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Research Report

Genome-wide Analysis of Iron-Regulated Transporter 1 (*IRT1*) Genes in Plants

Recep Vatansever[1], Ertugrul Filiz[2], and Ibrahim Ilker Ozyigit[1*]

[1]*Department of Biology, Faculty of Science and Arts, Marmara University, 34722, Goztepe, Istanbul, Turkey*
[2]*Department of Crop and Animal Production, Cilimli Vocational School, Duzce University, 81750, Cilimli, Duzce, Turkey*

*Corresponding author: ilkozyigit@marmara.edu.tr

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Abstract. Iron (Fe) is an essential micronutrient required in a number of biological processes in plant species. Fe transporters are a type of broad-range metal transporter and have different families functioning in different compartments. This study focused on iron-regulated transporter 1 (*IRT1*), which are mainly responsible for Fe uptake from root, in 17 selected plant species with an emphasis on *Brachypodium distachyon*, *Chlamydomonas reinhardtii*, *Solanum lycopersicum* and *Populus trichocarpa* species. All IRT1 proteins were observed to belong to the ZIP (PF02535) protein family with eight transmembrane (TM) domains, and have a similar molecular weight (33.86-42.72 kDa, except for *C. reinhardtii* with 65.83 kDa) and amino acid length (324-408 aa, except for *C. reinhardtii* with 639 aa), with *pI* values of 5.31-7.16. The sub-cellular localization of these proteins was predicted to be the plasma membrane. Similar exon numbers were also detected with most genes having 2-3, except for *C. reinhardtii* (5), *Physcomitrella patens* (5) and *Vitis vinifera* (4). In a phylogenetic tree, monocot-dicot separation was not observed in main groups but some subgroups included only monocot or dicot proteins. Predicted interaction partner analysis of AtIRT1 (AT2G30080.1) pointed to main interaction partners either directly related with iron transport or that of other metal ion. The results of this study provide a theoretical reference for elucidating the structural and biological role of *IRT1* genes/proteins in plant species.

Additional key words: chelate, micronutrient, redox reaction, reactive oxygen species, transmembrane topology

Introduction

Iron (Fe) is one of the abundantly required essential micronutrients in plants. It is needed for a number of biological processes such as photosynthesis, respiration, chlorophyll biosynthesis, DNA synthesis, nitrogen fixation and hormone production. Besides, it has a function as structural component for heme, Fe-sulfur cluster and other Fe-binding sites (Vert et al., 2002; Kobayashi and Nishizawa, 2012). Plants need 10^{-9}-10^{-4} M of Fe concentrations to maintain a healthy growth. Insufficient uptake or deficiency causes interveinal chlorosis in leaves and reduces crop production. Although iron is abundantly found in nature, it is not directly available by plants since it is slightly soluble as the Fe^{3+} form under the presence of oxygen at physiological pH (Kim and Guerinot, 2007). Therefore, transport of iron requires either chelation of Fe^{3+} or acidification of the rhizosphere to reduce it to the Fe^{2+} form (Briat and Lobreaux, 1997). In addition, chemical properties of iron make it a suitable element for redox reaction but when it exists in large quantities and in a free state, it has a tendency to generate reactive oxygen species (ROS) (Marschner, 1995). Therefore, plants need to tightly regulate the uptake, utilization, intracellular compartmentalization, storage and partitioning of iron at cellular and molecular levels (Briat et al., 1995; Vert et al., 2002; Kobayashi and Nishizawa, 2012). Two different strategies have been acquired by graminaceous and non-graminaceous plants for acquisition of iron (Römheld and Marschner, 1986). In non-graminaceous plants, ferric chelates are reduced at the root surface, and formed ferrous ions are absorbed through the root plasma membrane. This process is accompanied by protons and phenolic compounds excreted from the roots to rhizosphere in order to increase the ferric ions solubility and/or reduce the capacity of ferric Fe on the root surface (Kobayashi and Nishizawa, 2012; Thomine and Vert, 2013). In graminaceous plants, uptake of iron depends on biosynthesis and secretion of mugineic acid family phytosiderophores (MAs), which are synthesized from S-adenosyl-L-methionine (SAM) pathway (Ma et al., 1999; Bashir et al., 2006; Ueno et al., 2007) through three consecutive enzymatic reactions of nicotianamine

synthase (NAS), nicotianamine aminotransferase (NAAT) and deoxymugineic acid synthase (DMAS) enzymes (Higuchi et al., 1999; Takahashi et al., 1999; Bashir et al., 2006). The synthesized MAs are secreted into the rhizosphere (Nozoye et al., 2011), which solubilize Fe (III) and form Fe (III)-MA complexes. Then, iron is taken up into root cells through the Yellow Stripe 1 (YS1) and Yellow Stripe 1-like (YSL) transporters (Inoue et al., 2009; Lee et al., 2009a, b; Thomine and Vert, 2013). Additionally, some plant species like rice represent both types of iron uptake mechanisms; by ferrous transporter (OsIRT1) as in non-graminaceous plants and Fe (III)-DMA uptake by OsYSL15 transporter (Inoue et al., 2009; Lee et al., 2009a, b) as in graminaceous plants.

Several plant metal transporters responsible for the transport of metal into cytosol have been identified, including zinc-regulated transporter (ZRT), iron-regulated transporter (IRT)-like protein (ZIP), natural resistance-associated macrophage protein (NRAMP) family and oligo peptide transporters (OPTs) family (Colangelo and Guerinot, 2006). ZRT, IRT-like protein (ZIP) family is a broad range plant metal transporter, in addition to Fe^{2+}, carrying various divalent metals such as zinc (Zn^{2+}), manganese (Mn^{2+}) and cadmium (Cd^{2+}) (Guerinot, 2000; Colangelo and Guerinot, 2006). ZIP proteins are 309-476 long amino acid residues with eight potential transmembrane (TM) domains, and they have a similar membrane topology, in which amino- and carboxyl-ends of protein located on the outside surface of plasma membrane. ZIP proteins also contain a variable cytoplasmic region between transmembrane domains 3 and 4, in which there is a potential metal-binding domain rich in histidine residues (Guerinot, 2000). Additionally, transmembrane domain IV contains the most conserved region of ZIP protein family, with an amphipathic helix containing a thoroughly conserved histidine residue. It is also reported that this histidine residue along with an adjacent semi polar residue might be a part of intramembranous metal binding site (Eng et al., 1998). NRAMP family is also a broad range plant metal transporter carrying Fe^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Al^{3+} (Thomine et al., 2000; Nevo and Nelson, 2006; Xia et al., 2010). This family consists of 10-12 transmembrane (TM) domains and has a consensus transport sequence between TM-8 and TM-9 domains shared with other ion channels and transporters (Cellier et al., 1995). OPT family can transport amino acid-containing compounds and their derivatives, and metals such as Mn, Zn, Cu, Ni, Cd and Fe (Schaaf et al., 2004; Yen et al., 2001).

When taken into account that Fe transporters are a type of broad-range metal transporters and have different families functioning in different compartments, plants are required to have a efficiently coordinated iron uptake system in roots in order to preserve the homeostasis and physiological processes properly. Therefore, this study focused on iron-regulated transporter 1 (IRT1), which are mainly responsible for Fe uptake from root, in 17 selected plant species with an emphasis on *Brachypodium distachyon*, *Chlamydomonas reinhadrtii*, *Solanum lycopersicum* and *Populus trichocarpa* species. In this context, physicochemical properties, conserved motif sequences, localization and transmembrane (TM) topologies of IRT1 proteins were determined; exon/intron organization of *IRT1* genes was analyzed; a phylogenetic tree of IRT1 proteins was constructed and interaction partners were evaluated. The results of this study will be a theoretical reference for understanding of structural and biological role of *IRT1* genes/proteins in various plants species, and this will pave the way for better understanding iron trafficking at root.

Materials and Methods

Sequence Retrieval of IRT1 Genes/proteins

A reference IRT1 protein sequence with Q38856.2 accession number was obtained from the protein database of NCBI (Romiti, 2010). Then, this sequence was queried in Phytozome (Goodstein et al., 2012) database against the proteome datasets of 17 selected plant species, including *Arabidopsis thaliana, Brachypodium distachyon, Brassica rapa, Chlamydomonas reinhardtii* (green alga)*, Cucumis sativus, Eucalyptus grandis, Glycine max, Gossypium raimondii, Medicago truncatula, Oryza sativa, Phaseolus vulgaris, Physcomitrella patens* (moss)*, Populus trichocarpa, Solanum lycopersicum, Sorghum bicolor, Vitis vinifera* and *Zea mays*. Then, obtained 211 genes filtered for a cut-off of $\leq e^{-10}$ threshold value; redundant sequences were removed and later analyzed by using TMHMM (Krogh et al., 2001) server for their transmembrane (TM) domain numbers and for known topology (Figure 1). This reduced the gene number to 26 for 17 species, including 1 gene for *A. thaliana*; 1 gene for *B. distachyon*; 1 gene for *B. rapa*; 2 genes for *C. reinhardtii*; 1 gene for *C. sativus*; 1 gene for *E. grandis*; 3 genes for *G. max;* 1 gene for *G. raimondii;* 2 genes for *M. truncatula;* 2 genes for *O. sativa;* 2 genes for *P. vulgaris;* 2 genes for *P. patens;* 2 genes for *P. trichocarpa;* 1 gene for *S. lycopersicum;* 1 gene for *S. bicolor;* 2 genes for *V. vinifera* and 1 gene for *Z. mays*. These genes renamed as specified in Table 1.

Physico-chemical, Subcellular Localization and Conserved Motif Analysis of IRT1 Proteins

Physico-chemical properties (sequence length, molecular weight and isoelectric point) of IRT1 proteins were analyzed by using Expasy's ProtParam server (Gasteiger et al., 2005). The conserved motif analysis was performed by using MEME tool (Timothy et al., 2009) with following parameters; maximum number of motifs to find, 10; minimum width of motif, 6 and maximum width of motif, 50. The sub-cellular locations of IRT1 proteins were predicted by CELLO server (Yu et al.,

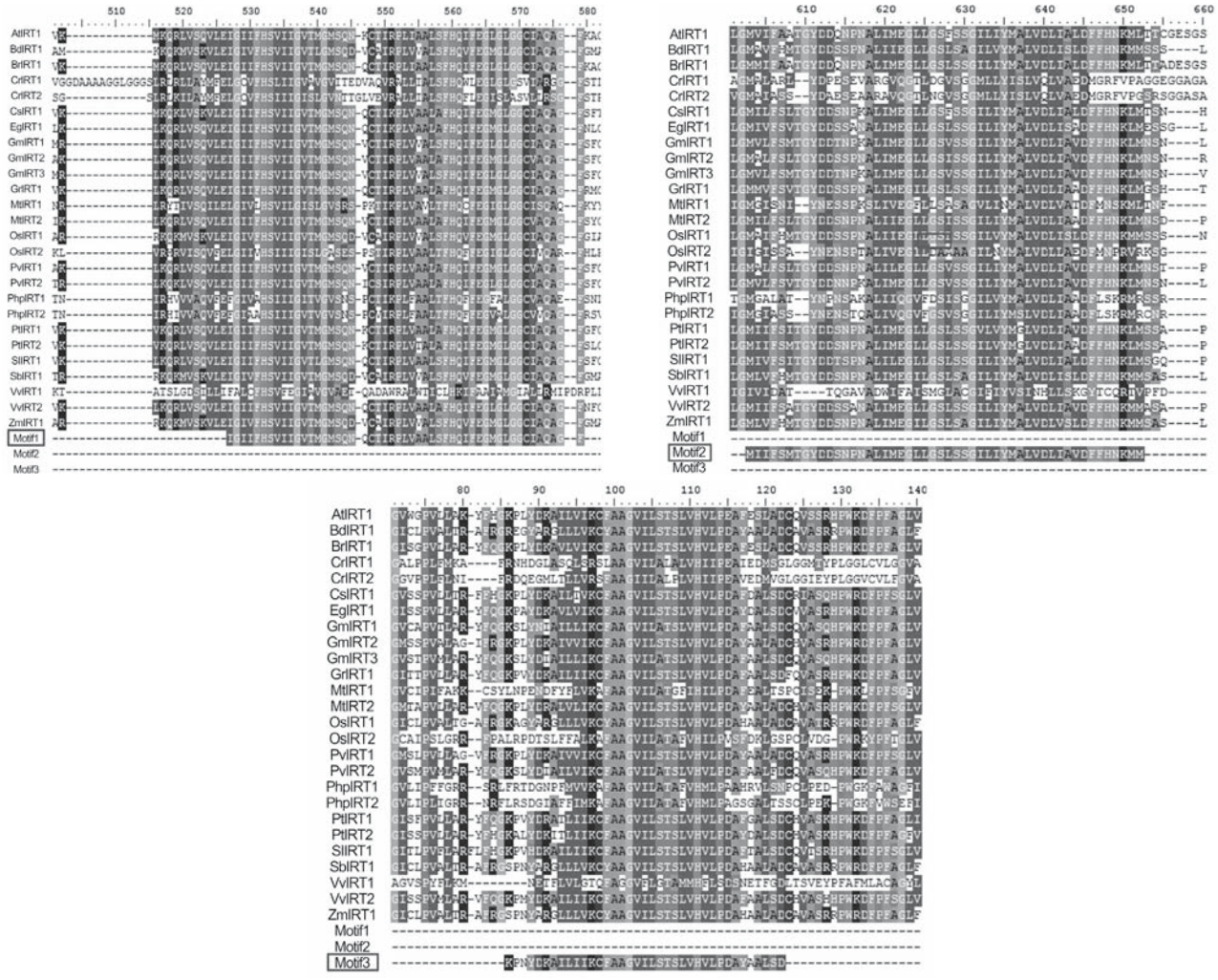

Fig. 1. Alignment of regions containing the three most conserved motifs in proteins of the ZIP family from 17 plant species.

2006). Domain analysis was performed by using pfam database (Sonnhammer et al., 1997). Also, exon/intron and upstream/downstream organization of *IRT1* genes were determined by using GSDS server (Guo et al., 2007). Gene expression data of *IRT1* genes at anatomical part and developmental stage level was obtained from Genevestigator database (Hruz et al., 2008).

Phylogenetic and Interaction Partner Analysis of IRT1 Proteins

IRT1 sequences were aligned by ClustalW (Thompson et al., 1994) and phylogenetic tree was constructed by MEGA 5 (Tamura et al., 2011) with maximum likelihood (ML) method for 1.000 replicates bootstrap value. The interaction partners of IRT1 proteins were predicted by using STRING server and cytoscape (Smoot et al., 2011; Franceschini et al., 2013).

Results and Discussion

Physico-chemical Properties and Subcellular Localization of IRT1 Proteins

All identified IRT1 proteins belong to ZIP (PF02535) protein family with eight transmembrane (TM) domains, and have a similar molecular weight (33.86-42.72 kDa, except for *C. reinhardtii* with 65.83 kDa) and amino acid length (324-408 aa, except for *C. reinhardtii* with 639 aa), with a *pI* value of 5.31-7.16. The sub-cellular location of these proteins was predicted as plasma membrane (Table 1). According to Guerinot (2000), ZIP proteins are 309-476 long amino acid residues with eight potential transmembrane (TM) domains. Thus, the identified IRT1 proteins in this study comply with the general physico-chemical properties of this family.

Expression Profile Analysis of *IRT1* Genes

To investigate the main expression tissues of *IRT1*, thereby

Table 1. Physico-chemical properties, gene organization and predicted sub-cellular location of IRT1 proteins in 17 plant species

Gene name	Gene Identifier (Phytozome)	EST Accession/ Identity (%)	Exon number	Protein length (aa)	MW (kDa)	pI	Pfam family	TMD	Predicted sub-cellular location
AtIRT1	AT2G30080	DR270051.1/ 394/396(99%)	2	341	36.02	5.71	Zip	8	Plasma Membrane
BdIRT1	Bradi2g34560	GT760473.1/ 427/430(99%)	2	399	42.16	5.95	Zip	8	Plasma Membrane
BrIRT1	Brara.E01313	EX113443.1/ 567/579(98%)	2	337	35.62	5.34	Zip	8	Plasma Membrane
CrIRT1	Cre07.g355100	AV630909.1/ 204/204(100%)	5	639	65.83	6.13	Zip	8	Nuc./Extracell./P. Membrane
CrIRT2	Cre13.g573950	No significant similarity found	5	408	41.83	5.78	Zip	8	Plasma Membrane
CsIRT1	Cucsa.139350	EG701610.1/ 397/495(80%)	2	334	35.68	5.73	Zip	8	Plasma Membrane
EgIRT1	Eucgr.B02234	HS054661.1/ 325/325(100%)	2	332	35.12	6.12	Zip	8	Plasma Membrane
GmIRT1	Glyma.11G169300	BU544153.1/ 538/561(96%)	3	326	34.77	5.81	Zip	8	Plasma Membrane
GmIRT2	Glyma.14G196200	CO981854.1/ 734/750(98%)	2	324	33.86	6.35	Zip	8	Plasma Membrane
GmIRT3	Glyma.18G060300	BU544153.1/ 571/575(99%)	3	328	35.03	5.96	Zip	8	Plasma Membrane
GrIRT1	Gorai.004G232700	CO128552.1/ 866/866(100%)	2	325	34.61	7.16	Zip	8	Plasma Membrane
MtIRT1	Medtr5g071990	BG449910.1/ 490/492(99%)	2	318	33.94	5.68	Zip	8	Plasma Membrane
MtIRT2	Medtr6g007687	EV260975.1/ 703/705(99%)	3	349	38.28	7.09	Zip	8	Plasma Membrane
OsIRT1	LOC_Os05g07210	CB634836.1/ 524/528(99%)	2	395	41.32	6.34	Zip	8	Plasma Membrane
OsIRT2	LOC_Os05g39540	CX105772.1/ 458/458(100%)	3	362	37.89	5.97	Zip	8	Plasma Membrane
PvIRT1	Phvul.006G070200	FE707806.1/ 652/839(78%)	3	328	34.96	5.53	Zip	8	Plasma Membrane
PvIRT2	Phvul.008G259200	FE707806.1/ 921/926(99%)	2	325	33.91	6.11	Zip	8	Plasma Membrane
PhpIRT1	Phpat.006G078700	BJ159856.1/ 473/473(100%)	5	367	39.55	6.51	Zip	8	Plasma Membrane
PhpIRT2	Phpat.016G001900	DC945718.1/ 712/717(99%)	2	330	34.87	6.58	Zip	8	Plasma Membrane
PtIRT1	Potri.001G279300	DT479218.1/ 712/786(91%)	2	337	35.95	6.85	Zip	8	Plasma Membrane
PtIRT2	Potri.009G074100	DT479218.1/ 778/786(99%)	2	335	35.71	6.03	Zip	8	Plasma Membrane
SlIRT1	Solyc05g053370.2	FS189824.1/ 445/446(99%)	2	328	35.27	6.12	Zip	8	Plasma Membrane
SbIRT1	Sobic.009G057700	EC889051.2/ 767/836(92%)	2	408	42.72	5.95	Zip	8	Plasma Membrane
VvIRT1	GSVIVG01014656001	CF513051.1/ 603/649(93%)	4	322	34.42	5.31	Zip	8	Plasma Membrane
VvIRT2	GSVIVG01024696001	EC925436.1/ 741/743(99%)	2	335	35.64	6.85	Zip	8	Plasma Membrane
ZmIRT1	GRMZM2G034551	EC889051.2/ 807/807(100%)	2	396	41.52	6.15	Zip	8	Plasma Membrane

TMD indicates transmembrane domain number; AtIRT1 stands for *A. thaliana*; *BdIRT1* for *B. distachyon*; *BrIRT1* for *B. rapa*; *CrIRT1, 2* for *C. reinhardtii*; *CsIRT1* for *C. sativus*; *EgIRT1* for *E. grandis*; *GmIRT1-3* for *G. max*; *GrIRT1* for *G. raimondii*; *MtIRT1, 2* for *M. truncatula*; *OsIRT1, 2* for *O. sativa*; *PvIRT1, 2* for *P. vulgaris*; *PhpIRT1, 2* for *P. patents*; *PtIRT1, 2* for *P. trichocarpa*; *SlIRT1* for *S. lycopersicum*; *SbIRT1* for *S. bicolor*; *VvIRT1, 2* for *V. vinifera* and *ZmIRT1* for *Z. mays*

its potential location and function, we have analyzed the gene expression profiles of the *IRT1* gene in *A. thaliana* and *S. lycopersicum* using Genevestigator. At anatomical part level, *Arabidopsis IRT1* have been identified to be expressed in flowering parts and in many root-related structures such as roots, primary roots, root tip, lateral root, maturation zone, radicle, root phloem companion cell etc. During the development, *Arabidopsis IRT1* was mainly expressed in seedling, germinated seeds and young rosette stages (Suppl. 1 Fig.1). Moreover, Fe-deficiency in *Arabidopsis* plants has greately affected the expression profile of *IRT1*. Under iron deficiency, the *IRT1* gene has been identified to be up-regulated with high fold-change (Suppl. 1 Fig. 2). In tomato (*S. lycopersicum*), *IRT1* has also demonstrated the similar tissue expression pattern to that of *Arabidopsis*, with an expression mainly in root, seedling and flowering parts (Suppl. 1 Fig. 3). The expression profiles of *Arabidopsis* and tomato plants indicated that *IRT1* could mainly function in root and flowering parts of plants. This also supports the function of IRT1 transporters at root for Fe uptake . Overall, general physico-chemical properties of identified IRT1 proteins and expression profile data analysis of *IRT1* genes in *Arabidopsis* and tomato supported that identified proteins could possibly function in Fe uptake at root.

Conserved Motif Analysis

Most conserved 10 motif sequences of IRT1 proteins were detected in 17 selected plant species. The analysis showed that motif 1 (IGIIFHSVIIGVTMGMSQNQCTIRPLVAALSFHQIFEGMGLGGCIAQAGF), motif 2 (MIIFSMTGYDDSNPNALIMEGLLGSLSSGILIYMALVDLIAVDFFHNKMM) and motif 3 (KPNYDKAILIIKCFAAGVILSTSLVHVLPDAYAALSD) were related with ZIP (PF02535) protein family and shared by all species while motif 4 (CQVASRHPWKDFPFAGLVTLIGALLALLVDLTASSHVEHHQ), motif 5 (ARACRDGQAATHLKTISIFVIFVTSVIGICSPVLLARYFQG), motif 6 (GTTAYMCFMFSVTTPMGIILG), motif 7 (KKACFIALTLGSASMSILALW), motif 8 (EEERREEELVKLKQRLVSQVLE), motif 9 (YTPVETQEKE) and motif 10 (PCLPEKPWRKFPWSGFITMLAAIGTLIMDTIATEYYMNRPQKQHG) were not related any protein family (Fig. 3B). Guerinot (2000) reported that transmembrane domain IV contains the most conserved region of ZIP protein family, with an amphipathic helix containing a thoroughly conserved histidine residue. This indicated implicitly that motif 1-3 may locate in transmembrane domain IV. To support this,

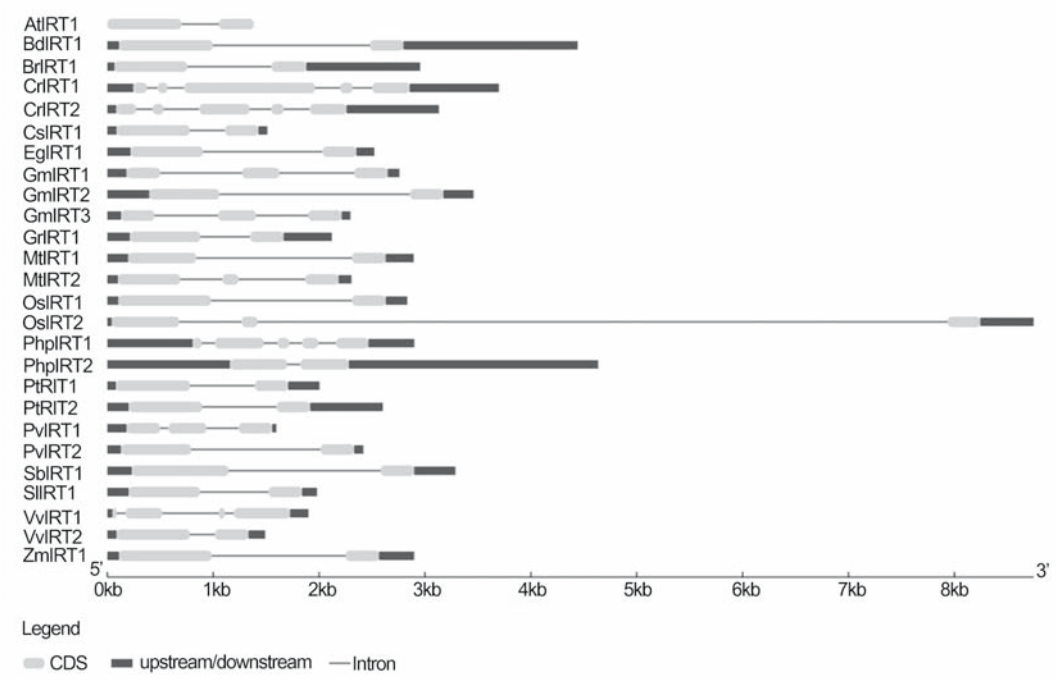

Fig. 2. Exon-intron organization of IRT genes. Exon, intron and upstream/downstream regions were indicated as filled yellow boxes, lines, and red boxes, respectively.

we checked the thoroughly conserved histidine residues in motif 1-3. In motif 1, two rows of thoroughly conserved histidine residues were detected (Fig. 1A); in motif 2, one row of histidine residues with partially disrupted by other residues detected (Fig. 1B), and in motif 3, one row of thoroughly conserved histidine residues detected (Fig. 1C). Thus, motif 1 and 3 were the two motifs with thoroughly conserved histidine residues. When membrane topologies of IRT1 proteins for transmembrane domain IV analyzed, motif 1 seemed more potential candidate for most conserved region in IRT1s. ZIP proteins also contain a variable cytoplasmic region between transmembrane domains 3 and 4 (Guerinot, 2000). This variable region was also clearly observed in transmembrane topology analysis in *B. distachyon*, *C. reinhadrtii*, *S. lycopersicum* and *P. trichocarpa* (Suppl. 2).

Exon/intron Organization of *IRT1* Genes

Gene organization analysis showed that *AtIRT1* has 2 exons; *BdIRT1* has 2 exons; *BrIRT1* has 2 exons; *CrIRT1,2* have 5 exons; *CsIRT1* has 2 exons; *EgIRT1* has 2 exons; *GmIRT1-3* have 3, 2 and 3 exons, respectively; *GrIRT1* has 2 exons; *MtIRT1,2* have 2 and 3 exons, respectively; *OsIRT1,2* have 2 and 3 exons, respectively; *PvIRT1,2* have 3 and 2 exons, respectively; *PhpIRT1,2* have 5 and 2 exons, respectively; *PtIRT1,2* have 2 exons; *SlIRT1* has 2 exons; *SbIRT1* has 2 exons; *VvIRT1,2* have 4 and 2 exons, respectively and *ZmIRT1* has 2 exons (Fig. 2). Exon numbers of species usually varied between 2 and 3, except for *C. reinhardtii,* and one member of *P. patents* and *V. vinifera.* Exon/intron variations (loss or gains) in these species might have occurred during the development of orthologous genes (Rogozin et al., 2005), and may be related with the species-specific functional diversities. Koralewski and Krutovsky (2011) report that shorter exons increase the potential for alternative splicing (AS) and genomic complexity but AS rate does not always correlate with the species complexity. When the data in Fig. 2 was analyzed, it was observed that *CrIRT1* and *2*, *MtIRT2, OsIRT2, PhpIRT1* and *VvIRT1* genes include some relatively shorter exons in comparison with other species but there was no relationship between exon length and genomic complexity observed since two lower plants *C. reinhardtii* and *P. patents* contained relatively shorter exons. In general, this shows that reliability of obtained results is directly related with the number of species analyzed. Therefore, studies regarding the evolutionary development of exons/introns; their contribution to speciation and species complexity necessitate to analyze the large number of inter-and-intra species sets.

Phylogenetic Analysis and Interaction Partners of IRT1 Proteins

The constructed phylogenetic tree was arbitrarily divided into four main groups, namely A, B, C and D. Group A contained four monocot (*O. sativa, B. distachyon, S. bicolor* and *Z. mays*) and 9 dicot species (*G. max, P. vulgaris, M. truncatula, C. sativus, E. grandis, V. vinifera, S. lycopersicum, P. trichocarpa* and *G. raimondii*); group B included one

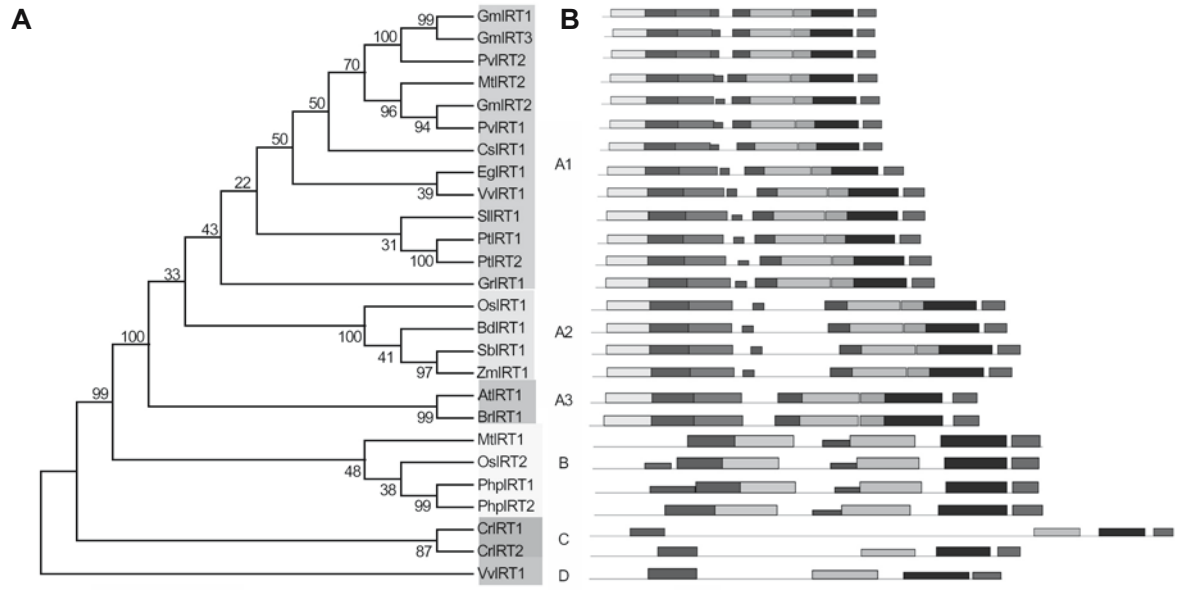

Fig. 3. Phylogenetic analysis (A) and schematic diagram of conserved motifs of IRT1 proteins in 17 plant species. Each motif is represented with a box of different color: motif 1, cyan; motif 2, blue; motif 3, red; motif 4, pink; motif 5, yellow; motif 6, green; motif 7, turquoise; motif 8, black; motif 9, dark green; and motif 10, gray (B).

monocot (*O. sativa*), one dicot (*M. truncatula*) and one lower plant (*P. patens*); group C only contained lower plant *C. reinhardtii* and group D contained only dicot species *V. vinifera* (Fig. 3A). The group A further subdivided into three subgroups named as A1, A2 and A3. Subgroups A1 and A3 contained dicot species whereas subgroup A2 included monocot species. Subgroup A2 separated with the highest bootstrap value (100%) from dicots (A1 and A3), suggesting that monocot *IRT1* genes diverged during the split of monocot and dicot in the course of phylogenetic development. Although 11 of 13 dicot species clustered in subgroup A1, only two dicots (*B. rapa* and *A. thaliana*) diverged from other dicots as subgroup A3 and one member of *V. vinifera* as group D, proposing that some genomic forces (insertion, deletion etc.) may affect the gene structure of *IRT1*s. In group B, distribution of species, including one monocot, one dicot and one moss may be derived of their higher species-specific genetic divergence of *IRT1* genes. Green alga *C. reinhardtii* had its own cluster as group C, with a high bootstrap value of 87%. In addition, species that sharing group A represented more similar conserved motif pattern than that of group B, C and D (Fig. 3B). Overall, phylogenetic data (Fig. 3A) were in agreement with the results of conserved motif analysis (Fig. 3B).

Predicted Interaction Partner Analysis

Predicted interaction partner analysis was performed for AtIRT1/ZIP6 (AT2G30080.1) protein. Ten putative interaction partners were found, including HMA4, AT1G51610, AtCDF1, ZAT, NAS3, AT3G20870, AT3G08650, NAS4, KEA3 and NRAMP2 for AtIRT1 (Fig. 4). Among these interaction partners, nicotianamine synthase (NAS) 4 is required for proper response to iron deficiency and distribution of iron (Koen et al., 2013). Perturbation studies showed that the *NAS4* gene is significantly up-regulated under Fe-deficiency, supporting its possible function in iron deficiency and its distribution (Suppl. 1 Fig. 4). Nicotianamine synthase (NAS) 3 functions as a sensor for iron status within the plant and involved in the transport of iron (Inoue et al., 2003). Although Fe-deficiency has led to slightly induce the up-regulation of *NAS3* at root, it is mainly down-regulated in leaf and shoot as response to Fe-deficiency (Suppl. 1 Fig. 5). This implicitly may indicate the presence of a relationship between cellular iron concentration and *NAS3* expression. NRAMP2 is an iron transporter protein functioning in iron metabolism in plants (Gruenheid et al., 1999). In addition, NRAMP family is a kind of broad range plant metal transporter carrying Fe^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Al^{3+} (Thomine et al., 2000; Nevo and Nelson, 2006; Xia et al., 2010). Under Fe-deficiency, the expression of *NRAMP2* gene did not show any significant change (Suppl. 1 Fig. 6). Thus, its expression could be related with the presence of cellular iron or iron related-uptake of other metals. AT3G20870 and AT3G08650 are ZIP family proteins functioning in metal ion transport (Wang et al., 2011). AtCDF1/ZAT are metal ion transmembrane transporters mediating Zn ion homeostasis in plants (Bloß et al., 2002). AT1G51610 (MTPC4) is a cation efflux family protein functioning in sequestration of excess metal in the cytoplasm into vacuoles to regulate metal homeostasis (Mäser et al., 2001). HMA4 is a cadmium ion transmembrane transporter,

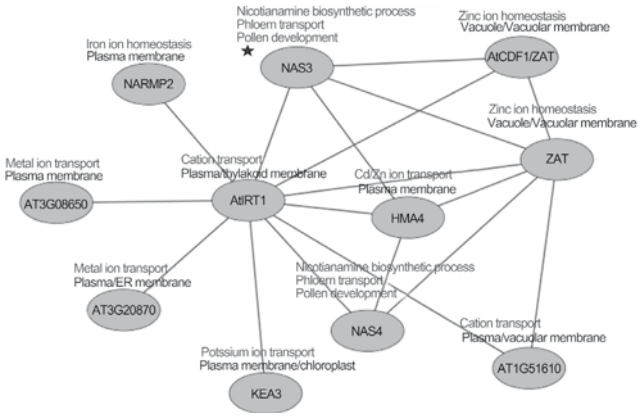

Fig. 4. Predicted interaction partners of AtIRT1 protein. Interactome was generated using Cytoscape for STRING data. Interaction partner analysis was performed for 10 putative interactors of *Arabidopsis* IRT1 (AT2G30080.1), including HMA4, AT1G51610, AtCDF1, ZAT, NAS3, AT3G20870, AT3G08650, NAS4, KEA3 and NRAMP2. Genes in interactome are annotated with gene ontology (GO) terms for biological process and cellular component. Biological process and cellular component terms are written in red and blue, respectively. Asterisk (*) indicates the no cellular component data available for GO terms.

and involved in cadmium and zinc transport (Miyadate et al., 2011). KEA3 is a potassium ion transmembrane transporter (Kunz et al., 2014). Although *AT3G20870*, *AT3G08650, AtCDF1/ZAT, AT1G51610 (MTPC4), HMA4 and KEA3* genes have been induced upon iron-deficiency, no significant differentially expression pattern has been demonstrated (Suppl. 1 Fig. 7-12). However, presence of their expression under Fe-deficiency indicates that these genes could be in a cross-talk with Fe-metabolism in a way. Moreover, all the interaction partners of *Arabidopsis* IRT1 are ion transporter proteins. When taken into account that many metal ion transporters are a type of broad-range metal transporter (Guerinot, 2000) carrying a range of metal ions, it is understandable that AtIRT1 proteins have an interaction with different types of transporter proteins.

Conclusion

In this study, we have performed a comparative analysis of iron-regulated transporter 1 (IRT1) proteins in several plant species. The results of this study will be a valuable theoretical knowledge for understanding the structural and biological role of *IRT1* genes/proteins in various plants species. However, since many metal ion transporters are broad-range carriers, carrying a range of metal ions simultaneously, a genome-wide structural and functional analysis of these transporters is needed to elucidate the real physiological process in metal ion uptake, transportation and storage. In addition, since the reliable homologous crystallographic structures for IRT1 proteins are not currently available, this hinders the construction of reliable 3D models and metal ion-transporter docking studies.

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