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Sequence analysis and expression of the IRT1 gene under iron deficiency in amaranth (Amaranthus mangostanus L.)

Yikun Wang (910365451@qq.com)
CAAS IARRP: Chinese Academy of Agricultural Sciences Institute of Agricultural Resources and Regional Planning
Kai Wang
Meng You
Hongli Fan
https://orcid.org/0000-0001-8742-9210

Research Article

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Abstract

Aims

Amaranth (*Amaranthus mangostanus* L.) is a hyperaccumulator that is effective in remediating soil cadmium (Cd) pollution. The *iron-regulated transporter 1* (*IRT1*) gene, which encodes the iron (Fe) transporter protein, plays a crucial role in Cd uptake in plants, and its expression is induced by Fe deficiency. But its role in amaranth remains unknown.

Methods

In this study, the *IRT1* gene from amaranth was cloned for sequence analysis and functional prediction using bioinformatics methods. A hydroponic experiment was performed to study amaranth Cd uptake and its expression under Fe deficiency with treatments of + Fe, −Fe, + Fe + Cd, and − Fe + Cd.

Results

A partial *AmIRT1* cDNA sequence encoding 185 amino acids was obtained. Protein structure prediction revealed with almost the entire Pfam ZIP functional domain within the sequence. Functional prediction indicated that the protein was a transmembrane (TM) ion transporter with three TM domains. Using homology comparison and phylogenetic tree analysis, we found that the *AmIRT1*-encoded protein showed the highest homology with Arabidopsis IRT proteins and clustered with IRT proteins from other plants. The shoot and root Cd concentrations increased by 9.6% and 10.9%, respectively, in − Fe treatment compared with + Fe treatment. Cd accumulation was also observed to increase. Notably, *AmIRT1* gene expression increased under both − Fe and − Fe + Cd treatments, but did not change under + Fe and + Fe + Cd treatments.

Conclusions

The structure and properties of the *AmIRT1*-encoded protein were similar to those of ZIP family members in other plants. Its expression can be increased by regulating Fe nutrition to improve amaranth Cd uptake.

Introduction

Cadmium (Cd), a major soil pollutant, causes health risks to humans and animals because of its high toxicity (Zou et al. 2021; Yu et al. 2016). Cd contamination has occurred in many agricultural soils due to human activities such as mining and fertilization. This problem has drawn more attention recently, as Cd pollution now accounts for the highest proportion of all heavy metal pollution in China (Wen et al. 2020; Chen et al. 2021). Cd-contaminated soils have been remediated using different effective methods (Haider et al. 2021), which mainly include physical, chemical, and biological remediation methods (Rezaei et al.

2021; Shi et al. 2020; Liang et al. 2022). Among these, the biological remediation method of phytoremediation is considered a clean, cost-effective, and environmentally friendly technique to remove Cd from contaminated soils (Zou et al. 2020; You et al. 2022). *Thlaspi caerulescens* (Zheng, et al. 2022), *Arabidopsis* (Meng et al. 2022), *Viola baoshanensis* (Zhuang et al. 2005), and *Solanum nigrum* (Dai et al. 2022) are recognized as Cd hyperaccumulators. However, the application of these plants is limited because of their low biomass, and planting them in many areas is difficult due to their regionality. In contrast, *Amaranthus mangostanus* is a Cd hyperaccumulator with the advantages of rapid growth, high biomass, and easy cultivation (Chi et al. 2019). Although amaranth is considered a highly effective phytoremediation plant, its metal accumulation mechanism is still poorly understood.

Cd is a non-essential element and does not have a highly selective protein channel (Zhang, et al. 2022). It enters plants through the absorption channels of essential elements such as calcium (Ca), iron (Fe), manganese (Mn), and zinc (Zn) (Clemens et al. 2013; Zhang et al. 2020). Because Cd uptake is a complex process, the center of focus is Cd absorption channels in plant roots (Liu et al. 2022). Several channel proteins are involved in Cd absorption, such as Zn/Fe-regulated transporter-like proteins (ZIPs) (Han et al. 2022), natural resistance-associated macrophage proteins (NRAMPs) (Chen et al. 2021), heavy metal-transporting ATPases (Zheng, et al. 2022), ATP-binding cassettes (Brunetti et al. 2015), and cation exchangers (Hu et al. 2019). Among these, ZIPs have received more attention. Many members of the ZIP family are mainly located in the plasma membrane of roots. They are not only involved in transmembrane (TM) Fe, Mn, and Zn transport (Lin et al. 2009) but also play an important role in Cd transport. ZIP family members, such as ZIP1, ZIP2, ZNT1, and iron-regulated transporter 1 (*IRT1*), have been isolated and identified from plants (Kupper and Kochian 2010; Wu et al. 2020; Zhu et al. 2012).

The *IRT1* gene has been cloned in Arabidopsis, rice, tobacco, and other plants (Bahmani et al. 2019; Dong et al. 2023; David et al. 1996). Its sequence and functions have also been confirmed in various plants, where it is mainly expressed in the root exoderm. *IRT1* gene expression in roots is induced by Fe deficiency. In Arabidopsis, Fe deficiency promotes *IRT1* gene expression to meet the plant demand for Fe, while also increasing Cd concentration in the plant (Vert et al. 2002). However, studies on *AmIRT1* are few, and its sequence information, specific functions, and expression under Fe deficiency remain unclear.

Previous studies found that the amaranth cultivar ‘Tianxingmi’ could enrich more Cd than 23 other amaranth cultivars (Zou et al. 2020; Fan and Zhou 2009). Therefore, we selected ‘Tianxingmi’ to conduct a preliminary study of the cloning and expression of the *IRT1* gene in amaranth. The rapid amplification of cDNA ends (RACE) method was used to clone a partial cDNA sequence of the amaranth *IRT1* gene. Bioinformatics methods were used to analyze the cDNA sequence and function. We conducted a hydroponic experiment to study Cd uptake in amaranth under Fe deficiency and analyzed *IRT1* gene expression by Northern blotting to elucidate the molecular mechanism of Cd accumulation in amaranth. Our results can contribute to the understanding of the *IRT1* gene sequence and function in amaranth and provide a theoretical basis for regulating *IRT1* gene expression to improve the efficiency of amaranth in the remediation of Cd-contaminated soil.

Materials methods

Plant materials and growth conditions

Amaranthus mangostanus L. cv. Tianxingmi was selected for this experiment after screening 23 amaranth cultivars, because it had the highest Cd uptake. Amaranth seeds were provided by the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China. These seeds were sown in vermiculite–peat after disinfection with 5% H_2O_2 (v/v). After having grown a leaf (about 3 weeks), seedlings that grew well were selected and transferred to polyethylene seedling trays with modified Johnson nutrient solution, and covered with polyethylene lids to protect the nutrient solution from light. The composition of the nutrient solution was 1.2 mM KNO_3, 0.8 mM $Ca(NO_3)_2$, 0.1 mM $NH_4H_2PO_4$, 0.2 mM $MgSO_4$, 50.0 mM KCl, 12.5 mM H_3BO_3, 1.0 mM $MnSO_4$ and $ZnSO_4$, 0.4 mM $CuSO_4$, 0.1 mM Na_2MoO_4, and 0.1 mM $NiSO_4$. For Fe treatment (+ Fe), 20 mM Fe-EDDHA (N, N0-ethylenebis-[(2-hydroxyphenyl)-gly) was added, and for Fe deficiency treatment (− Fe), no Fe-EDDHA was added. MES ($C_6H_{13}NO_4S$) buffer was added to both + Fe and − Fe treatments to a final concentration of 1.0 mM. KOH was used to adjust the pH of the nutrient solution to 5.5. During the plant growth period, the nutrient solution in each tray was refreshed every five days. Plants were grown in a controlled environment chamber under 14 h light at 30°C/10 h dark at 20°C, a relative humidity of 60–70%, and a luminous flux density of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Experiment design and sampling

After 15 days of growth, four treatments were established: +Fe (Fe alone treatment), −Fe (Fe deficiency treatment), + Fe + Cd (Fe with Cd treatment), and − Fe + Cd (Cd without Fe treatment). The Cd concentration was 2.5 mg/L. Samples were taken 6 days after growing in the four treatments. The samples were rapidly frozen in liquid nitrogen and stored in an ultra-low temperature refrigerator at − 80°C.

RNA extraction

RNA of amaranth roots was extracted according to Lombi et al. (2000). Amaranth roots (0.15 g) were ground in liquid nitrogen and placed in a centrifuge tube treated with diethyl pyrocarbonate (DEPC). Subsequently, 1 mL of TRIzol reagent was added, and the tube was shaken for 30–60 s; 0.2 mL of chloroform was added, mixed (the tube was turned upside down and straight again), and the tube was placed in an ice bath for 5 min, centrifuged for 10 min (12000 rpm, 4°C), and the sediment was removed. Thereafter, 0.6 volume of isoamyl alcohol was added, and the tube was kept to stand at room temperature for 10 min, centrifuged for 10 min (12000 rpm and 4°C), and the sediment was removed, washed with 70% ethanol, dried, and stored in 50 μL DEPC water at − 80°C.

Cloning of the *AmIRT1* gene

Primer design

According to the published nucleotide sequence of the *IRT* gene in Arabidopsis, tomato, rice, and wheat in GenBank, two nested primers *IRT-3-1* and *IRT-3-2* were designed using DNAMAN software, and these primers were synthesized at Shanghai Sangon Company, Shanghai, China.

IRT-3-1

5'-CAT CAA ATG TT(C/T) GAA GG(A/C/G) ATG G-3'

IRT-3-2

5'-CTT GG(C/T) GG(C/T) AT(C/T) CT(A/C/T) CAG G-3'

Based on the 3' end sequence of the isolated *IRT* gene, three gene-specific primers were designed, and three rounds of polymerase chain reaction (PCR) amplification were performed to isolate the 5' end of the amaranth *IRT1* gene (Cd hyperaccumulating variety).

IRT-5-1

5'-AAG CAA GCA CAT AAC AGC-3'

IRT-5-2

5'-AGG TCC CTTGAAATC TGA AGC-3'

IRT-5-3

5'-CCA ATC ACT ATC AAT GCT GTTG-3'

Cloning of the 3' end sequence of *AmIRT1*

To synthesize first-strand cDNA, total RNA extracted from amaranth roots was used as the template for reverse transcription and adaptor-dT (5'-GATTTCTGTCCGACGACTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3') was used as a primer. The reagents used in the reverse transcription reaction were purchased from TaKaRa. Using the newly synthesized cDNA from amaranth roots as a template, two primers *IRT-3-1* and *IRT-3-2* were paired with adaptor-dT primers, and two rounds of PCR amplification were performed to clone the 3' end sequence of the *IRT1* gene.

Cloning of the 5' end sequence of *AmIRT1*

Total RNA extracted from amaranth roots was used as the template for reverse transcription to synthesize first-strand cDNA, and *IRT-5-1* was used as a primer. Using the newly synthesized cDNA from amaranth roots as a template, two primers *IRT-5-2* and *IRT-5-3* were paired with adaptor-dT primers, and two rounds of PCR amplification were performed to clone the 5' end sequence of the *IRT1* gene.

Partial sequence of *AmIRT1* cDNA

The 3' and partial 5' end sequences of the cloned *AmIRT1* gene were spliced using DNAMAN software to obtain a partial *AmIRT1* cDNA sequence.

Northern blotting

Electrophoresis, mode conversion, hybridization, and development in Northern hybridization were based on the methods of Sambrook et al. (1989).

Plant Cd analysis

Plant samples were digested with a HNO_3–$HClO_4$ mixture to analyze the total concentrations of Cd. The elements were analyzed using ICP-MS (Optima 8300, Perkin Elmer, USA). Standard reference rice GBW10045a (GSB-23a) was used to confirm the analytical quality and verify the accuracy of the data during the analysis.

Bioinformatics analysis

NCBI BLASTp software (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) was used to analyze the amino acid sequence of the protein encoded by the partial sequence of the *AmIRT1* gene (hereafter *AmIRT1*-encoded protein). SMART software (http://smart.embl-heidelberg.de/) was used to perform the functional prediction of the *AmIRT1*-encoded protein. The isoelectric point (pI), molecular weight, and amino acid composition were analyzed using ExPASy. (http://www.expasy.org) The protein secondary structure was predicted using SOPMA (the self-optimized prediction method with alignment). Phosphorylation sites of the *AmIRT1*-encoded protein were predicted using NetPhos 3.1 (https://services.healthtech.dtu.dk/services/NetPhos-3.1/). The TM domains of the *AmIRT1*-encoded protein were analyzed using Phobius (https://phobius.sbc.su.se/). Plant-mPLoc (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/) was used for subcellular localization prediction of the *AmIRT1*-encoded protein. GenBank was used to search for proteins that had the highest homology with the *AmIRT1*-encoded protein. Homology comparison was performed using DNAMAN software. The phylogenetic tree was constructed using MEGA 4.1 software.

Results

3' and partial 5' end sequences of *AmIRT1*

We searched for the known *IRT* genes in plants using NCBI BLAST software, and compared the homologous sequences of these genes. Two conserved regions were found. Based on this result, we designed two nested annexing primers *IRT-3-1* and *IRT-3-2* [5'-CAT CAA ATG TT(C/T) GAA GG(A/C/G) ATG G-3' and 5'-CTT GG(C/T) GG(C/T) TG(C/T) AT(C/T) CT(A/C/T) CAG G-3'].

For reverse transcription and synthesis of first-strand cDNA, total RNA was extracted from amaranth roots and used as a template, and adaptor-dT (5'-GATTTCTGTCCGACGACTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3') was used as a primer. Using this cDNA as a template, degenerate *IRT-3-1* and *IRT-3-2* were paired with adaptor-dT primers for nested PCR amplification. Finally, a specific fragment of approximately 400 bp was amplified and recovered for sequencing. The sequencing results showed that the fragment length was 432 bp (Fig. 1a).

According to the 3' end sequence of the obtained amaranth *IRT1* gene, three nested specific primers were designed: *IRT-5-1*, *IRT-5-2*, and *IRT-5-3* (5'-AAG CAA GCA CAT AAC AGC-3', 5'-AGG TCC CTTGAAATC TGA AGC-3', and 5'-CCA ATC ACT ATC AAT GCT GTTG-3'). Reverse transcription and PCR amplification were performed. Specific bands appeared at approximately 400 bp. The sequencing results showed that the fragment length was 386 bp (Fig. 1b).

AmIRT1 cDNA sequence

The 3' and partial 5' end sequences of the cloned *AmIRT1* gene were spliced using DNAMAN software to obtain a partial *AmIRT1* cDNA sequence with a length of 662 bp, encoding 185 amino acids (Fig. 2a). The pI and molecular weight were 5.49 and 19848.38, respectively. Among the amino acid residues, the percentage of leucine was the highest at 13.0%, followed by valine at 9.2%. The two least abundant amino acid residues were histidine and tryptophan at 1.6% and 0.5%, respectively (Fig. 2b). Phosphorylation site prediction analysis results showed that the *AmIRT1*-encoded protein had 12 possible phosphorylation sites, including seven serine sites, three threonine sites, and two tyrosine sites (Fig. 2c). Protein secondary structure prediction analysis results showed that the α helix, β turn, and random coil of the *AmIRT1*-encoded protein corresponded to 51.63%, 2.17%, and 29.89% (Fig. 2d).

Prediction of the structure and function of the *AmIRT1*-encoded protein

The ZIP family includes Zn transporters and many metal transporters usually with a conserved Pfam ZIP functional domain (Natasha et al. 1998). The amino acid sequence of the *AmIRT1*-encoded protein contained almost the entire Pfam ZIP functional domain (Fig. 3a). Functional prediction of the *AmIRT1*-encoded protein showed that it had a typical Pfam ZIP domain, starting from the seventh amino acid and ending at the 181st amino acid, which consisted of 179 amino acids (Fig. 3b). Thus, the function of the *AmIRT1*-encoded protein might be similar to ZIP family proteins. Subcellular localization prediction analysis of plant proteins indicated that it was a plasma membrane-localized protein (Fig. 3c). The protein showed three TM domains (positions: 93–112; 122–144; 164–181) (Fig. 3d), indicating that it was a TM ion transporter.

Homology comparison of the *AmIRT1*-encoded protein and other IRT-like proteins

By using GenBank, we searched for proteins with the highest homology with the *AmIRT1*-encoded protein and found many ZIP family proteins. We then selected nine proteins with the highest score, and used DNAMAN software to perform a homology comparison of these proteins. The *AmIRT1*-encoded protein

was closely related to At_cation_zinc_transporter, AtIRT1, AtIRT2, At_putative_IRT, CsIRT, MsIRT, Os_iron_transporter_Fe3, OsIRT1, and PsIRT. The highest homology was with At_cation_zinc_transporter (63.04%), and the lowest homology was with OsIRT1 (53.70%). The high homology indicated a close genetic relationship, which could further explain the functional similarity.

Phylogenetic tree analysis of the *AmIRT1*-encoded protein and other IRT-like proteins

A dendritic branch graph can be used to represent the genetic relationship between various species or genes. We performed a phylogenetic tree analysis of the *AmIRT1*-encoded protein and nine proteins. The phylogenetic tree analysis results showed that the *AmIRT1*-encoded protein clustered with the Arabidopsis zinc transporter, Arabidopsis iron transporter IRT1, cucumber IRT, *Begonia microphylla* IRT1, pea IRT, Arabidopsis IRT1, Arabidopsis IRT2, rice trivalent iron transporter, and rice IRT1. Thus, these members might have similar functions.

Cd uptake and *AmIRT1* gene expression

Amaranth plants were treated with − Fe, +Fe, −Fe + Cd, and + Fe + Cd. Fe deficiency increased Cd uptake in amaranth (Table 1). Compared with + Fe treatment, the shoot and root Cd concentrations were increased by 9.6% and 10.9%, respectively, by − Fe treatment. The root Cd changes were significant. In addition, the shoot and root Cd accumulation increased by 1.9% and 11.7%, respectively.

RNA was extracted from amaranth roots, and *AmIRT1* gene expression was analyzed by Northern blotting (Fig. 6). Under − Fe and − Fe + Cd treatments, the expression of two IRT-like genes in amaranth increased, whereas under + Fe and + Fe + Cd treatments, their expression did not change. Thus, *AmIRT1* gene expression was induced by exogenous Fe deficiency stress.

Table 1
Cd concentration and accumulation under ± Fe treatments

Treatment	Cd concentration (mg/kg)		Cd accumulation (μg/plant)	
	shoot	root	shoot	root
−Fe	198 ± 12a	851 ± 22a	506 ± 10a	291 ± 9a
+Fe	179 ± 7a	758 ± 16b	496 ± 8a	257 ± 8a

Discussion

Many techniques have been recently proposed for improving phytoremediation efficiency. In addition, many gene cloning methods have emerged with the continuous development of biotechnology (Goldberg 2001). These methods help to gain a deeper understanding of how genes in hyperaccumulators regulate their expression and improve the efficiency of pollutant remediation. Furthermore, several studies have

combined methods of biotechnology with phytoremediation (Xu et al. 2018). This combination helped to understand the uptake process of heavy metals by hyperaccumulators and the improvement of phytoremediation efficiency by the molecular method (Ozyigit et al. 2020). For example, researchers transferred heterologous target genes from bacteria, plants, or animals into plants and fully expressed them, effectively improving the tolerance and accumulation ability of these plants to heavy metals (Daghan et al. 2013; Eapen and D'Souza 2005). A study showed that transferring the yeast metallothionein gene into cauliflower increased Cd tolerance and conferred the plant with Cd accumulation ability (Hasegawa et al. 1997). Another example of the combination of biotechnology and phytoremediation methods is the regulation of root transporters in hyperaccumulators. Zhu et al. (2020) demonstrated that BTS knockdown-upregulated IRT1 also facilitated Cd uptake by roots, thereby increasing the Cd concentration in plant tissues. Thus, BTS knockdown increased Cd accumulation in plants by more than one fold (Zhu et al. 2020). He et al. (2017) found that plants overexpressing *IRT1* accumulated higher levels of Cd than wild-type plants. Thus, IRT1 might be responsible for the uptake of these metals, as IRT1 protein expression was increased in these plants. The *IRT1* gene was first described in Arabidopsis, which mediates the uptake of metals such as Fe, Zn, and Cd (Korshunova et al. 1999; Zhu et al. 2012). Therefore, from these examples, we understand how gene expression regulates plant uptake of heavy metals.

In the present study, we also combined biotechnology and phytoremediation methods, isolated a partial cDNA sequence of the *IRT1* gene from amaranth, a recognized metal hyperaccumulator, and studied *IRT1* gene expression. We thus have a preliminary understanding of the amino acid sequence of the *AmIRT1*-encoded protein. In addition, by using the RACE method, we cloned the 3' end (432 bp) and partial 5' end (386 bp) sequences of amaranth *AmIRT1* cDNA. After splicing the 3' and partial 5' end sequences, the resulting sequence encoded a protein with 185 amino acids. Furthermore, we determined pI and molecular weight, and predicted the phosphorylation sites and protein secondary structure (Fig. 2). Phosphorylation can synthesize ATP and improve energy. Phosphorylation site prediction analysis results showed that the *AmIRT1*-encoded protein had 12 sites that were phosphorylated, including seven serine sites, three threonine sites, and two tyrosine sites. Secondary structure prediction of the *AmIRT1*-encoded protein showed that the α helix, β turn, and random coil accounted for 51.63%, 2.17%, and 29.89%, respectively (Fig. 2d).

We performed a BLAST analysis to understand the function of the protein. The ZIP family plays an important role in metal uptake and accumulation in plants (Guerinot 2000). Because the *IRT1* gene is a member of the ZIP family, we performed a BLAST search with it in other plants. By genome-wide identification and analysis, 12, 20, and 30 ZIP family members have been identified in maize, lettuce, and peanut, respectively. Most of them contain eight TM regions and conserved metal ion-binding domains that play an important role in metal transport (Tao and Lu 2022). pI is between 5.00 and 9.28, and the length of ZIPs is 309–476 amino acids (Fu et al. 2021). The IRT1 protein belongs to the ZIP family and is a broad-range metal ion transporter in plants, transporting ions such as Zn, Mn, Fe, and Cd (Korshunova et al. 1999). It has been cloned in yeast, Arabidopsis, and many other plants, but less in amaranth. In this study, we cloned the partial cDNA sequence of the *AmIRT1* gene, and the online BLAST analysis results

showed that the amino acid sequence of its protein included almost the complete Pfam ZIP functional domain. Further functional prediction of the protein showed that it had a typical Pfam ZIP domain. We used TM region prediction and subcellular localization prediction to make preliminary predictions of the properties and functions of the *AmIRT1*-encoded protein. Three TM domains were found in this protein, indicating that it was a TM ion transporter. Subcellular localization prediction analysis of plant proteins showed that the *AmIRT1*-encoded protein was a plasma membrane-localized protein. The high similarity of different genes indicated that their functions were similar. Pence et al. (2000) compared ZNT1 with ZIP family proteins in Arabidopsis and found that it showed significant sequence homology with ZIP4 and IRT1. Their findings indicated that ZNT1 was a Zn transporter (Pence et al. 2000). Zhong et al. (2019) found that the amino acid sequence of ZIP2 in watercress (*Nasturtium officinale*) was similar to that of ZIPs in other plants. Among these plants, *Capsella rubella* had the highest similarity, 92% (Zhong et al. 2019). In our study, *AmIRT1* was closely related to *At_cation_zinc_transporter*, *AtIRT1*, *AtIRT2*, *At_putative_IRT*, *CsIRT*, *MsIRT*, *Os_iron_transporter_Fe3*, *OsIRT1*, and *PsIRT*, and the highly similar sequences of the proteins of these members were primarily located in the TM region. We found that the *AmIRT1*-encoded protein had low similarity with ZIP family proteins in other plants. The highest homology was with Arabidopsis (63.04%), and the lowest homology was with rice (53.70%). Multiple sequence alignment or phylogenetic tree analysis can reveal the evolutionary relationship between gene sequences and determine their homology. The phylogenetic tree analysis results showed that the *AmIRT1* gene clustered with *IRT* genes from other plants (Arabidopsis, dicotyledons, and monocotyledons). Thus, the *AmIRT1*-encoded protein might have functions similar to IRTs from Arabidopsis, dicotyledons, and monocotyledons. Therefore, it can be concluded from the sequence and phylogenetic tree analyses that the *AmIRT1*-encoded protein is a member of the ZIP family despite its low homology with other plants. Furthermore, the *AmIRT1*-encoded protein transports divalent metal ions and is located in the plasma membrane.

Different members of the ZIP family are differentially expressed in various tissues under various external stresses. The expression of many ZIPs is induced by Zn or Fe deficiency (Verbruggen et al. 2009; Lilay et al. 2020). Fe is a well-known nutrient that interacts with Cd, and it can affect Cd uptake by three mechanisms. First, Fe oxides can reduce the bioavailability of Cd in soil by converting Cd from available Cd to Fe/Mn oxide-bound Cd, which also plays an important role in Cd immobilization (Li et al. 2021; Muehe et al. 2013). Second, Fe plaque is deposited on the root surface, mainly in the form of ferrihydrite and goethite (Zhang et al. 2022). It can also effectively alleviate Cd toxicity in rice (Liu et al. 2008). The amount of Cd in Fe plaque can significantly influence Cd uptake and accumulation in rice. Third, Cd can enter plants by the Fe-uptake pathway because Fe is chemically similar to Cd (Zhao and Wang 2019). Fe uptake by dicotyledons primarily depends on the reduction of ferric chelates by ferric chelate reductase and the subsequent acquisition of the resulting Fe^{2+} into root cells by IRT1 (Sytar et al. 2021). Therefore, Fe competes with Cd for transporters and reduces potential Cd uptake and translocation in plants. This antagonistic interaction between Fe^{2+} and Cd^{2+} may contribute to increased Cd toxicity in soil. Several studies have shown that an increased exogenous supply of Fe results in decreased Cd uptake by plants such as Arabidopsis (He et al. 2017). Our previous study reached a similar conclusion that Cd uptake

increased under Fe deficiency. Compared with + Fe treatment, the shoot and root Cd concentrations were increased by 9.6% and 10.9%, respectively, by − Fe treatment, while Cd accumulation also increased in amaranth (Table 1). This previous study confirmed that plant Cd uptake increased because of increased transporter expression. The transporter is induced by Fe deficiency and can improve plant tolerance to Fe deficiency, while enhancing Cd uptake and transport and increasing the Cd concentration in plants. For example, adding Fe to the culture medium reduced the Cd concentration of rice roots and shoots by 26–65% and 9–683%, respectively, and the expression levels of *OsIRT1*, *OsNRAMP5*, and *OsNRAMP1* significantly increased (Hussain et al. 2020). In our study, *IRT1* gene expression significantly improved in the absence of Fe treatment compared with Fe treatment, which is similar to *AtIRT1* and *OsIRT1* gene expression. This result confirmed that *AmIRT1* gene expression was induced by Fe deficiency.

Conclusion

We cloned the *IRT1* gene in amaranth by the RACE method and performed sequence and functional analyses using bioinformatics methods. The structure and properties of the *AmIRT1*-encoded protein were similar to those of ZIP family members in other plants. Its expression was enhanced under Fe deficiency, resulting in more Cd accumulation. Thus, the *AmIRT1*-encoded protein is also a root ion transporter and its expression can be increased by regulating Fe nutrition to improve amaranth Cd uptake.

Declarations

CRediT authorship contribution statement

Yikun Wang: Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. Kai Wang: Data curation, Formal analysis. Meng You: Data curation, Formal analysis. Hongli Fan: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

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Figures

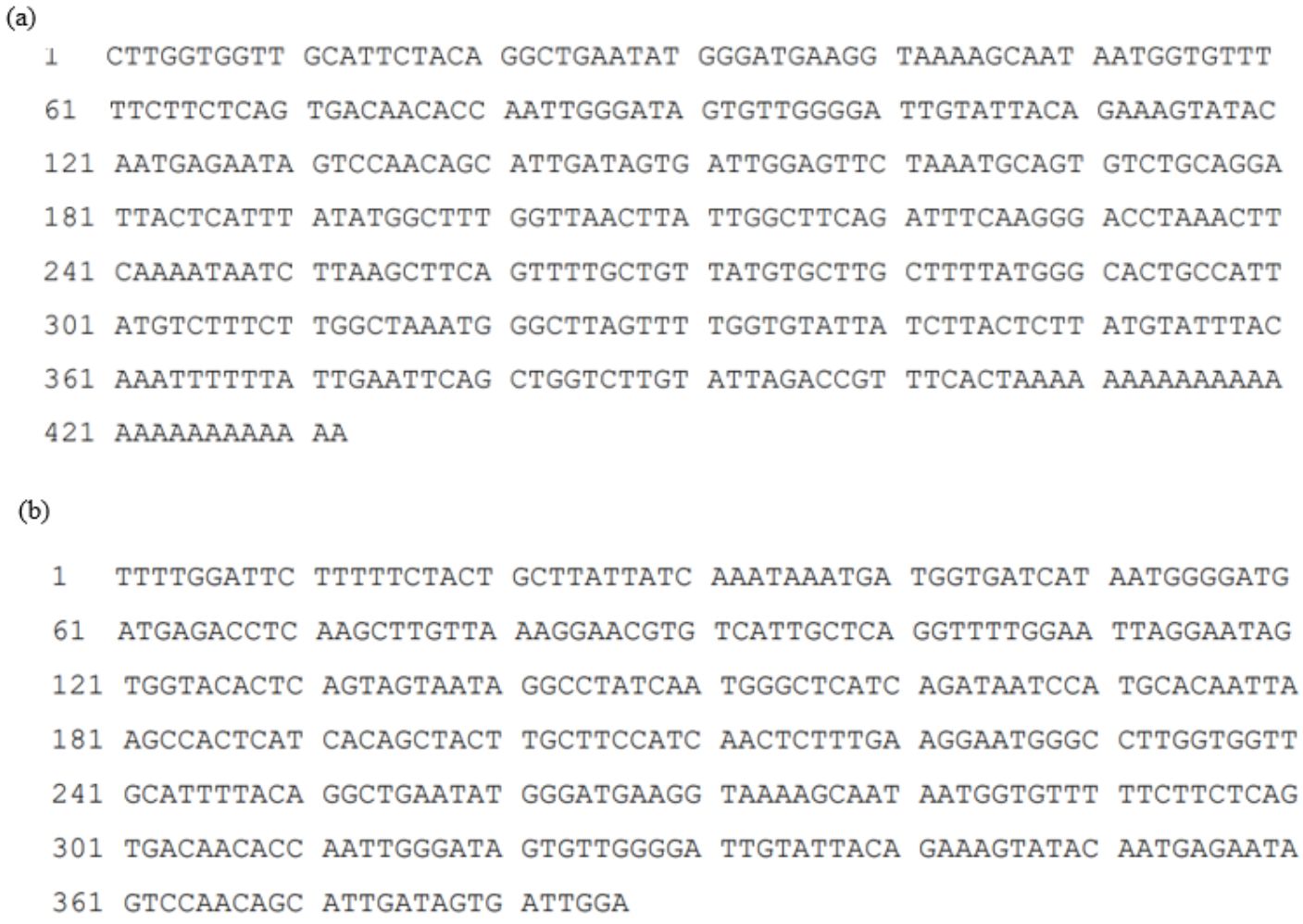


Figure 1

3' end sequence (a) and partial 5' end sequence (b) of *AmIRT1*cDNA.

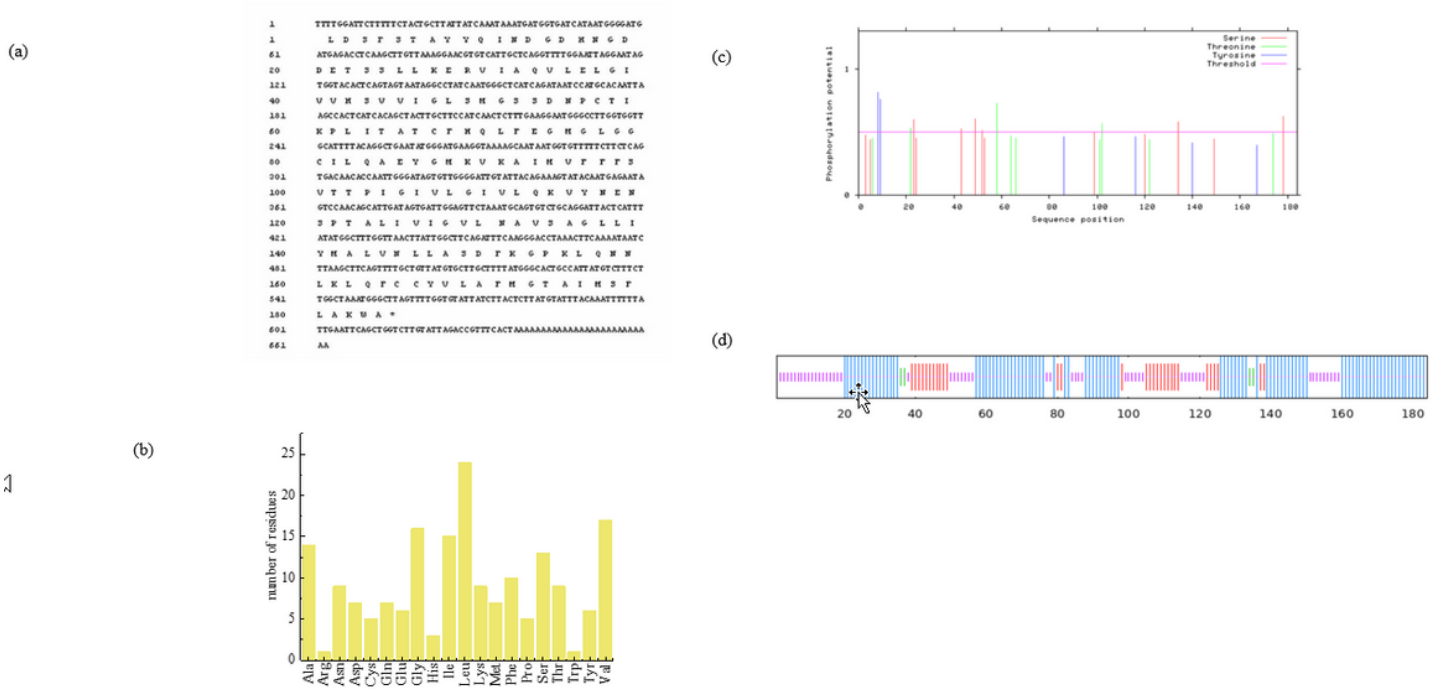


Figure 2

(a) *AmIRT1* cDNA and deduced amino acid sequences, (b) prediction of phosphorylation sites of the *AmIRT1*-encoded protein, (c) amino acid composition analysis of the *AmIRT1*-encodedprotein, and (d) protein secondary structure prediction results.

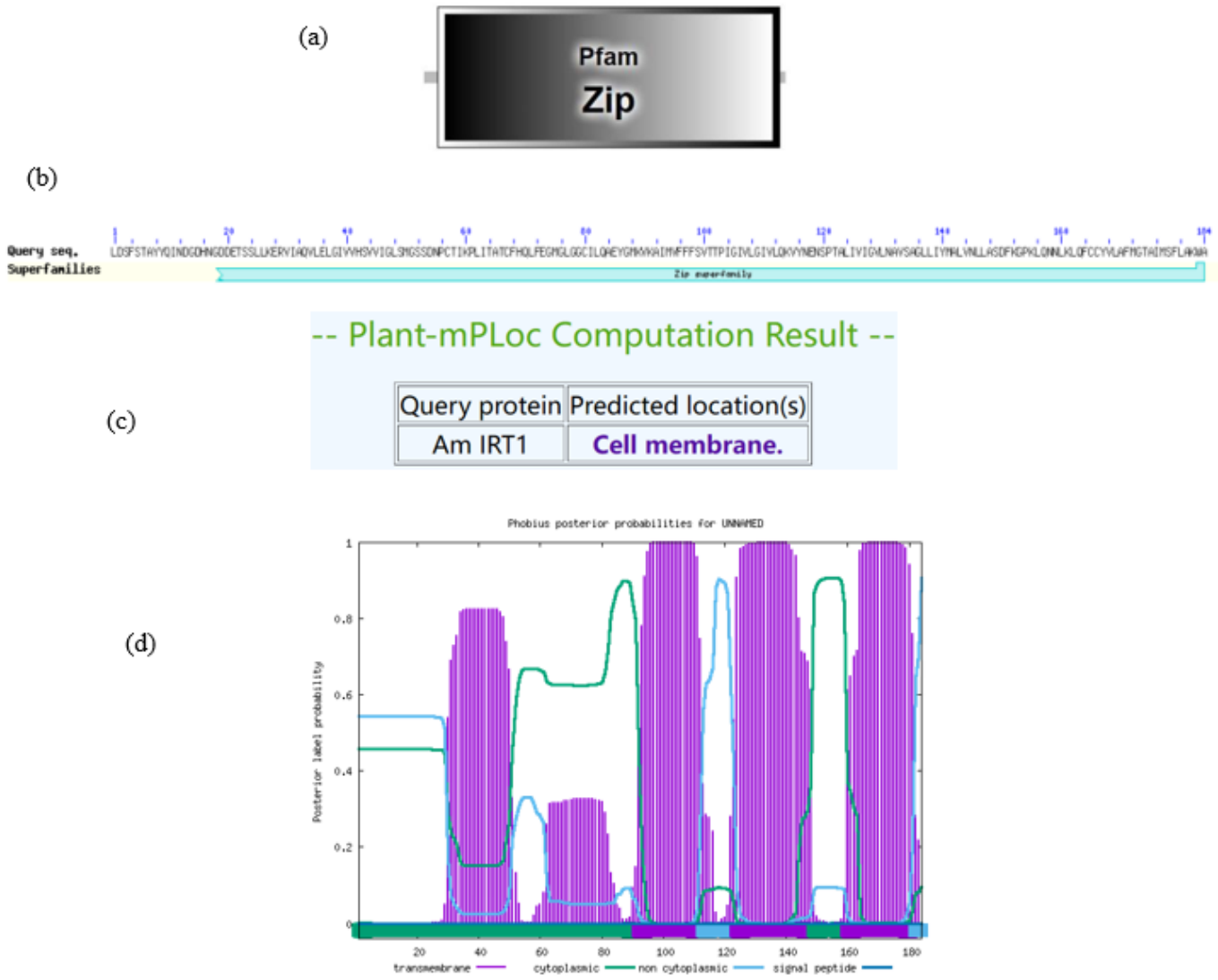


Figure 3

Functional domain of the *AmIRT*-encoded protein predicted by SMART (a) and BLASTp software (b), (c) subcellular localization prediction of the *AmIRT1*-encoded protein by Plant-mPLoc, and (d) transmembrane region prediction of the *AmIRT*-encoded protein.

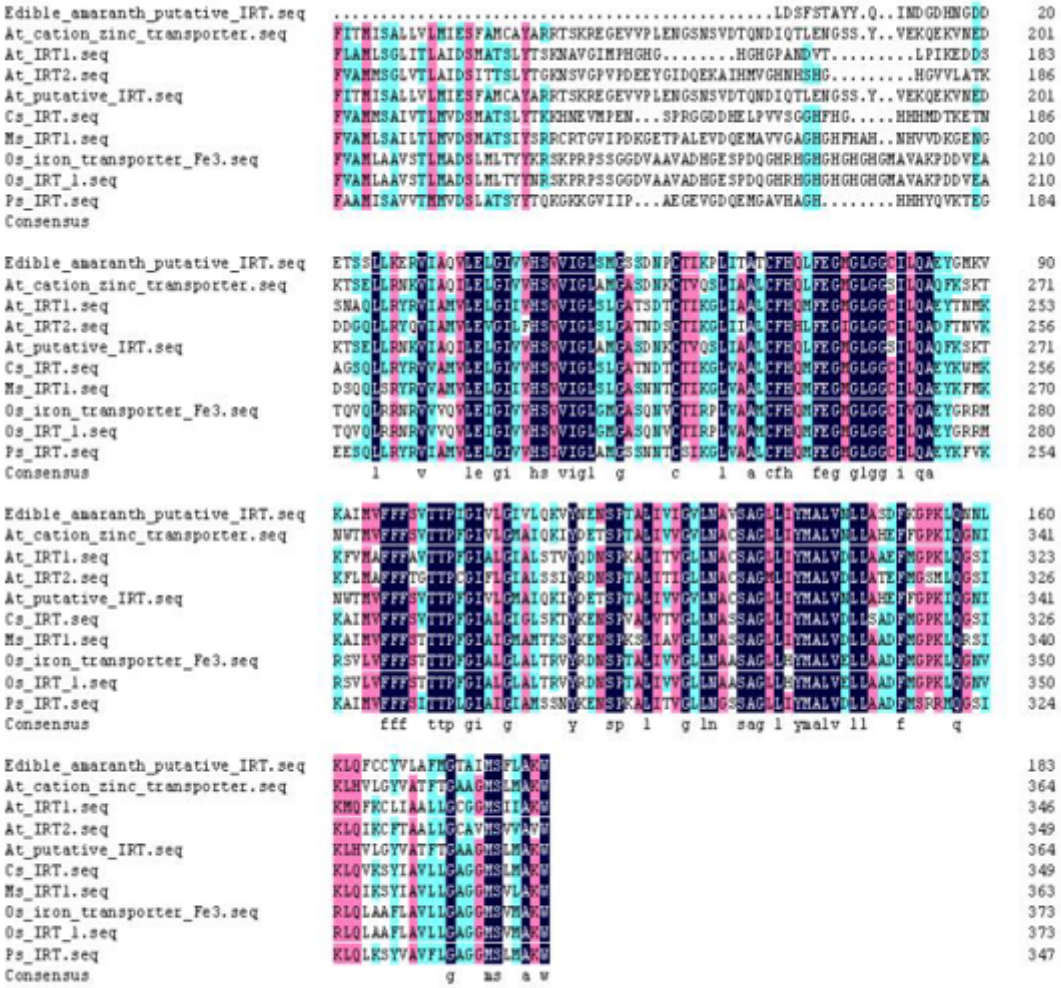


Figure 4

Comparison of the amino acid sequence of the *AmIRT1*-encoded protein with that of other IRT proteins.

Note: IRT amino acid sequences from top to bottom: amaranth *IRT*-encoded protein, Arabidopsis zinc transporter, Arabidopsis IRT1, Arabidopsis IRT2, Arabidopsis putative IRT, cucumber IRT, *Begonia microphylla* IRT1, rice trivalent iron transporter, rice IRT1, and pea IRT.

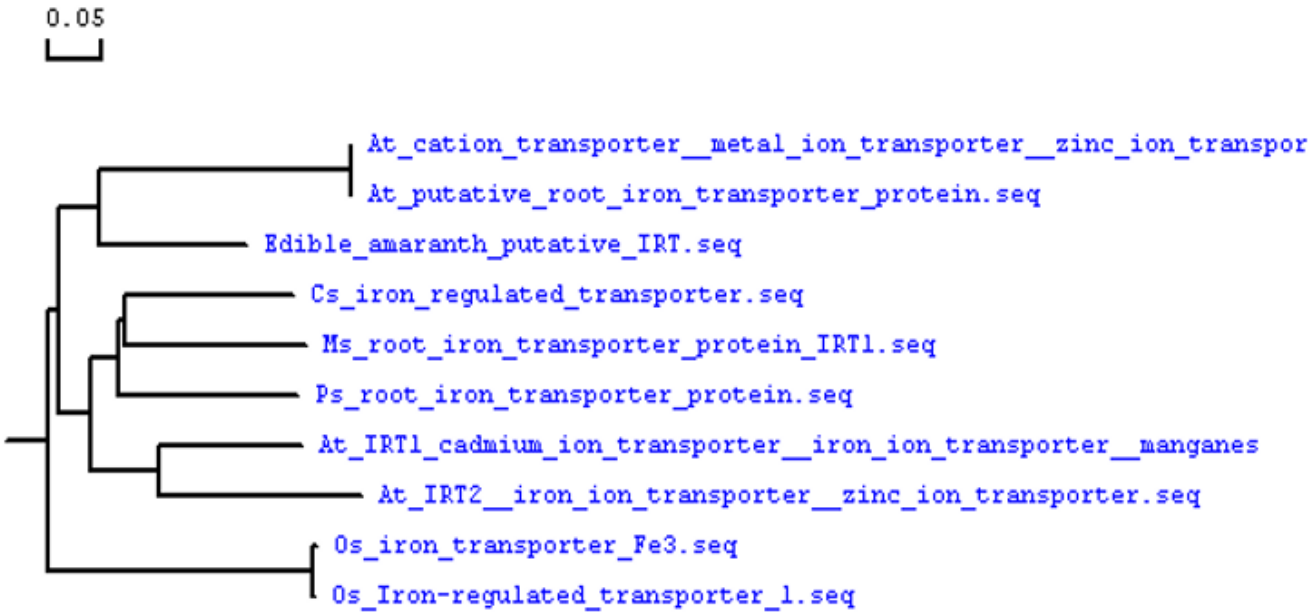


Figure 5

Phylogenetic tree analysis of several IRTproteins.

Note: Sequences from top to bottom: Arabidopsis zinc transporter, Arabidopsis iron transporter IRT1, amaranth *IRT*-encoded protein, cucumber IRT, *Begonia microphylla* IRT1, pea IRT, Arabidopsis IRT1, Arabidopsis IRT2, rice trivalent iron transporter, and rice IRT1.

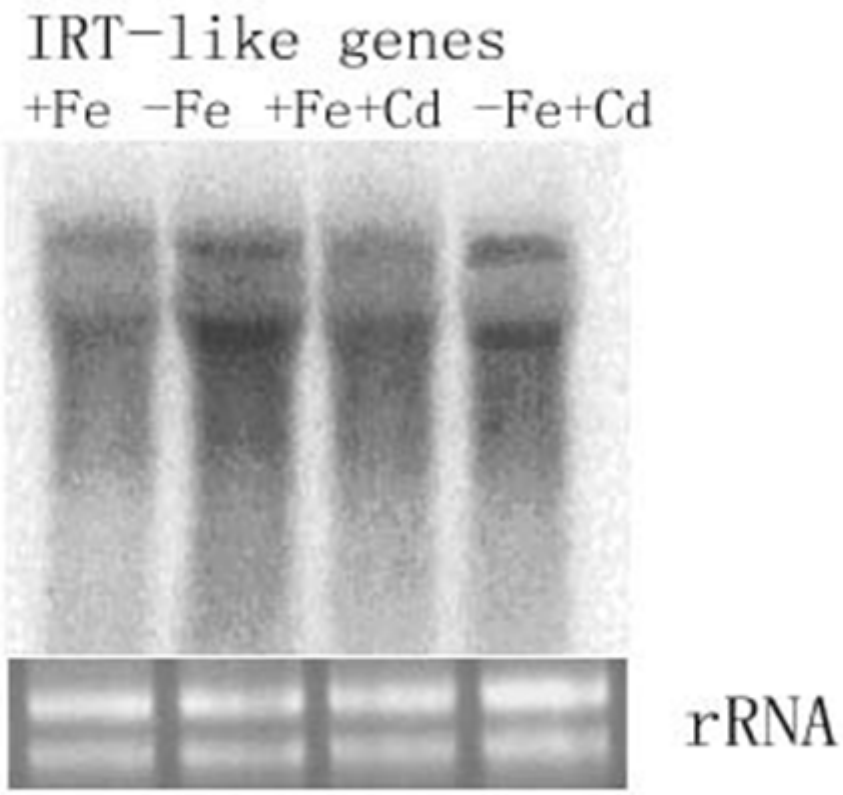


Figure 6

Analysis of expression patterns of *AmIRT1* by Northern blotting.

Note: +Fe: Fe alone treatment, −Fe: Fe deficiency treatment, +Fe+Cd: Fe with Cd treatment, and −Fe+Cd: Cd without Fe treatment