture (4:1, v/v) at 200°C until the digestion was completed. The diluted supernatant was determined to quantify the concentrations of mineral elements using an inductively coupled plasma mass spectrometry (ICP-MS; NexIONTM 350X, PerkinElmer).

**Transcriptome Analysis**

For the low Fe treatment, the 7-d-old uniform seedlings after seed germination were hydroponically grown under Fe-sufficient and low-Fe solution for 10 days until sampling. The shoots and roots of the aforementioned *Triticum aestivum* plants were harvested, and three independent biological replicates were used for each treatment. Pre-chilled Trizol (Takara Bio Inc, Kusatsu, Shiga, Japan) was used to isolate total RNA, following which the RNA integrity number (RIN) was assessed. Samples of RNA with the RIN values > 8.0 were obtained to construct strand-specific cDNA libraries, which were further employed for the paired-end sequencing (read length = 150 bp) on a lane of an Illumina Hiseq 4000 platform. The fragments per kilobase of exon model per million mapped reads (FPKM) values were normalized to quantify the gene expression abundances, and both false discovery rate and P values < 0.05 were used to identify the differentially expressed genes (DEGs) [55]. PANTHER (http://www.pantherdb.org/data/) [56] and Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.kegg.jp/) [57], respectively, were used to perform gene ontology (GO) and pathway enrichment analysis of the DEGs. Heat maps showing differential gene expression were delineated using a Multi experiment Viewer (http://www.tm4.org/mev.html) [58].

**Reverse transcription-quantitative polymerase chain reaction assays**

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays were performed to validate the accuracy of transcriptome sequencing data. After removing genomic DNA from RNA samples with RNase-free DNase I, total RNA was used as RT templates for cDNA synthesis using the PrimeScriptTM RT Reagent Kit Eraser (Perfect Real Time; TaKaRa, Shiga, Japan). The RT-qPCR assays were performed to detect relative gene expression using SYBR®Premix Ex TaqTM II (TliRNaseH Plus) (TaKaRa, Shiga, Japan) using a Bio-Rad C1000 Touch Thermal Cycler of CFX96TM Real-time PCR detection system.

The RT-qPCR program was as follows: 95°C for 3 min, 40 cycles of 95°C for 10 s, and 60°C for 30 s. The melting curve was plotted as follows to analyze the primer gene-specificity: 95°C for 15 s, 60°C for 1 min, and 60-95°C for 15 s (+0.3°C/cycle). The expression data of target genes were normalized using BnaEF1-α [59], and the relative gene expression levels were calculated according to the 2$^{-\Delta\Delta CT}$ method [60]. Each sample contained three independent biological replicates.

**Gene co-expression network analysis**
The gene co-expression networks were constructed to identify gene interactions and locate core genes that connect most neighboring genes involved in the response to low Fe stress in *Triticum aestivum*. For each pair of genes, the threshold of the Pearson correlation value was set according to the default settings (http://plantgrn.noble.org/DeGNServer/Analysis.jsp), and then gene co-expression networks were constructed by CYTOSCAPE 3.2.1 (Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Canada) (http://www.cytoscape.org/) [61].

**Statistical analysis**

Statistical Productions and Service Solutions 17.0 (SPSS, Chicago, IL, USA) was used to perform statistical tests. One-way analysis of variance followed by Tukey's honestly significant difference (P < 0.05, P < 0.01, and P < 0.001) multiple comparison tests were used to determine the significant differences.

**Results**

**The morphological response of wheat to low Fe stress**

The typical symptom of low Fe in plants is the thin green veins on the pale green leaves. As the symptoms worsen, the leaves become thinner and white (or pale yellow), and brown spots and bad tissue appear on the leaves. After the leaves mature, the boundary between the green veins and the light green or light yellow mesophyll becomes more obvious. Our results showed that under low Fe stress, the growth and development of the root system of wheat were inhibited, and the root hair density became higher (Fig. 1A, C). Leaves were pale yellow or whitish, young leaves were chlorosis, and there was obvious visible inter-vein yellowing on the leaves (Fig. 1A, B).

The biomass of wheat was measured and found that under low Fe stress, the fresh weight and dry weight of the shoots were reduced by about 1.2 times, and the fresh weight and dry weight of the roots were reduced by about 1.3 times and 1 time, respectively (Fig. 2A, B). However, the relative water content of shoots showed no significant difference under different treatments (Figure. 2C). Determination of photosynthetic pigment content in wheat found that under low Fe stress, the concentrations of total chlorophylls (including chlorophyll a and b) and carotenoids (including xanthophyll and carotene) were significantly reduced (Fig. 2D, F). The ratio of the concentration of chlorophyll a to chlorophyll b was increased significantly under low Fe stress, which was also a sign of plant adaptation to adversity stress (Fig. 2E). In addition, low Fe stress reduced the ratio of total chlorophyll concentration to carotenoid concentration (Fig. 2E). Then we measured the ion leakage of the root system and found that there was no difference in ion leakage rate under different treatment conditions (Fig. 2G).

The damage of organelles was observed by transmission electron microscope, and the intracellular ultrastructure of the morphological difference between the control and the Fe-deficiency stress was studied (Fig. 3A). Our results showed that under normal Fe supply condition, chloroplast was well developed, complete in structure, convex lens-shaped, rich in content, many thylakoids, clear and complete basal lamella, and chloroplast membrane, and multiple basal lamellas, neatly arranged. However, under the low Fe condition, the chloroplast deformed and became smaller and the chloroplast membrane was blurred; the lamella system could not be stacked to form basal particles, and most of the basal lamellas disappeared and became blurred.

Root growth is determined by the balance between cell division and cell elongation [15]. To study the role of Fe in the growth and development of the root system, we observed the cell size of the meristematic zone and elongation zone in the root system grown under normal or low Fe stress (Fig. 3B). The meristem size was calculated as the area where isodiametric cells extend from the quiescent center (QC) to the cell, which was twice the length of the immediately preceding cell [16]. The boundary of the transition zone is different in each cell type, so in all the analyses performed here, the cortical cell file was used to define the boundary [15]. Our results showed that compared with the control, the number
of cells in the meristematic zone of the root system under Fe-deficient condition was significantly reduced, and the length of the cells in the elongation zone was significantly shorter (Fig. 3B-D).

Under the low Fe treatment, the root-to-shoot ratio was reduced but the difference was not significant. It could be inferred that the low Fe stress inhibited the growth of the shoots higher than that of the roots (Fig. 4A). Subsequently, the specific effects of low Fe stress on the growth of the wheat root system were analyzed. Low Fe stress was significantly reduced root-related parameters, including total root length, main root length, root surface area, root volume, number of root tips, average root diameter, and lateral root length (Fig. 4B-H). Then we measured the root activity of wheat and found that the root activity of wheat seedlings decreased significantly under low Fe stress, indicating that low Fe stress reduced the ability of roots to absorb Fe (Fig. 4I). These results indicated that the growth and development of wheat seedlings under low Fe stress were severely inhibited.

**Physiological response of wheat to low Fe stress**

To further understand the physiological response of wheat to low Fe stress, the contents of some osmotic adjustment substances that may participate in the regulation of low Fe stress resistance were tested. Under low Fe stress, the Pro concentration in the shoots and roots was increased significantly, but the concentration of MAD was only increased significantly in the shoots (Fig. 5A, D). The content of MDA is a sign of plasma membrane peroxidation. Our results showed that compared with the control, there was no significant difference in the concentration of MAD in roots under the low Fe stress, which indicated that the low Fe stress did not cause peroxidative damage to the plasma membrane (Fig. 5D). Under the stress of low Fe, the concentrations of OFR and \( \text{H}_2\text{O}_2 \) in the shoots and roots were significantly higher than those of the control condition (Fig. 5B, C). The results showed that low Fe stress accumulated more ROS.

**Fe deprivation caused ROS accumulation**

When plants are stressed by Fe deficiency, they will produce a large amount of ROS, which will cause oxidative damage. To analyze the effect of low Fe on the accumulation of ROS in wheat plants, DAB and NBT staining were performed on the shoots and roots of wheat after different treatments to observe the accumulation of \( \text{H}_2\text{O}_2 \) and \( \text{O}_2^- \) (Fig. 6A, B). The results showed that compared with normal Fe treatment, the DAB staining and NBT staining colors of wheat plant shoots and roots under low Fe stress were dark brown or dark blue, respectively, indicating that the shoots under low Fe stress, the concentrations of \( \text{H}_2\text{O}_2 \) and \( \text{O}_2^- \) in the roots and roots were significantly higher than those of the control (Fig. 6A, B). These research results showed that wheat plants produced more ROS after low Fe treatment. This result was consistent with the conclusion of Fig. 5B and C, that was, low Fe stress increased the accumulation of ROS.

**Effect of low Fe stress on mineral nutrient elements in wheat**

Inductively coupled plasma mass spectrometry (ICP-MS) is used to determine the ion expression profile of control and low Fe stress. ICP-MS quantitative analysis showed that the Fe\(^+\) concentration in the shoots and roots of wheat was significantly reduced under the low Fe stress treatment (Fig. 7B). Subsequently, we tested the concentrations of other metal cations, including Cu\(^{2+}\), Mn\(^{2+}\), Zn\(^{2+}\), Mg\(^{2+}\), Na\(^+\), Ca\(^{2+}\), Cd\(^{2+}\), and K\(^+\) (Fig. 7). In general, the response of low Fe stress to Cu\(^{2+}\), Mg\(^{2+}\), Na\(^+\), Cd\(^{2+}\), and K\(^+\) was similar. Under low Fe stress, the concentrations of Cu\(^{2+}\), Mg\(^{2+}\), Na\(^+\), Cd\(^{2+}\), and K\(^+\) in the shoots did not change significantly; on the contrary, the concentrations of these cations increased significantly in the roots (Fig. 7A, E, F, H, I). Under low Fe stress, the Mn\(^{2+}\) concentration was increased significantly in the shoots and roots (Fig. 7C). Unlike the above cations, the concentrations of Zn\(^{2+}\) and Ca\(^{2+}\) did not change significantly in the shoots and roots (Fig. 7D, G).

**Transcriptional response of wheat to low Fe stress**


To understand the molecular basis of Fe tolerance in wheat seedlings under low Fe stress, an RNA-seq library was established using the shoots and roots of low Fe seedlings as materials. The correlation between gene expression levels in samples is an important indicator for testing the reliability of experiments. In this study, under the same treatment, the Pearson correlation coefficient between each pair of biological replications was mostly higher than 0.90, indicating that the similarity between samples in the transcriptome was very high (Figure S1). RNA-Seq generated more than 1,145,14 million Raw reads (Table 1). Among these reads, the GC content of libraries was about 54.00%. After quality control, 1,134,550,000 Clean reads were obtained, the average error rate of sequencing bases was less than 0.0249%, the Q20 value was more than 98.03%, and the Q30 value was more than 94.06%. Among them, 7,592-10.23 million Clean reads were located in the wheat genome (Table 1). Principal component analysis showed that there were significant differences in the expression patterns between different treatments and different wheat tissues (Fig. 8A). 2,861 genes were identified as differentially expressed (FDR <0.05, fold change $\geq$ 2). There were a total of 137 differentially expressed genes (DEGs) in the shoots and roots (Fig. 8B). The number of DEGs in the shoots was relatively small (378), under Fe-deficient stress, 313 genes were up-regulated and 65 genes were down-regulated (Fig. 8B, C). There were a large number of DEGs in the roots (2,619), under low Fe stress, there were 1,589 up-regulated genes and 1,030 down-regulated genes (Fig. 8B, D).

To obtain the functional information of DEGs, GO annotation analysis was performed on DEGs, and DEGs were classified into three types: biological process (BP), cell component (CC), and molecular function (MF). GO entries with p-value < 0.001 were considered to be significantly enriched. In the shoots and roots under the stress of low Fe, BP was mainly enriched in the metabolic process and cellular process (Fig. 8E); CC was mainly enriched in the cell part and membrane part (Fig. 8E); in the MF, binding and catalytic activity were the two most abundant GO terms (Fig. 8E). The KEGG annotation divided the pathways that DEGs participate in into five categories: Cellular Processes, Environmental Information Processing, Genetic Information Processing, Metabolism, and Organismal Systems. Most DEGs in Cellular Processes were annotated to Endocytosis pathway, most DEGs in Environmental Information Processing were annotated to MAPK signaling pathway–plant pathway, most DEGs in Genetic Information Processing were annotated to Protein processing in endoplasmic reticulum pathway, most of Metabolism DEGs were annotated to the Phenylpropanoid biosynthesis pathway, and most DEGs in Organic Systems were annotated to the Plant-pathogen interaction pathway (Fig. 8F).

To further deepen our understanding of the functions of these DEGs, we conducted GO enrichment analysis (Fig. 8G). Among them, GO terms with p-value < 0.001 were considered to be significantly enriched. We found that most DEGs were related to plant metabolism and biosynthesis processes, such as aspartic acid biosynthetic process, nicotianamine synthase activity, nicotianamine metabolic process, nicotianamine biosynthetic process, nicotianamine aminotransferase activity, and L- methionine biosynthetic process, etc. These results indicated that the synthesis of Fe chelator and Fe transport was regulated to maintain Fe homeostasis. Significantly enriched GO terms related to ion transport and ion homeostasis included iron ion transmembrane transport, intracellular sequestering of iron ion, sequestering of metal ion and sequestering of iron ion, etc. The above results indicated that the transporter played an important role during the low Fe period. In addition, the GO terms related to photosynthesis, such as photosynthesis, light harvesting in photosystem I, were also significantly enriched, indicating that low Fe affected the photosynthesis of wheat, which was closely related to the decrease of chlorophyll content in the shoots (Fig. 2D, E, F). These results emphasized the importance of membrane and/or membrane-localized metal ion transporters and regulatory and metabolic proteins in the stress of low Fe.

The KEGG database was used to further determine the pathways involved in the response of wheat to low Fe stress (Fig. 8H). Studies have shown that in plants exposed to abiotic stress, the accumulation of amino acids was believed to have a beneficial effect in the process of plant stress adaptation [17-19]. Most of the DEGs were involved in amino acid metabolism pathways, such as Alanine, aspartate and glutamate metabolism, Tyrosine metabolism, Cysteine, and methionine metabolism and Phenylalanine metabolism, and other KEGG pathways, which indicated that amino acid metabolism and protein synthesis played an important role in the response of wheat to Fe deficiency stress. DEGs were
also involved in pathways related to photosynthesis, such as Photosynthesis − antenna proteins and Carotenoid biosynthesis, which indicated that low Fe had a significant impact on the photosynthesis of wheat. In addition, Phenylpropanoid biosynthesis, MAPK signaling pathway-plant, and other KEGG pathways were also significantly enriched (Fig. 8H).

Transcriptional response of Fe transport-related genes under low Fe stress

GO enrichment indicated that the synthesis of Fe chelator and Fe transport played an important role during low Fe stress. Among many DEGs, genes related to Fe ion homeostasis are the key genes for wheat tolerance to low Fe. Therefore, we analyzed the expression of genes related to Fe uptake and transport under Fe-deficiency stress. Figure S2 showed a molecular model of genes involved in Fe absorption and transport in plant roots (Figure S2A), chloroplasts, mitochondria, and vacuoles (Figure S2B). Transcription results analysis showed that most of the key genes involved in strategy I uptake of Fe, such as Fe\(^{3+}\) chelate reductase (FRO), iron-regulated transporter (IRT), natural resistance-associated macrophage protein (NRAMP), and Yellow stripe-like (YSL), were significantly up-regulated under Fe-deficiency stress. Among them, TaFRO2-2A (Figure S3A) and TaIRT1a-4A (Figure S3E), TaNRAMP2-4A (Figure S3B), and TaYSL15a-6D (Figure S3C) were up-regulated by 3 times, 6 times, 5 times, and 70 times, respectively, which might play a decisive role in the process of Fe uptake by plants.

In strategy II, the expression abundance of genes involved in plant Fe vector synthesis and Fe uptake and transport-related genes was significantly up-regulated. The process of synthesis and secretion of plant Fe siderophores is very complicated and requires the participation of 13 enzymes, but there are mainly four key enzymes: methionine synthetase (SAM), Nicotinamide synthase (NAS), Nicotinamide aminotransferase (NAAT), and Deoxyergate synthase (DMAS). Transcription analysis showed that these four key enzyme genes were significantly induced under low Fe stress. Among them, TaSAM3-6B (Figure S3D), TaNAS1c-6D (Figure S3I), TaNAAT1b-1B (Figure S3G), and TaDMAS1-4A (Figure S3F) had the most differential expression fold changes, which were up-regulated by 3 times, 3105 times, and 2718, and 24 times, respectively. The GO enrichment results also found that the GO terms related to nicotinamide (NA) synthesis were significantly enriched under low Fe stress (Fig. 8G). It might be related to the up-regulation of the expression abundance of the four key enzyme genes mentioned above, SAM, NAS, NAAT, and DMAS. And the synthesis of plant Fe carrier is related to the methionine salvage pathway because SAM is the key precursor for the synthesis of malic acid substances (MAs), which are produced by methionine. Transcriptome KEGG enrichment analysis also showed that methionine metabolism, L-methionine biosynthetic process, and L-methionine salvage from the S-adenosylmethionine pathway were significantly enriched (Fig. 8H). The above results indicated that amino acid metabolism played an important role in wheat response to low Fe. In strategy II, genes involved in Fe\(^{3+}\) transport such as transporter of mucenatic acid (TOM) (Figure S3J) and multidrug and toxin efflux family (MATE) (Figure S3K) were significantly up-regulated under low Fe stress. Among them, the differential expression of TaTOM-2B and TaMATE-4A changed the most, up-regulated by 2 times and 20 times, respectively. It was speculated that it might play a key role in the transport of iron from the xylem to neighboring cells. Oligopeptide transporter (OPT) is a polypeptide transporter located on the plasma membrane of plant epidermal cells. Transcriptome analysis showed that it was significantly induced by low Fe, and the differential expression of TaOPT3-5B was significantly up-regulated by low Fe by 44 times (Figure S3M), which might play a key role in the process of transmembrane transport of Fe\(^{3+}\)-PS complexes near epidermal cells to plants.

The process of vacuolar iron transporter ginseng (VIT) for vacuolar membrane positioning and vacuole loading of Fe into seed embryos not only promotes the development of seeds but is also crucial for seed germination. NRAMP is located on the vacuole membrane, mediates the transport of Fe from the vacuole of the seed embryo to the cytoplasm, and participates in the supply of Fe during seed germination. Transcriptome results showed that the expression abundance of most VIT genes was significantly down-regulated under low Fe stress, especially TaVIT2-5B was down-regulated by 3 times (Figure S3R), which might play a key role in promoting seed development. NRAMP3/4 was significantly induced by
low Fe stress, and TaNRAMP3-7D was up-regulated and expressed 3 times, which might play an important role in the transport of Fe from the vacuole of the seed embryo to the cytoplasm (Figure S3B). Mitochondrial m-type thioredoxin in chloroplast (ATM) is mainly involved in the process of exporting Fe-S to mitochondria, thereby maintaining the balance of Fe ion in mitochondria. Transcriptome analysis found that TraesCS5A02G110300 had the highest expression abundance in ATM, which might play an important role in maintaining Fe ion balance in mitochondria (Figure S3H). Permease in chloroplasts (PIC) is the first protein identified to participate in chloroplast Fe transport. Its expression is not regulated by Fe and is a constitutively expressed protein. Transcriptome analysis showed that PIC was significantly induced by low Fe in the shoots, and TraesCS1A02G165700 had the highest expression abundance (Figure S3L). Iron efflux transporter ferroportin (FPN) located in the chloroplast can regulate the intracellular Fe content by participating in the transport of NA or Fe-NA complex into the chloroplast. Transcriptome results showed that FPN was significantly induced by low Fe in roots, and TaFPN1-7A was significantly up-regulated by 60 times (Figure S3P), which might play a key role in maintaining Fe homeostasis in chloroplasts under low Fe stress. Nonintrinsic ABC protein (NAP) is located on the inner chloroplast membrane. It encodes a non-plasma membrane-localized nucleotide-binding domain subunit of the ABC transporter. Transcriptome analysis showed that NAP was significantly induced by low Fe in the shoots, and TraesCS3A02G047200 was significantly up-regulated. This result indicated that it might be part of the chloroplast Fe-ABC transport complex and played an important role in regulating the dynamic balance of Fe in the chloroplast (Figure S3Q). Another contributor to promoting the transfer of Fe ion into the chloroplast is mitoferrin-like, which contains chloroplast transit peptides and is mainly located in rosette leaves. Transcriptome results showed that the expression abundance of most mitoferrin or mitoferrin-like under low Fe stress decreased significantly, and the expression levels of TraesCS6D02G228200 and TraesCSU02G183100 were significantly down-regulated. It was speculated that they might play an important role in maintaining Fe content in chloroplasts (Figure S3N, O). The above results emphasized the importance of Fe ion uptake and transport proteins and regulatory and metabolic proteins in the stress of low Fe.

Involved in the transcriptional response of photosynthesis-related genes under low Fe stress

The GO enrichment analysis showed that GO terms related to photosynthesis, such as photosynthesis, light harvesting in photosystem I, were significantly enriched (Fig. 8G). And the KEGG enrichment analysis also showed that Photosynthesis – antenna proteins and carotenoid biosynthesis KEGG pathways were significantly enriched under low Fe stress (Fig. 8H), which indicated that low Fe significantly affected the photosynthesis of wheat plants. Many genes related to photosynthesis, such as photosynthetic antenna protein and key genes in the biosynthesis of carotenoids were identified (Figure S4). Through KEGG pathway analysis, a total of 33 genes encoding photosynthesis-antenna proteins (Figure S4A) and 39 genes encoding carotenoid biosynthesis (Figure S4B) were screened, and their expression levels were shown in Figure S4. The results showed that the expression levels of most genes involved in the photosynthetic antenna protein pathway and carotenoid biosynthetic pathway were significantly induced by low Fe (Figure S4A, B). The ratio of the concentration of chlorophyll a to chlorophyll b under low Fe stress was significantly higher than that under the control condition (Fig. 2E), which was a characteristic of plants adapting to adversity stress. Among them, chlorophyll an oxygenase (CAO), chlorophyll (ide) b reductase (CBR), chlorophyll synthase (CS), and chlorophyllase play an important role in the conversion between chlorophyll a and chlorophyll b (Figure S4C). Among the DEGs involved in photosynthesis, we found that the genes encoding these enzymes showed an increased expression pattern under low Fe stress (Figure S4C). There are two synthetic pathways for plant carotenoid substances: MEP pathway and MVA pathway. These two pathways generate geranylgeranyl diphosphate (GGPP), the direct precursor substance for carotenoid synthesis. GGPP generates the first carotenoid substance phytoene. After dehydrogenation, cyclization, hydroxylation, epoxidation, etc., it is transformed into other carotenoids. Transcriptome analysis showed that the expression of key enzyme genes in the process of carotenoid syntheses such as phytoene synthase (PSY), β-carotene hydroxylase (β-OHase), zeaxanthin epoxidase (ZEP), 9-cis-epoxycarotenoid dioxygenase (NCED), LUT5, and violaxanthin de-epoxidase (VDE) was significantly inhibited under low Fe stress (Figure S4D). And we found that Chlorophyll a/b binding proteins (Figure S4E), Photosynthesis II reaction proteins (Figure S4F), RuBisCo subunit binding proteins (Figure S2G), Mg²⁺ chelatase (Figure S4H), and Ribulose
biphosphatase carboxylase/oxygenase activase (Figure S4I) were significantly down-regulated under low Fe stress. These results all indicated that the severe degradation of wheat chlorophyll and photosynthesis was inhibited under low Fe stress.

**Transcriptional response of other ion transporters to low Fe stress**

Among the numerous DEGs, genes related to ion homeostasis are the key genes for wheat resistance to Fe-deficiency stress. Fig. 9A showed a molecular model of the key genes responsible for regulating K⁺, Na⁺/Cl⁻, and Ca²⁺ transport. Transcription analysis showed that most of the K⁺ transporter genes, including the K⁺ efflux transporter gene KEA located on the chloroplast, the vacuolar K⁺ inflow transporter gene KCO, the plasma membrane-located K⁺ inflow transporter gene AKT/KAT and HKT, and the K⁺ efflux gene SKOR was up-regulated under low Fe stress (Fig. 9B). The expression of Na⁺/H⁺ antiporter gene NHX, especially NHX2 involved in the Na⁺ compartment in the vacuole, and SOS1/NHX7 regulated the outflow of Na⁺ in the cell, were significantly up-regulated. To avoided cell damage due to excessive accumulation of Na⁺ in the cell (Fig. 9C). NHD could regulate the efflux of Na⁺, and its expression level was significantly up-regulated under low Fe stress, especially the expression of TaNHD1-5B was significantly up-regulated by 2 times, which might help reduce chloroplast damage caused by excessive Na⁺ (Fig. 9C). The chloroplast-localized bile acid: Na⁺ cotransporter (BASS) could regulate Na⁺ influx, and its expression level was significantly inhibited by low Fe stress, especially the expression level of TaBASS1D-1D was significantly down-regulated by 3 times (Fig. 9C). In addition, low Fe stress also induced the expression of most ALMT genes, which were related to the transport of Cl⁻ in vacuoles, especially the expression of TaALMT12-1B was significantly up-regulated by a factor of 2 under low Fe stress (Fig. 9C). Subsequently, the gene expression related to Na⁺/Ca²⁺ transport was studied, including CXX, CAX, ANXD, GLR, and CNGC. The expression levels of most genes changed significantly under low Fe stress. This result indicated that these genes might be involved in ion homeostasis under low Fe stress (Fig. 9D). For example, under low Fe stress, TaCCX expression was increased, TaCAX expression was decreased, and TaANXD expression was increased. This might be an important reason for reducing cytoplasmic Na⁺ and increasing cytoplasmic Ca²⁺ concentration (Fig. 9D). Most of the plasma membrane-localized NSCC, including GLR and CNGC, were up-regulated under low Fe stress (Fig. 9D). This might be an important way for Ca²⁺ and Na⁺ to enter the cytoplasm under low Fe stress (Fig. 9D). In addition, under low Fe stress, the expression levels of genes PHT2;1 (Fig. 9E) and COPT (Fig. 9F) involved in the uptake and transport of Pi and Cu²⁺ by roots were significantly down-regulated, while genes involved in Mg²⁺ uptake and transport were significantly induced by low Fe stress (Fig. 9G). The above results all indicated that the maintenance of ion homeostasis in cells was of great significance for plants to respond to low Fe stress.

**Expression profile of cell cycle-related genes and ROS metabolism-related genes under low Fe stress**

To study whether the inhibitory effect of Fe deficiency on root growth is related to the degree of cell division and differentiation, we combined the transcriptome to analyze the rich expression of cell cycle-related genes (Figure S5A). Cyclin and cyclin-dependent kinase belong to cell cycle control proteins, which play a key role in the process of mitosis [20]. LRP (lateral root primordia) is specifically expressed in adventitious roots and lateral root primordia. It is a transcription factor that regulates root elongation and has strong tissue specificity [20]. Transcriptome analysis showed that under low Fe stress, the expression abundance of these genes was significantly down-regulated (Figure S5A). It indicated that the shortening of roots under low Fe stress might be due to the decrease in the division frequency of cells in the meristematic zone and the shortened cell elongation in the elongation zone.

According to the results in Fig. 5 and Fig. 6, we combined the transcriptome to analyze the expression of key enzyme genes in the ROS metabolic pathway (Figure S5B, C). The respiratory burst oxidase homologous gene (RBOH) encodes NADPH oxidase and plays a key role in the synthesis of ROS [21]. Under low Fe stress, 11 RBOH differentially expressed genes were significantly up-regulated in shoots or roots (Figure S5B). The expression of SOD, CAT, and PDH genes in most
shoots and roots increased under low Fe stress, which might be necessary for ROS clearance under low Fe stress (Figure S5C).

**Gene co-expression network analysis**

In allohexaploid wheat, multiple copies of genes are ubiquitous. Therefore, the identification of core genes is an important prerequisite for understanding the molecular mechanisms of important agronomic traits. The systematic analysis of the transcriptional response of genes related to Fe uptake and transport under low Fe stress will help us to fully understand the adaptation mechanism of plants to Fe-deficiency stress. To identify the core members of genes related to Fe uptake and transport, we constructed a gene co-expression network (Figure S6A). The results showed that some Fe\(^{2+}\) absorption and transport related genes \(TaIRT1b-4A\) and plant siderophores related genes such as \(TaNAS2-6D, TaNAS1a-6A, TaNAS1-6B\) and \(TaNAAT1b-1D\) were identified as core target genes, which might play a key role in plant response to low Fe stress (Figure S6A).

**Discussion**

Worldwide, low Fe stress severely inhibits plant growth, yield, and crop quality. Allohexaploid wheat is a major food crop and is highly sensitive to the stress of low Fe. Analyzing the physiological and molecular mechanisms of wheat tolerance to low Fe stress is an effective way to cultivate low Fe wheat varieties.

**Transcriptomics-assisted analysis of morphophysiological responses of wheat to low Fe stress**

In higher plants, low Fe is characterized by chlorosis between the veins of young leaves, while mature leaves remain green due to Fe immobility [22]. Our research results showed that under low Fe stress, wheat seedlings had obvious plant damage, including yellowing of leaves (Fig. 1) and inhibition of root growth (Fig. 1, 3). Although Fe is not a component of chlorophyll, it is indispensable in the synthesis of chlorophyll precursors. It is also an important indicator for studying wheat growth characteristics, physiological changes, and Fe nutritional status [23]. The degree of leaves xanthosis caused by low Fe in crops is mainly expressed by chlorophyll content because chlorophyll content is an effective indicator for judging whether crops are lacking Fe [24, 25]. As a functional indicator of photosynthetic pigment and light response [26], the concentration ratio of chlorophyll a and chlorophyll b under low Fe stress was significantly lower than the control condition (Fig. 2D), indicating that low Fe stress had a more obvious inhibitory effect on chlorophyll a. In addition, the concentration of total chlorophyll and carotenoids were decreased under low Fe stress (Fig. 2D, F), which also indicated that chlorophyll was degraded or damaged photosynthesis under low Fe stress [27]. Whether the stress of plant nutrient deficiency or physiological nutrient deficiency can cause the metabolic disorder of its leaves, severe nutrient deficiency will affect the organ structure of the plant, resulting in changes in the cell and subcellular morphology of plant leaves. Previous studies in rice [28] and *Arabidopsis thaliana* [29] found that the reduction or number of chloroplasts would inhibit the photosynthetic rate, and the destruction of the chloroplast structure would limit the photosynthetic rate. In this study, low Fe stress caused chloroplast separation and chlorophyll degradation, which might further lead to photosynthesis inhibition (Fig. 2D-F, Fig. 3A). The analysis of DEGs showed that the photosynthesis-related KEGG pathway was highly enriched (Fig. 8H), and the genes related to the chlorophyll biosynthesis pathway were significantly down-regulated under low Fe stress (Figure S4). The root system is an organ for wheat to absorb essential mineral elements for growth and development. Because root growth is related to cell division and elongation rate, our analysis of the root system of wheat showed that under low Fe stress, cell division was reduced, resulting in a reduction in the elongation of meristem cells, which was related to the observed reduction in growth (Fig. 2B, C).

Some research results show that under the stress of low Fe, the content of ROS and osmotic adjustment substances change significantly [30]. When adversity stress forces the production of a large amount of ROS in plant cells, it will cause damage to macromolecular substances and other components in the cell, hindering the normal metabolism and growth of
plants, and even death [31]. Our results showed that under the condition of low Fe, the concentration of OFR and H$_2$O$_2$ were increased significantly (Fig. 5B, C), which initiated membrane lipid peroxidation and damages the structure of the cell membrane system. However, the ion leakage rate (Fig. 2G) and MAD content (Fig. 5D) in the root system were not different from those of the control, indicating that the accumulation of ROS did not cause great damage to the cell membrane system in the wheat root system. The content of osmotic regulators such as Pro and MAD accumulated in the cells in a large amount, thereby regulating the osmotic potential of the cells and further maintaining the normal metabolism of the cells (Fig. 5A, D).

Transcriptomics-assisted analysis of ion response of wheat to low Fe tress

Fe homeostasis in plants is jointly participated by a variety of Fe ligands, transporters, and regulatory factors. Usually, Fe is combined with ligand for transportation or storage to reduce the risk of free Fe producing toxic ROS [32]. NA and Deoxysarcosine (DMA) are Fe chelating agents and are responsible for the acquisition and transport of Fe. NA has long-distance and cellular Fe transport functions in higher plants [33–35]. DMA is mainly used for the long-distance transportation of Fe in the xylem and phloem [36, 37]. We also found that GO terms related to NA and DAM synthesis were significantly enriched in our transcriptome (Fig. 8G). In addition, expressions of key enzymes in NA and DAM synthesis pathways, such as SAM, NAS, NAAT, and DMAS, were significantly up-regulated (Figure S3D, I, G, F), which was consistent with previous results in wheat [38–40] and rice [33–35]. Previous studies have shown that the expression abundance of genes related to Fe uptake and transport in mitochondria [41, 42], chloroplasts [43, 44], and vacuoles [45] are significantly up-regulated under Fe-deficiency stress. This conclusion was consistent with our transcriptome results (10H-R). The increased expression of these genes in young leaves under low Fe tress might be to unload more Fe into mesophyll cells to meet the normal absorption and utilization of Fe by young leaves. But when the Fe content in the cell exceeds the limit, the plant can transport Fe$^{2+}$-chelates into the organelles to store Fe such as vacuoles, avoiding cell toxicity. Studies have shown that when AtVTL1, AtVTL2, or AtVTL5 is expressed under the 35S promoter in the background of nramp3/nramp4 or vit1-1, in the two mutants [46], AtVTL1, AtVTL2 or AtVTL5 can restore root growth. In yeast, the VIT homologous Ca$^{2+}$-Sensitive-Cross-Complementer (CCC1) protein is a vacuolar Fe transporter that transports Fe to the vacuole under condition of excess Fe [45]. The pastatin promoter is used on cassava for overexpansion, and the expression of atvit1 gene is positively correlated with the increase of Fe concentration in storage roots. This result shows that the VIT gene is involved in mediating the vacuolar retention of Fe [47]. The above results all indicate that the dynamic balance of Fe in cells is essential for the normal development of plants.

Conclusions

Fe not only plays an important role in plant physiological functions but also plays an important role in maintaining various cell processes. In the past few decades, more and more progress has been made in understanding how plants adapt to low Fe in the soil. However, how to observe the Fe dynamics in plants is still a significant challenge. Although there is a lot of information indicating that many genes are responsible for the absorption of Fe from the soil, the transportation from roots to shoots, the storage in cells, and even the regulation of transcription and post-transcriptional levels. However, further research is still needed to reveal the further connection and integration between Fe-deficient signaling pathways and developmental and physiological networks. This study analyzed the morphological and physiological changes of the shoots and roots under low Fe tress and combined the transcriptome to analyze the expression profiles of genes, which related to Fe uptake and transport, photosynthesis, and other ion transporter-related genes. Finally, key genes affecting plant response to low Fe stress were identified. In summary, these results might provide excellent genetic resources for the apparent regulatory mechanism of wheat resistance to low Fe tress and crop genetic improvement.

Abbreviations
ATM: Mitochondrial m-type thioredoxin in chloroplast; BASS: chloroplast-localized bile acid; BP: biological process; CAO: chlorophyll an oxygenase; CBR: chlorophyll (ide) b reductase; CC: cell component; CCC1: Ca²⁺-Sensitive cross-Cross-Complementer; CS: chlorophyll synthase; CV: Coefficient of Variation; DAB: 3'3’-Diaminobenzidine; DEGs: differentially expressed genes; DMA: Deoxysarcosine; DMAS: Deoxyergate synthase; Fe: iron; FIT: fer-like iron deficiency-induced transcription factor; FPKM: fragments per kilobase of exon model per million mapped reads; FPN: Iron efflux transporter ferroportin; FRO: Fe³⁺ chelate reductase; GGPP: geranylgeranyl diphosphate; GO: Gene Ontology; H⁺-ATPase: proton ATPase; H₂O₂: hydrogen peroxide; ICP-MS: Inductively coupled plasma mass spectrometry; IRT: Iron-regulated transporter; KEGG: Kyoto Encyclopedia of Genes and Genomes; LRP: lateral root primordia; MATE: Multidrug and toxin efflux family; MDA: malondialdehyde; MF: molecular function; NAAT: Nicotinamide aminotransferase; NAP: Nonintrinsic ABC protein; NAS: Nicotinamide synthase; NBT: Nitro blue tetrazole; NCED: 9-cis-epoxycarotenoid dioxygenase; NRAMP: Natural resistance-associated macrophage protein; OFR: superoxide anion; OPT: Oligopeptide transporter; PIC: Permease in chloroplasts; Pro: proline; PS: phytosiderophore; PSY: phytoene synthase; QC: quiescent center; RBOH: respiratory burst oxidase homologous gene; RIN: RNA integrity number; RT-qPCR: Reverse transcription-quantitative polymerase chain reaction; SAM: Methionine synthetase; TOM: Transporter of magnetic acid; TTC: tetrazolium; VDE: violaxanthin de-epoxidase; VIT: Vacuolar iron transporter ginseng; YSL: Yellow stripe-like; ZEP: zeaxanthin epoxidase; β-OHase: β-carotene hydroxylase.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files]. All the data and materials that are required to reproduce these findings can be shared by contacting Dr. Ying-peng Hua (yingpenghua@zzu.edu.cn).

Plant Ethics

We declare that the collecting of plant material is allowed by the Convention on the Trade in Endangered Species of Wild Fauna and Flora: https://www.cites.org/, and plant material was collected with permission in accordance with national and international guidelines.

In this study, all the bread wheat (Triticum aestivum L.) cultivar zhengmai 1860 were obtained from the Henan Academy of Agricultural Sciences (Zhengzhou, Henan Province, China).

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

Ying-peng Hua, Jin-yong Huang, Ting Zhou, and Jin-yong Huang planned and designed the research and analyzed data. Ying-peng Hua and Yue Wang performed the experiments. Yue Wang wrote the original manuscript. Ying-peng Hua reviewed and edited the manuscript. All authors read and approved the final manuscript.

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References


Tables
Table 1  Statistics of wheat transcriptome sequencing data under low Fe stress

<table>
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<tr>
<th>Sample</th>
<th>Raw reads</th>
<th>Clean reads</th>
<th>Mapped Reads</th>
<th>Error rate(%)</th>
<th>Q20(%)</th>
<th>Q30(%)</th>
<th>GC content(%)</th>
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<td>HIR1</td>
<td>103,367,072</td>
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<td>98.24</td>
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<td>LIR1</td>
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<td>LIR3</td>
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</table>

Note: HIR (Hight Iron Root), HIS (Hight Iron Shoot), LIR (Low Iron Root), LIS (Low Iron Shoot). 1, 2, and 3 respectively represent different biological repetitions. Error rate (%) is the average error rate of sequencing bases corresponding to the quality control data. Q20% is the proportion of the nucleotide quality value larger than 20; Q30% is the proportion of the nucleotide quality value larger than 30; GC content (%) is proportion of guanidine and cytosine nucleotides among the total nucleotides.

Figures
Figure 1

The morphological response of wheat to low Fe stress. (A) The overall phenotype of wheat plants under normal Fe (50 mm) and low Fe (2 mm) conditions for 10 days. Bar: 5 cm. (B) Wheat leaves treated with different Fe concentrations. Bar: 2 mm. (C) Wheat roots treated with different Fe concentrations. Bar: 2 mm.
Figure 2

Physiological response of wheat to low Fe stress. (A) fresh weight, (B) dry weight, (C) relative water content (%), (D) pigment concentration, (E) ratio of Chl a/Chl b and Chl (a+b)/Chl (x+c), (F) Chlorophyll concentration, (G) ion leakage. Data are means (±SD), n=5. Using single-way ANOVA analysis, difference* means significant difference (*P<0.05; **P<0.01; ***P<0.001).
Figure 3

Microscopic characteristics of wheat plant ultrastructure under low Fe stress. (A) Chloroplast structure, (B) Effect of low Fe stress on the cells in the meristem and elongation zone of wheat roots. Bar: 50 \( \mu \)m, 25 \( \mu \)m, 2.5 \( \mu \)m; (C) The length of the meristem, (D) Cell length in elongation region. Using \( t \) test analysis, difference* means significant difference (*\( P<0.05; **P<0.01; ***P<0.001 \)).
Figure 4

Effect of low Fe stress on the growth of wheat root system. (A) root/shoot ratio, (B) total root length (cm), (C) primary root length (cm), (D) root surface (cm²), (E) root volume (cm³), (F) root tip numbers, (G) average root diameter (mm), (H) lateral length (cm), (I) root activity (μg TPF g⁻¹ FW h⁻¹). Data are means (±SD), n=5. Using t test analysis, difference* means significant difference (*P<0.05; **P<0.01; ***P<0.001).
Figure 5

Effect of low Fe stress on osmotic adjustment substances in wheat. (A) proline (Pro), (B) superoxide anion (OFR), (C) hydrogen peroxide (H$_2$O$_2$), (D) malondialdehyde (MDA). Data are means (±SD), n=5. Using single-way ANOVA and Tukey’s HSD test analysis, difference* means significant difference (P <0.05). Data are means (±SD), n=5. Using single-way ANOVA analysis, difference* means significant difference (*P<0.05; **P<0.01; ***P<0.001).

Figure 6

Effect of low Fe stress on ROS accumulation in wheat. (A) Effect of low Fe on H$_2$O$_2$ accumulation in wheat leaves and roots. The bar in the leaves is 2mm, the bar in the roots is 5mm. (B) Effect of low Fe on O$_2^-$ accumulation in wheat leaves and roots. The bar in the leaves is 2 mm, the bar in the roots is 5 mm.

Figure 7
Ion analysis of wheat plants under different Fe treatment conditions. (A) Cu, copper; (B) Fe, iron; (C) Mn, manganese; (D) Zn, zinc; (E) Mg, magnesium; (F) Na, sodium; (G) Ca, calcium; (H) Cd, cadmium; (I) K, potassium. Data are means (±SD), n=5. Using single-way ANOVA analysis, difference* means significant difference (*P<0.05; **P<0.01; ***P<0.001).

Figure 8

Analysis of wheat plant transcriptome sequencing data under different Fe concentration treatments. (A) principal component analysis of differentially expressed genes (DEGs) (A), venn diagram analysis (B), shoot volcano diagram (C) and root volcano diagram (D) in the control (normal Fe: 50 μm) and treatment (low Fe: 2 μm), the difference between stem (S) and root (R) is significant. The number of differentially expressed genes is listed in parentheses. GO annotation (E), KEGG pathway (F), GO enrichment (G) and KEGG enrichment (H) analysis of all DEGs in the shoots and roots between the control and treatment conditions. For G and H, the size of the circle indicates the number of DEGs, and the enrichment factor indicates the degree of enrichment of the KEGG pathway involving DEGs. For transcriptome sequencing, selected uniform wheat plants after germination, half of which were transplanted into a nutrient solution with normal Fe concentration for cultivation, and half were transplanted into a nutrient solution with low Fe for cultivation, and samples were taken 10 days later.

Figure 9

Differential expression map of other ion transport genes in wheat plants under low Fe stress. (A) Molecular model of genes responsible for the transport of other cations. Differential expression profiling of the genes involved in the transport of potassium (K⁺) (B), sodium (Na⁺)/chlorion (Cl⁻) (C), calcium (Ca²⁺) (D), phosphorus (Pi) (E), copper(Cu²⁺) (F), and magnesium (Mg²⁺) (G) ions. For transcriptome sequencing, selected uniform wheat plants after germination, half of which were transplanted into a nutrient solution with normal Fe concentration for cultivation, and half were transplanted into a nutrient solution with low Fe for cultivation, and samples were taken 10 days later. The heat map shows the gene expression level indicated by the TPM value. Differentially expressed genes that show higher expression levels under the control (normal Fe: 50 μm) and treatment (low Fe: 2 μm) are indicated by asterisks.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.Correlationanalysisofrootandrootsamples.pdf
- FigureS2.MolecularmodelofFeabsorptionandtransportrelatedgenes.pdf
- FigureS3.DifferentialexpressionprofilesogenesrelatedtoFeabsorptionandtransportinwheatplantsunderlowFestress.pdf
- FigureS4.DifferentialexpressionprofileofphotosynthesisrelatedgenesinwheatplantsunderlowFestress.pdf
- FigureS5.HeatmapofexpressionofgenesinvolvedincellcycleandROSmetabolism.pdf
- FigureS6.CoexpressionnetworkanalysisofFeabsorptionandtransportrelatedgenesunderlowFestressconditions.pdf