Generation of the wheat mutants with high iron (Fe) and expression analysis of the responsive genes involved in iron accumulation and ethylene response

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Abstract

Wheat (*Triticum aestivum* L.) is one of the most important staple crops, feeding one-third of the global population. Among the mineral nutrients, iron (Fe) is one of the necessary trace elements for human body. Iron deficiency will lead to various diseases. Although some progress has been made on the Fe biofortification aimed at increasing the levels of the essential mineral in the staple crop, little is known about the molecular regulations of the Fe accumulation-associated gene expressions in the wheat plant. In the present study, two wheat mutant lines with high ferric contents were identified. Transcriptomic analysis indicated that the differentiated expression genes (DEGs) were involved in different biological processes such as catalytic reaction, translation, biologic regulations, biosynthesis of secondary metabolites, and stress signaling. Further study revealed that the responsible genes for Fe homeostasis such as *TaFIBP* (encoding a ferric ion binding protein), *TaZIP29* (a ZRT/IRT-like protein (ZIP) encoding gene) and *TaYSL1* (wheat homologue of the *Arabidopsis* yellow stripe-like protein encoding gene) were highly induced when treated with exogenous Fe in the wheat mutant lines. In addition, it was observed that the ethylene sensitivity of the wheat mutant lines with high iron were obviously altered. And, the transcript levels of the ethylene responsive genes (*TaERF1*, 2, 3, 4, 6) were significantly affected in the wheat mutant lines, suggesting that ethylene may participate in regulation of the iron accumulation in wheat. All these results advance our understanding of the molecular regulations in the Fe accumulation of wheat.

Introduction

Iron is one of the necessary trace elements for human body. Iron deficiency will lead to malnutrition, decreased body immunity, retardation of growth, and other diseases (Briat et al. 2015). In plants, iron participates in various biological processes such as chlorophyll synthesis, mitochondrial respiration, nitrogen assimilation, production and scavenging of reactive oxygen species (ROS), and enzymatic activities (Hansch and Mendel 2009). Fe deficiency dramatically affects plant growth, formation, and productivity. Plants absorb Fe from soil. In soil, iron availability is often low due to its low solubility. To adapt to Fe-deficient conditions, plants have developed multiple strategies to absorb Fe. Strategy I occurs in non-graminaceous monocots and dicots, while strategy II exists in graminaceous monocots (Römheld and Marschner, 1986; Kobayashi and Nishizawa, 2012). When exposed to a Fe-deficient environment, the Strategy I plants usually promote the Fe absorbing by enhanced ferric reductase activity, acidification of the rhizosphere and up-regulation of Fe^{2+} transporters (Curie and Briat, 2003; Hell and Stephan, 2003; Romera and Alcántara, 2004), while the Strategy II plants often increased the secretion of phytosiderophores (PSs) to form a Fe^{3+} -MA complex (Kobayashi and Nishizawa, 2012).

In past years, numerous studies had identified the responsible genes in Fe assimilation and transportation. For example, it was found that *AtYSL1* (a member of the *YELLOW STRIP-LIKE* family) participates in Fe loading and transport in *Arabidopsis* (Le Jean et al. 2005; Waters et al. 2006; Chu et al. 2010). In addition, it was reported that *AtOPT3*, a member of the oligo-peptide transporter family, also regulates seed Fe accumulation in *Arabidopsis*. Lack of *AtOPT3* causes decreased seed Fe accumulation

(Stacey et al. 2008). In plants, ferritins were thought to sequester Fe, and buffer against the excess of Fe (Roschzttardtz et al. 2013).

Plant hormones and small molecular regulators play important roles in responses to iron deficiency (Yang et al., 2013). For instance, auxin, brassinosteroids, and gibberellins (GAs) and others have been reported to be involved in the regulation of Fe deficiency-induced physiological processes in plants. Several lines of evidence suggested that auxin is involved in plant root growth and the expression of Fe-uptake genes under Fe deficiency condition (Jin et al., 2011; Giehl et al., 2012; Ivanov et al., 2012; Rai et al., 2015). Qi et al (2012) reported that OsARF12, a transcription factor that activates the auxin response gene, affected Fe accumulation and distribution in rice. Xu et al (2014) provided evidence indicating that *OsABCB14* is involved in both auxin transport and Fe homeostasis in rice. In addition, Liu et al (2015) reported that auxin signaling is involved in the regulation of Fe-deficiency induced impairment of photosynthetic activity and shoot growth in rice. Brassinosteroids are also involved in Fe homeostasis in rice (Wang et al., 2015). In a GA-deficient *Arabidopsis* mutant, expression of the genes associated with Fe-deficiency responses was up-regulated (Matsuoka et al., 2014). Wild et al (2016) reported that the spatial distribution of GA-regulated DELLA growth repressors in *Arabidopsis* roots was adapted to the root system architecture and the Fe-uptake machinery to the plant's Fe demand. Recently, Wang et al (2017) provided evidence supporting that GA was in mediation of Fe homeostasis by negatively regulating Fe transport and translocation in rice. Moreover, a study showed that jasmonate responsive genes were also up-regulated in rice when subjected to Fe deficiency (Kobayashi et al., 2016).

The phytohormone ethylene also participated in the regulation of Fe accumulation in plants. Early studies suggested that ethylene may not be involved in the regulation of Fe-deficiency responses by strategy II plants (Romera et al., 1999; Romera and Alcántara, 2004). Wu et al (2011) showed that ethylene regulates expression of the genes associated with Fe acquisition in rice plants. In *Arabidopsis*, it was reported that EIN3 can physically interact with and stabilize FIT1 (Lingam et al. 2011). The *Arabidopsis* AtERF72 negatively regulates Fe-deficiency responses by binding to the promoters of several Fe-deficiency responsive genes (Liu et al. 2017). In addition, it was found that the rice *ERF* genes are transcriptionally regulated under Fe overload stress conditions (dos Santos et al. 2013). A recent study showing that lack of *ERF95* or *EIN3* reduces seed Fe accumulation in *Arabidopsis*, while overexpression of *ERF95* could rescue Fe accumulation in the *ein3* loss-of-function mutant (Sun et al. 2020).

Bread wheat (*Triticum aestivum* L.) is cultivated on more land than any other crop. So far, most studies have been focused on the Fe biofortification aimed at increasing the levels of the essential mineral in the staple crop. For instance, it was reported that over-expression of the rice (*Oryza sativa* L.) nicotianamine synthase 2 (*OsNAS2*) gene led to an accumulation of higher concentrations of grain Fe, Zn, NA (nicotianamine) and DMA (2'-deoxymugineic acid) in transgenic plants (Beasley et al., 2019). Overexpression of *TaVIT2* in wheat and barley resulted in more than twice as much iron in white flour fractions (Connorton et al., 2017). Overexpression of *TpSnRK2.10* or *TpSnRK2.11* in dwarf Polish wheat significantly increased Fe accumulation in roots and Cd accumulation in roots and shoots (Wang et al.,

2019). Thus, there is an urgent need to explore more about molecular regulations of the Fe accumulation-associated gene expressions in the wheat plant.

Previously, we used EMS (ethylmethanesulfonate) mutagenesis to generate a mutant library from the popular wheat variety 'Luyuan 502' in China (Ma et al. 2021). By screen of the mutant plants, a number of salt-tolerant lines with high crop yield were obtained from the study. Further analysis from the mutant plants in the levels of mineral ions led to identification of two lines with high ferric contents. The presence of the wheat mutant lines with high iron provided a good example to explore changes of endogenous genes responsible for iron accumulation at a transcriptome scale in wheat. In the present study, the iron responsive gene expression, ethylene sensitivity and ethylene signaling-regulated gene expressions were examined in the mutant plants. These results undoubtedly advance our understanding about the molecular regulations of Fe accumulation in the crop.

Materials And Methods

Plant material and growth condition

The bread wheat cultivar Luyuan502 (approved by the Chinese National Crop Variety Examination and Approval Committee, 2011) was used for experiments. Seeds of 'Luyuan502' were sterilized in 2% NaClO for 10 min, washed in sterile water for 3 min. After washing 5 times, the seeds were placed in the petri dish filled with two layers of sterile filter paper. The surface sterilized seeds were incubated at room temperature. The petri dishes were then incubated in the growth chamber at 25°C under 16-h light/ 8-hour dark for 24 h or 6 d.

Measurement of iron content

The surface sterilized seeds were dried in an oven at 65°C, and then crushed in a grinder. The fine particles of the samples passed through a 1 mm sieve were collected. 1.00 g of each sample was mixed in a tube containing 10 mL HNO₃ and 2 mL HClO₄, and placed at 160°C overnight until the solution is clear and transparent. 10 mL deionized water was added into the tube, and mixed well. The iron content of the samples was measured using an inductively coupled plasma emission spectrometer (ICP-OES, PerkinElmer).

Ethylene sensitivity assays

Surface sterilized seeds in 2% NaClO were placed in the petri dish filled with two layers of sterile filter paper and supplemented with sterile ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) at 300 µM. The petri dishes were wrapped in aluminum foil and incubated for 24 h or 6 d at room temperature. Ethylene insensitive mutants were picked up from the screen by measuring seedling growth and root length of the etiolated seedlings. Three replicates were taken, and at least 10 seeds were examined in each replicate.

RT-qPCR analysis

Total RNA was isolated from 200 mg of plant tissue using a RNAiso Plus (Takara, Japan). cDNA synthesis was performed using M-MLV reverse transcriptase (Takara, Japan). RT-qPCR was performed using Applied Biosystems QuantStudio 5 (Thermo Fisher, USA). Gene-specific primers used for RT-qPCR were provided in Table S1. Sequence information of the wheat genes and their accession numbers were obtained from Wheat@URGI (<https://wheat-urgi.versailles.inrae.fr/>). The *TaACTIN* (GenBank: AB181991) was used as the internal control. Each RT-qPCR was carried out with at least three biological replicates in the experiments.

Transcriptome sequencing

The 2-week-old seedlings of the wheat (*Triticum aestivum* L.) cultivar 'Luyuan502' and the mutant lines *LHFe-1* and *LHFe-2* were used for sequencing by Genedenovo Biotechnology Co., Ltd (Guangzhou, China). Three biological replicates were used in each sample. The sequencing library was sequenced by Illumina HiSeqTM 2500, and the libraries were generated using NEBNext[®] UltraTM RNA Library Prep Kit for Illumina[®] (NEB, USA). The short reads were filtered by an alignment tool (Bowtie2 2.2.8) (Langmead and Salzberg 2012). The obtained reads were then aligned to the reference genome (<http://plants.ensembl.org/index.html>) (Kim et al. 2013; Bolser et al. 2015). The transcripts were annotated and identified according to Trapnell et al (2010, 2012).

The genes with fold change ≥ 2 and False Discovery Rate (FDR) < 0.05 were defined as significant Differentially Expressed Genes (DEGs). The Gene Ontology (GO) analysis (<http://www.geneontology.org/>) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (<http://www.genome.jp/kegg/>) were constructed, respectively.

Results

Generation and identification of salt tolerant wheat mutant lines with high iron

To generate stress-tolerant wheat plants, the bread wheat cultivar 'Luyuan502' was mutagenized by EMS as previously described (Ma, et al. 2021). In brief, the EMS-mutagenized seeds were sown, grown and screened in a saline-alkali soil field (0.3% - 0.7% salinity). A large number of the M1 lines were harvested. Among them, the salt tolerant wheat lines were selected for further study mainly based on their increased yields by 20% - 30% in the M₂ - M₅ generations (Ma et al. 2021). Next, the iron contents of the salt tolerant wheat mutant lines were measured. Two lines, *LHFe-1* and *LHFe-2* generated from 'Luyuan502' possessed remarkably higher iron content than the wild types and other mutant lines (Fig. 1). This observation implied that the genes responsible for iron assimilation, transportation, and accumulation may be altered in the mutant plants.

Transcriptomic profiling of the differentiated expression genes in the wheat mutant lines with high Fe

The presence of the wheat mutant lines with high iron (Fe) provided a good example to explore the change of endogenous genes responsible for iron accumulation at a scale of transcriptome in wheat. The wheat mutant line *LHFe-1* and *LHFe-2* were chosen for transcriptome analysis using the wild type 'Luyuan502' as a control. The 2-week-old seedlings of the wheat plants were collected and used for RNA-seq analysis. Based on the comparisons between WT and each of the wheat mutant lines, the transcripts with fold change ≥ 2 and FDR < 0.05 were defined as significant DEGs. A venn diagram of DEGs in the comparisons of WT-vs-*LHFe-1*, WT-vs-*LHFe-2* showed 509 DEGs overlapped (Fig. 2; Table S2). The GO and KEGG enrichment analysis indicated that the overlapped DEGs were mostly affected and mainly involved in cellular activities such as catalytic reaction, translation and biologic regulations, biosynthesis of secondary metabolites, metabolism of amino acid, lipid and carbohydrate, and environmental stress signaling pathways (Fig. 2; Table S2). Although a large number of DEGs were picked up from the transcriptome analysis, the key genes responsible for the iron accumulation were largely unknown in the wheat plant.

Expression profiles of iron responsive genes in the wheat mutant lines with high iron

We next examined the responsive expression levels of the wheat orthologs which were likely related with Fe homeostasis in the other plants. *TaYSL 1* (accession number: TraesCS2B01G407900) is the wheat homologue of *Arabidopsis AtYSL 1* that encodes a yellow stripe-like protein and participate in seed iron loading via transport of Fe-nicotianamine chelate (Le Jean et al. 2005; Waters et al. 2006; Chu et al. 2010). Analysis of the *TaYSL 1* gene expression revealed that the *TaYSL 1* transcript levels was obviously induced by iron in the wheat mutant lines, and the levels was higher than that of WT after treatments. In contrast, the iron-induced expression of the *TaYSL 1* transcripts was not detected in the wild type (Fig. 3). This result implied that the ability of iron transport in the wheat mutant lines with higher iron was better than the WT. Similarly, *TaZIP29*, a member of the ZRT/IRT-like protein (ZIP) encoding gene family that may be involved in the transport of non-transferrin bound iron (accession number: TraesCS6D02G153800) in plants (Bogdan et al. 2016), was highly induced after EDTA-Fe treatment for 24 h. Although endogenous levels of the *TaZIP29* transcript were lower in the wheat mutant lines than that of WT, the EDTA-Fe treatment could enhance the expressions of *TaZIP29* in most of the samples (Fig. 3), suggesting that the *TaZIP29* may participate in the iron accumulation in wheat plants. In addition, it was found that *TaFIBP*, encoding a ferric ion binding protein (accession number: TraesCS1A01G019900), exhibited a dramatic increase at 24 h after a low-level period (0 - 12 h) among the plants (Fig. 3). Compared to the wild type (WT), the wheat mutant lines (*LHFe-1*; *LHFe-2*) showed higher levels of the *TaFIBP* transcripts at different treatment time points, indicating that high iron contents in the mutant lines may be attributed to the iron-induced *TaFIBP* expression in wheat plants.

Interestingly, it was found that the wheat homologue *TaRTH* (accession number: TraesCS5A02G445300) of *Arabidopsis AtRTE1* mainly involved in the ETR1 ethylene receptor signaling (Zheng et al. 2017), displayed obvious iron induction when treated with EDTA-Fe for different time in wheat plants (Fig. 3). And, the transcript levels of *TaRTH* in the wheat mutant lines were mostly higher than that of the wild

type. These results suggested that ethylene may mediate TaRTH to participate in iron accumulation in wheat plants.

Altered ethylene sensitivity of the wheat mutant seedlings with high iron

We examined the ethylene sensitivity of the wheat mutant seedlings with high iron. As previously described (Ma et al. 2021), the wheat seeds were soaked and cultivated in the petri dish supplemented with the ethylene precursor ACC at different concentrations (0, 100, 200, 300, and 400 μ M) for 48 h, and the longest seedling root lengths were measured. As shown in Figure 4, the seedling root lengths were decreased as ACC concentration increased. Compared to the WT, the seedling root lengths of the wheat mutant lines were obviously longer after the treatment with higher ACC concentrations (200, 300 and 400 μ M), indicating that the ethylene sensitivity of the wheat mutant lines with high iron were significantly decreased at seedling stage (Fig. 4a,b).

Expression profiles of ethylene responsive genes in the wheat mutant lines with high iron

Ethylene sensitivity alteration of the wheat mutant seedlings prompted us to investigate the change of transcript levels among the ethylene signaling-associated genes in the wheat mutant lines. The wheat *ETHYLENE RESPONSE FACTOR* (*ERF*) genes (*TaERF1*, 2, 3, 4 and 6) were chosen for examination of their transcript levels when treated with ACC as previously described (Ma et al. 2021), and the results indicated that transcript levels of the *TaERFs* were rapidly increased and peaked at 6 h, after then they decreased at 12 h in the wild type (LY502) (Fig. 5). In order to know whether these *TaERFs* were affected in the wheat mutant lines with high iron when treated with ACC, the transcript levels of the *TaERFs* were examined in the seedlings of the mutant lines treated with ACC. As shown in Figure 5, the transcript levels of the *TaERFs* in *LHFe-1* were also rapidly elevated, but unlike LY502, their peak appeared at 3 h except for *TaERF4*. In *LHFe-2*, the response patterns of the *TaERFs* were similar to those in LY502 except for *TaERF4* (Fig. 5).

In addition to the *TaERF1*, 2, 3, 4 and 6, two other genes upstream of the ethylene signaling pathway, *TaRTH* and *TaEIN2*, were also analyzed. As shown in Figure 5, their response patterns in LY502, *LHFe-1* and *LHFe-2* were similar to those of *TaERFs*. Curiously, the induction levels of *TaRTH* in the wheat mutant lines were significantly higher than that in LY502 (Fig. 5). These data suggested that the accumulation of iron in the mutants is likely to be related to the changes of ethylene response in the mutants.

Discussion

Does iron ion participate in ethylene signaling regulation?

Ethylene participates in various aspects of plant growth, development, and stress responses, such as seed germination, flowering, sex determination, fruit maturity, senescence, and stress responses (Dubois et al., 2018). It is believed that mineral ions are involved in ethylene response and signaling. In early studies, it was reported that the binding of ethylene to receptors is mediated by a copper cofactor

(Schaller and Bleecker 1995; Rodriguez et al. 1999; Wang et al. 2006). The *Arabidopsis RESPONSE TO ANTAGONIST1* (*RAN1*) encodes a copper-transporting P-type ATPase and was responsible for the cuprous ion delivery to ethylene receptors (Hirayama et al. 1999; Woeste and Kieber 2000). The loss-of-function *ran1* mutants showed constitutive ethylene responses and hypersensitivity to copper-chelating agents (Hirayama et al. 1999; Woeste and Kieber 2000; Binder et al. 2010). Further studies indicated that *RAN1* interacted with ANTIOXIDANT PROTEIN1 (*ATX1*), loss-of-function of *ATX1* showed ethylene hypersensitivity, while overexpression of *ATX1* exhibited tolerance to copper (Puig et al. 2007; Shin et al. 2012). In addition to copper, silver also serves as the cofactor for ethylene binding to ETR1 (Beyer 1976; Rodriguez et al. 1999; McDaniel and Binder 2012). In plants, silver is widely used as an inhibitor of ethylene responses. It was reported that ethylene binding to the ETR1 receptor with silver was ~30% of binding with copper (McDaniel and Binder 2012). Silver may have an effect on ethylene signaling outside of the ethylene binding pocket of the receptors (McDaniel and Binder 2012; Bakshi et al. 2015).

In contrast, little is known whether iron could participate in ethylene response and signaling. In the present study, it was noted that expression of the wheat *TaRTH* (accession number: TraesCS5A02G445300), an ortholog of the *Arabidopsis AtRTE1* which encodes an activator of the *Arabidopsis* ETR1 receptor (Resnick et al. 2006, 2008; Zheng et al. 2017), was obviously induced by iron in wheat plants (Fig. 3). In addition, the expression pattern of *TaRTH* was highly correlated with that of the ferric ion binding protein encoding gene *TaFIBP* (accession number: TraesCS1A01G019900) and *TaZIP29* (accession number: TraesCS6D02G153800) which encodes a ZRT/IRT-like protein (ZIP) involved in the transport of non-transferrin bound iron in plants (Bogdan et al. 2016) (Fig. 3). Although it is unclear whether *TaRTH* could directly interact with a ferric ion binding protein or an iron transporting protein, it is speculated that iron may mediate *TaRTH* to take a part in ethylene signaling in wheat plants.

Ethylene is involved in iron accumulation in plants

In past years, increasing data indicated that the gaseous plant hormone ethylene plays important roles in iron homeostasis in plants. Ethylene is perceived by receptors, and mediates downstream components to transduction into the transcription activator EIN3/EIL1 in the nucleus. EIN3 promotes expressions of the downstream *ERFs* and other ethylene-responsive genes, thus activating multiple ethylene responses. In *Arabidopsis*, it was reported that EIN3 can physically interact with and stabilize FIT1, a protein playing a major role in the regulation of Fe acquisition (Lingam et al. 2011), indicating that iron homeostasis could be ethylene-regulated. Later studies provided more evidence demonstrating the involvement of *ERFs* in iron homeostasis. For example, the *Arabidopsis AtERF72* could bind to the promoters of the Fe-deficiency responsive genes such as *IRT1*, *HA2* and *CLH1*, and negatively regulate Fe-deficiency responses (Liu et al. 2017). In rice, it was reported that seven *ERF* genes were transcriptionally regulated under Fe overload stress conditions (dos Santos et al. 2013). More recently, it was observed that a loss-of-function T-DNA insertion allele of *ERF95* (*erf95-1*) confers increased sensitivity to Fe deficiency during early seedling growth of *Arabidopsis* (Sun et al. 2020). The *Arabidopsis* *ERF95* is a transcriptional activator of *FER1*, and functioned downstream EIN3 to regulate seed Fe accumulation. In addition, their experiments showed that the abundance of messenger RNAs (mRNAs) from multiple *ACS* genes encoding 1-

aminocyclopropane-1-carboxylate (ACC) synthase also increased during seed maturation. It was proposed that ethylene plays an important role in the regulation of Fe accumulation in seed.

In wheat, we showed that high transcript levels of the ERF encoding genes (*TaERF1*, 2, 3, 4, 6) were detected in the wheat mutant lines with high iron, and the wheat ERF encoding genes were highly induced by exogenous ACC (Fig. 5). In addition, it was observed that ethylene sensitivity of the wheat mutant seedlings with high iron was obviously altered when treated with ACC. All these results suggest that ethylene may participate in regulation of the iron accumulation in wheat. Unfortunately, it is still unclear how ethylene is involved in the Fe homeostasis in wheat plants. Further experiments need to perform to examine whether the wheat ethylene signaling regulator TaRTH could mediate interaction with iron binding protein to regulate iron accumulation.

The genes responsible for iron accumulation in wheat

Interestingly, the wheat mutant lines with high iron from this study provided a good example to explore changes of the endogenous genes which may be likely responsible for iron accumulation at a scale of transcriptome in wheat. Comparisons in WT-vs-*LHFe-1* and WT-vs-*LHFe-2* gave 509 DEGs overlapped (Fig. 2; Table S2). Among them, 34 DEGs showing elevated levels were detected in the mutant lines, whereas the rest displayed down-regulated expressions in *LHFe-1* and *LHFe-2*. The GO and KEGG enrichment analysis indicated that the overlapped DEGs were involved in various processes, including cellular metabolism, catalytic activity, translation, biologic regulation, response to stimulus, and amino acid, lipid and secondary metabolite biosynthesis pathways. All these information provided a foundation for further exploration of the molecular regulations in Fe homeostasis of wheat.

Meanwhile, expression analysis of the known genes responsible for Fe homeostasis in wheat strongly supports that the ability of iron accumulation in the wheat mutant lines with higher iron was better than the WT (Fig. 3). For example, wheat *TaFIBP* encoded a ferric ion binding protein (accession number: TraesCS1A01G019900), and exhibited a dramatic increase at 24 h when treated with 1.5 mM EDTA-Fe (Fig. 3). Compared to the wild type (WT), the wheat mutant lines (*LHFe-1*; *LHFe-2*) showed higher levels of the *TaFIBP* transcripts at different treatment time points. As shown in Figure 3, a similar response was observed for the wheat *TaZIP29* which encodes a member of the ZRT/IRT-like protein (ZIP) and may participate in the transport of non-transferrin bound iron (accession number: TraesCS6D02G153800) in plants (Bogdan et al. 2016). Moreover, this study also analyzed the iron response of *TaYSL1* (accession number: TraesCS2B01G407900), a wheat homologue of *AtYSL1* that encodes the *Arabidopsis* yellow stripe-like protein and participate in seed iron loading via transport of Fe-nicotianamine chelate (Le Jean et al. 2005; Waters et al. 2006; Chu et al. 2010), and its expression was obviously induced by iron and the transcript levels were higher than that of WT (Fig. 3), suggesting that the iron transport in the wheat mutant lines with higher iron was better than the WT.

Declarations

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Author Contributions

NL, YL, JS, and XS performed the experiments. YY did data analysis and wrote a version of the manuscript. CHD designed the experiments and did revision of the manuscript.

All authors have read and approved the manuscript. We declared that the authors of this paper have no conflict of interest.

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Figures

Figure 1

The iron contents of the wild type and salt tolerant wheat mutant lines.

The seeds of different wheat lines were directly used to measure the iron contents. Error bars represented SD (n = 3). Different lowercase letters above the columns indicated the significant differences by Tukey's test ($P < 0.05$).

Figure 2

Comparisons of LY502-vs-LHFe-1 and LY502-vs-LHFe-1.

(A) The venn diagrams of DEGs in the comparisons of LY502-vs-LHFe-1 and LY502-vs-LHFe-1. (B) GO enrichment analysis of the DEGs in the comparisons. (C) KEGG enrichment analysis of the DEGs in the comparisons.

Figure 3

Expression profiles of iron responsive genes in LY502, *LHFe-1* and *LHFe-2*.

Two-week old wheat seedlings were treated with 1.5 mM EDTA-Fe for different times as indicated and then used for RNA isolation and gene transcript levels detection. TaACTIN was used as an internal control. Error bars represented SD (n = 3). The significant differences between the wild type and mutant wheats in each group were valued by Student's t test (** $P < 0.01$, * $P < 0.05$).

Figure 4

The wheat mutant lines with high iron were insensitive to ethylene.

(A) The root length of LY502, LHFe-1 and LHFe-2 treated with ACC at different concentrations. Error bars represented SD ($n = 3$). The significant differences between the wild type and mutant wheats in each

group were valued by Student's t test (** $P < 0.01$, * $P < 0.05$). (B) The root phenotypes of LY502, *LHFe-1* and *LHFe-2* treated with 300 μM ACC under dark conditions.

Figure 5

Expression profiles of Ethylene responsive genes in LY502, *LHFe-1* and *LHFe-2*.

Two-week old wheat seedlings were treated with 300 μM ACC for different times as indicated and then used for RNA isolation and gene transcript levels detection. *TaACTIN* was used as an internal control. Error bars represented SD ($n = 3$). Different lowercase letters above the columns indicated the significant differences in each line by Tukey's test ($P < 0.05$).

Supplementary Files

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