Current Journal of Applied Science and Technology

39(38): 14-23, 2020; Article no.CJAST.62826
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

In silico Identification and Analysis of Iron (Fe) Transporters in Various Plant Species

Akula Dinesh[1*] Ramya Rathod[1] and M. Sreedhar1

[1]Dept of Genetics and Plant Breeding, Regional Sugarcane and Rice Research Station, PJTSAU, Rudrur-503188, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author AD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RR and MS mentored and monitored the work and reviewed the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2020/v39i3831091
Editor(s):
(1) Dr. Alessandro Buccolieri, Università del Salento, Italy.
Reviewers:
(1) Paola Vellante, Italy.
(2) Maysaa Ali Abdul Khaleq, Al-Rasheed University College, Iraq.
(3) Krisztina Bela, University of Szeged, Hungary.
Complete Peer review History: http://www.sdiarticle4.com/review-history/62826

Original Research Article

Received 19 September 2020
Accepted 23 November 2020
Published 11 December 2020

ABSTRACT

In plants, Iron is an important micronutrient which is required for various processes like photosynthesis, respiration and for balanced redox potential. Iron has a significant role in human nutrition. Therefore, increasing the Fe content of economical parts through conventional, Molecular and/or Transgenic breeding will have dramatic impact on human health. Therefore, this study was aimed to identify and characterize homologues of OsIRT1, OsIRT2, OsVIT1, OsYSL2, OsYSL15 and OsYSL18 in 21 different plant species. The study revealed that, a total of 51 putative Fe transporter proteins homologues were identified which could be characterized with 555 to770 amino acids sequence length with 61 to 84.8 kDa molecular weight and pI of 8.2 to 9.4 having basic nature with 9 to15 TMD with an average of 13 TMDs. The sub-cellular localization of putative Fe transporters was predicted as the plasma membrane and all the identified Fe homologues contained oligopeptide transporter (OPT) domain proteins which play a significant to role in Fe transport and homeostasis. Further, the analysis led to identification of highly conserved residues in the putative Fe sequences which could be used as potential motif signatures in identification of new Fe transporter. The interactome analysis for oryza sativa OsYSL15 transporter showed putative

**Corresponding author: E-mail: Dine.a32@gmail.com;*

interaction with NAS2, NAS1, DMAS1, OsJ_32857, IRT1, IRO2, IDEF1, IRT2, IDI2 which are found to be directly involved in Fe transport from roots to grain. This study, elucidates the valuable theoretical knowledge about the Fe genes, protein features and assist in molecular manipulation of Fe transporters in various plants for developing high Fe in economical part.

Keywords: OsYSL2; OsYSL15 and OsYSL18; transmembrane domains; iron (fe) transporter; oligopeptide transporter; motifs.

1. INTRODUCTION

Iron is an essential micronutrient for plants, which plays a significant role in important processes like photosynthesis, respiration and chlorophyll biosynthesis, nitrogen fixation and also a component in Fe-binding sites [1]. Despite of soil with large depositions of Fe, plants cannot utilize it because most of Fe is present in insoluble Fe3+ form. Therefore, iron uptake by the plants requires chelation of Fe^{3+} or rhizosphere acidification to reduce it to Fe^{2+} form easy uptaken [2]. On the other side, Fe readily accepts and donates electrons making it essential for plants at the same time, producing toxic reactive oxygen species [3]. Therefore, plants have developed efficient mechanisms for Fe uptake from root to shoot and transfer to grain [4,5,6].

The various mechanisms developed for Fe acquisition in plant species have been characterized into two categories: Non-graminaceous plants includes strategy I and graminaceous plants consist of Strategy II [7]. In non-graminaceous plants, ferric chelates are reduced at the root surface by Ferric Chelate Reductase (FRO) gene which cause the insoluble form of Fe^{3+} to Fe^{2+} on the surface of root cells, and reduced ions are uptken by the plasma membrane of root [5]. In graminaceous plants, iron uptake involves biosynthesis and secretion of mugineic acid family phytosiderophores (MAs), which solubilize soil Fe. The phytosiderophores have high affinity for Fe^{3+} which bind efficiently and transport Fe to the interior of root cells. The MAs are synthesized from S-adenosyl-L-methionine (SAM) pathway by the involvement of three consecutive enzymatic reactions of nicotianamine aminotransferase (NAAT), nicotianamine synthase (NAS), and deoxymugineic acid synthase (DMAS) enzymes [8,9,10]. The synthesized MAs are rleased into the rhizosphere which solubilize Fe3+ and Fe^{3+}-MA complexes. Then, iron is taken up into root cells through the Yellow Stripe 1 (YS1) and Yellow Stripe 1-like (YSL) transporters [11,12]. Interestingly, rice uptakes Fe by ferrous transporter (OsIRT1) as in non-graminaceous plants and Fe (III)-DMA uptake by OsYSL15 transporter as in graminaceous plants [11].

Meanwhile, the OsYSL2 gene encodes nicotianamine transporter which is responsible for the phloem transport of Fe and manganese in the Fe (II)-NA and Mn(II)-NA and also responsible for long-distance transport into sink tissues [13,14]. The constitutive expression of OsYSL2 gene in rice under the control of a sucrose transporter (OsSUT1) had yielded polished seed with higher concentrations of Fe. This shows the importance of OsYSL2 gene in biofortification of rice crop. Likewise, another Fe (III)- DMA transporter gene, OsYSL18 which is found to be expressed in pollen and pollen tubes suggesting a role in phloem Fe transport. It was found that the YSL family belongs to oligo peptide transporters (OPTs) family [15].

The Oligopeptide Transporter (OPT) family was highly conserved among both prokaryotes and eukaryotes. The OPT family has two clades, the peptide transporters and the yellow stripe-type iron-complex transporters (YS) [15,16]. Peptide transporter homologues primarily transport oligopeptides and glutathione derivatives. In A. thaliana, glutathione influence the expression of iron uptake and transport related genes when grown under iron deficiency conditions. This study also confirmed that over-expression of glutathione enhances the tolerance to Fe deficiency. These OPTs were mainly found in plasma membrane that import substrates from the apoplasm and the external environment. They also play a important role in plant growth and development [15]. OPTs were predicted to have several signature motifs with 12 transmembrane domains.

Other than OPTs, iron-regulated transporter (IRT) like protein (ZIP), zinc regulated transporter (ZRT) and natural resistance-associated macrophage protein (NRAMP) family were also involved in metal transportation. ZIP family transports Fe^{2+}, Zn^{2+}, Mn^{2+}, Cd^{2+}, Ni^{2+}, and Co^{2+} [17]. Similarly, NRAMP localize in intracellular

vesicle or the plasma membrane which transports divalent metals. Likewise, IRT transporters generally localize to the plasma membrane [18,19].

From this, Fe transporters involve broad range of metal transporters with different family domains functioning in different ways to maintain the Fe homeostasis in plants. Thus, identification of new putative Fe transporters in different plant species and analyzing the molecular basis and topological information is carried out. To accomplish this, few functionally characterized Fe transporters *Viz*., OsIRT1 (Q75HB1), OsIRT2 (Q6L8G1), OsVIT1 (Q6MWE5), OsYSL2 (Q6H3Z6), OsYSL15 (Q6H3Z3) and OsYSL18 (Q941V3) were used for identification putative transporter in 21 plants species using sequence homology. The newly identified putative Fe transporters will be analyzed for conserved motif sequences, physicochemical properties, localization and transmembrane (TM) topologies and interaction partners. The results of this study will be a theoretical reference for understanding of structural and biological role of Fe transporters in various plants species, and this will pave the way for better understanding iron trafficking from source to sink in different plants.

2. MATERIALS AND METHODS

2.1 Retrieval of Fe Transporter Genes and Proteins

Seven *Oryza sativa* Fe transporter protein sequences such as OsIRT1 (Q75HB1), OsIRT2 (Q6L8G1), OsVIT1 (Q6MWE5), OsYSL2 (Q6H3Z6), OsYSL15 (Q6H3Z3) and OsYSL18 (Q941V3) were obtained from UniProtKB/Swiss-Prot database of NCBI (ncbi.nlm.nih.gov/). UniProtKB/Swiss-Prot database harbors the non-redundant, manually curated high-quality protein sequences [20]. These obtained sequences were used as queries in proteome datasets of 21 plant species including, *Ananas comosus, Arabidopsis thaliana, Brassica oleracea, Capsella grandiflora, Citrus clementina, Eucalyptus grandis, Carica papaya, Brachypodium distachyon, Brasica rapa, Glycine max, Gossypium raimondii, Medicago truncatula, Manihot esculenta, Daucus carota, Linum usitatissimum, Phaseolus vulgaris, Populus trichocarpa, Setaria italica, Solanum lycopersicum, Sorghum bicolor* and *Zea mays* in Phytozome database (phytozome.jgi.doe.gov/pz/portal.html) [21]. The homologous with E value equal to 0 was selected for further analysis. Later, redundant sequences were manually removed and a set 51 putative Fe transporter sequences were retrieved for subsequent analysis.

2.2 Sequence Analysis of Fe Transporter Proteins

Physico-chemical features of putative Fe transporter sequences were analyzed by ProtParam tool (http://web.expasy.org/protparam/) [22]. Sub-cellular localizations were predicted by CELLO server (http://cello.life.nctu.edu.tw/) [23]. Protein domain families were charecterised in NCBI-CD search [24]. Transmembrane domains (TMDs) were predicted by TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) [25]. Motif analysis was performed using MEMEtool (http://meme-suite.org/tools/meme) [26] with following parameters; maximum number of motifs to find, 5; minimum width of motif, 6 and maximum width of motif, 50. The Interactome network was generated by Cytoscape [27] using STRING data (http://string-db.org/) [28].

3. RESULTS AND DISCUSSION

3.1 Retrieval of Fe Transporter Genes and Proteins

The functionally characterized *Oryza sativa* Fe transporter protein such as OsIRT1 (Q75HB1), OsIRT2 (Q6L8G1), OsVIT1 (Q6MWE5), OsYSL2 (Q6H3Z6), OsYSL15 (Q6H3Z3) and OsYSL18 (Q941V3) were used as query sequence in protein dataset of Phytozome database to obtain the corresponding homologues in selected 21 plant species. In order to retrieve the very close homologues, the BLAST-p function with the E value equal to zero was used as selection criteria. None of the homologues for Fe transporters OsIRT1 and OsIRT2 and OsVIT1 had E-value equal to 0, which were discarded from further analysis. The homology search for OsYSL2, OsYSL 15 and OsYSL 18 showed presence of 54 unique putative Fe transporter among the 21 species. These included 3 genes from *Ananas comosus, 1* gene from *Arabidopsis thaliana, 1* gene from *Brassica oleracea, 1* gene from *Capsella grandiflora,1* genes from *Citrus clementina,* 1 gene from *Daucus carota, 3* genes from *Eucalyptus grandis,2* genes from *Carica papaya,* 10 genes from *Brachypodium distachyon, 2* genes from *Brasica rapa, 2* genes from *Glycine max, 2* genes from *Gossypium raimondii, 2* genes from *Medicago truncatula, 3* genes from *Manihot esculenta, 3* genes from

Linum usitatissimum, 1 genes from *Phaseolus vulgaris,3* genes from *Populus trichocarpa, 4* genes from *Setaria italica, 2* genes from *Solanum lycopersicum, 4* genes from *Sorghum bicolor* and 3 genes from *Zea mays* (Table. 1).

3.2 Sequence Analysis of Fe Transporter Genes and Proteins

A total of 54 Fe transporter homologues were identified in proteome datasets of 21 plant species by homology search. Based on the physio-chemical analysis of 54 proteins, 3 proteins *viz.,* Aco007552.1, Bradi3g01497.1.p and Brara.C03653.1.p were found to be unstable based on instability index which more than 40 which were excluded from further analysis. All identified Fe homologues contained oligopeptide transporter (OPT) domain proteins (Fig.1). Plant Oligopeptide Transporters (OPTs) comprise a small gene family (PF03169) which plays an important role in the mobilization of small peptides and secondary amino acids that can compound with metals. They were also reported to transport Fe in arabidopsis and oryza. In the present study, the putative Fe transporters contains 555 to 770 amino acids with 61 to 84.8 kDa molecular weight and pI of 8.2 to 9.4 with 9-15 transmembrane domain with an average of 13 TMDs (Table.1). The topological variations in TMDs may have arisen due to broad range activities of these family members. Earlier studies found that, OsYSL15 gene encodes protein of 672 amino acids and 16 transmembrane domains (TMs) with the N terminus located in the cytoplasm [11]. In another study, the analysis of the nine YSL family members using TMAP showed presence of 15 transmembrane domains [29,30]. The bioinformatic analysis of OsOPT proteins showed 12-16 transmembrane helices with molecular weights that ranging from 50 to 90 KDa. In another study, OPT gene analogues in Vitis and Populus ranged from 372 to 760 amino acids in length, with predicted pI ranging from 5.45 to 9.44 and polypeptides had 8-16 transmembrane helices and localized in plasma membrane. Our results were in accordance with the previously reported features of YSL family members and also OPT gene family which predicts the reliability of the newly identified Fe transporters.

All the new identified protein demonstrated a basic characteristic with a pI > 8. The compounds with pI > 7 are basic, and those with pI < 7 are acidic. The subcellular localizations of Fe transporter homologues were predicted as plasma membrane. The results were in accordance with the previous functionally characterized OsYSL15 transporters proteins which were found to be localized in Plasma membrane [31,32]. Therefore, we state that topological features and physicochemical properties of the newly identified putative Fe transporter are in accordnce with the general features of Fe transporters mentioned in literatures. Overall, we may report that in feature studies regarding the identification of Fe transporters in more plant species, OPT transporter family (cl14607) domain, 9 to 15 TMDs and basic nature of Fe transporters could be used in their characterization. Hence, overall the bioinformatic tools can be used to find the Fe transporter homologues in different plant genomes. Even then, wet-lab studies are needed to confirm the Fe transport activities of these identified proteins.

3.3 Conserved Motif Analysis

We have searched the most conserved five motif sequences in Fe transporter proteins using MEME tool (Fig.2). Motif 1(VSFVGLFSVVPLRKIMIIDYKLTYPSGTATAHLINSFHTPQGAKLAKKQV),Motif 2 (FQWFYTAGDGCGFSQFPTFGLKAYENRFYFDFSATYVGVGMICPHIVNIS) and Motif 3 (CGVMMNIVSTASDLMQDFKTGYLTLTSPRSMFVSQVIGTAMGCVISPCVF) were 50 amino acid residue long and shared by all the plant species. While, motif 4 (AYIFAPVLAFCNAYGCGLTDWNMASNYGKLAIFTIG) and Motif 5 (YDDQRRTEVFLKDQIPTWFAYAGYVAIAAISTITIPHMFHQ) were 36 and 41 amino acid sequences respectively. These motifs were also shared by all the plant species. The conserved domain analysis using NCBI CD search showed that all the consensus sequences belongs to OPT protein family (Fig.2). Overall, these sequences are highly conserved in all analyzed species; therefore, they could be used in characterization of Fe transporters in different plant species.

3.4 Predicted Interaction Partner Analysis

The protein functions in a can be known by Protein protein interactions (PPIs). The function of newly identified proteins can be predicted on the evidence of their interaction with a protein, whose function is already revealed. It was proved that more than 80% of proteins form complex to yield biological function to the proteins [33].

Moreover, detailed study of PPIs will help in modeling of functional pathways to understand the molecular mechanisms of cellular processes. With this view, to further characterize the potential interaction partners of the putative Fe transporters, we did ineractome analysis using STRING server. Protein sequence from *A. thaliana* (AT1G65730.1) (Fig. 3A) and *O. sativa* (OsYSL15) (Fig. 3B) ws used as representatives of dicots and monocots, respectively for interactome study. The analysis showed that, ten putative interactors *viz.,* OPT6, OPT1, OPT3, TOC34, TOC33, OPT8, OPT5, OPT9, OPT4 and NRMP3 proteins with medium to high confidence score were predicted for *A. thaliana* AT1G65730.1 protein. The AtOPT3, a member of the OPT family was found to be involved in Fe transport in arbidopsis and it is mainly up regulated under Fe deficiency conditions [34]. Further, opt3 knock down mutant exhibit a defect in Fe loading in seeds, showing it as a candidate for Fe biofortification [34]. With this, AtOPT3 plays a critical role in iron metabolism and accumuliton in seeds [34]. Another gene, OsOPT7 whose expression is 60-fold higher in shoots and roots of plants when grown under iron deficiency [35]. These results indicate that OsOPT7 also plays an important role in Fe transport in rice. Similarly, OsOPT1, OsOPT3, OsOPT4, OsOPT5, and OsOPT7 were found to transport ferrous and/or ferric iron chelated to nicotianamine which helps in Fe transport and homeostasis [36]. The functionally characterized gene, AtNRAMP3 expression is up regulated by iron deficiency and mediates in long□distance transport The loss of function of AtNRAMP3 gene led to increased accumulation of manganese and zinc in the roots, indicating its role in multiple ion transport. AtNRAMP3 and AtNRAMP4 transporters are responsible accumulation of Fe in cytosol during germination [18]. OPTs are most likely involved in Glutathione (GSH) transport and play direct as well as indirect roles in many GSH mediated processes, metal homeostasis [15]. Glutathione plays key role in mitigating biotic and abiotic stress by mediating redox potential of the cell. This GSH preserve cell redox homeostasis and improve internal iron availability. The present analysis revealed that all the proteins were predicted to interact with AT1G65730.1 protein directly or indirectly for iron transport and accumulation.

In *O. sativa*, NAS2, NAS1, DMAS1, OsJ_32857, IRT1, IRO2, IDEF1, IRT2, IDI2, OS04To444800-02 were found to be putative interaction partners. Under Fe deficiency situations, mediates uptake of Co, Cd, Mn and Zn. The IRT2 transporter which is highly similar to IRT1 and mainly capable of Fe and Zn transport in roots of *A. thaliana* under Fe-limited conditions. Similar to IRT1, the IRT2 also involves in transportation of Zn, Mn and Cd. Phytosiderophores are formed by the trimerization of S-adenosylmethionine to form one molecule of nicotianamine (NA) by Nicotianamine synthase (NAS). NA is a biosynthetic precursor of phytosiderophores and is thus a crucial component for iron (Fe) acquisition. Overexpression of HvNAS1has shown to incresse Fe and Zn up to three and two fold, respectively, in rice grains [37]. Likewise, OsNAS1, OsNAS2, and OsNAS3 were differentially regulated by Fe level and found to be involved in long distance transport of Fe. The knock-down of OsDMAS1 significantly up-regulates the genes involved in Fe uptake and homeostasis.

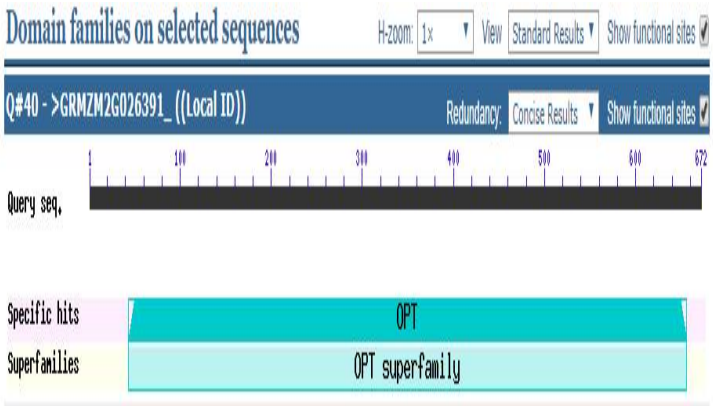

Fig. 1. The picture showing identification of OPT domain in GRMZM2G026391 protein sequence

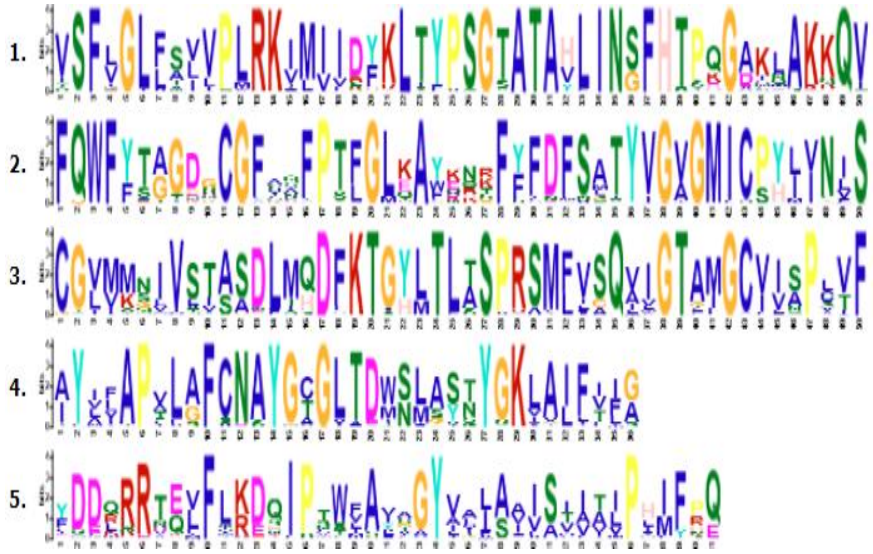

Fig. 2. Sequence logo of the five most conserved motifs of Fe transporter proteins in 21 plant species

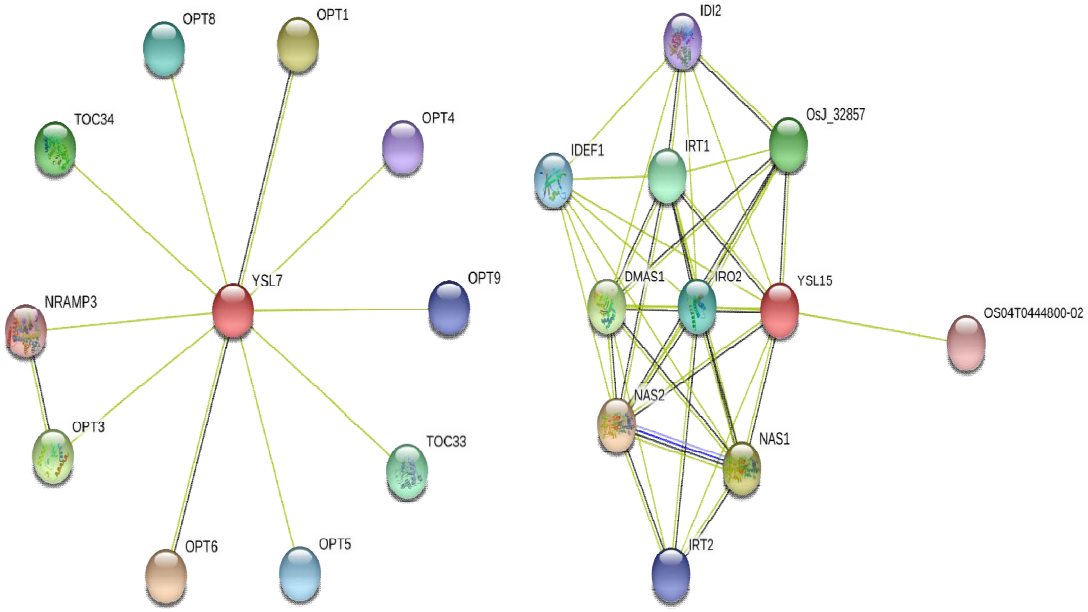

Fig. 3. Interaction partner analysis of Fe transporters of *A. thaliana* (AT1G65730.1) (A) and *O. sativa* (OsYSL15) (B) which were selected as representatives of dicots and monocots respectively. 10 potential interactors of both species were visualized. Stronger associations are represented by thicker lines

The gene OsIRO2, performs a crucial function of Fe uptake from soil and Fe translocation to grain during seed maturation [38]. OsIRO2 gene is induced under Fe starvation and is positively regulated by IDEF1. OsIRO2 also positively regulates various genes related to Strategy II, IDE1 and IDE2 [39]. The two rice transcription factors IDEF1 (IDE-binding factor 1) and IDEF2, which bind specifically to IDE1 and IDE2, respectively which regulate iron homeostasis related genes in rice during Fe deficiency.

Table 1. List of putative Fe transporters in 21 plant species and their gene and protein features

Sl. no	Gene ID (Phytozome)	Conserved protein domain family	sub cellular localization	Exon number	Protein length	MW (kDa)	pI	Instability index	TMD
1	Aco010337.1	OPT	Plasma Membrane	6	711	77.5	8.49	39.05	14
2	Aco010643.1	OPT	Plasma Membrane	7	682	74.6	8.75	36.06	15
3	AT1G65730.1	OPT	Plasma Membrane	4	688	75.7	9.17	33.7	14
4	Bol022433	OPT	Plasma Membrane	6	666	73.3	9.13	29.8	14
5	Bradi2g53950.2.p	OPT	Plasma Membrane	13	679	73.7	9	31	12
6	Bradi3g01520.1.p	OPT	Plasma Membrane	2	676	74.3	9	35	12
7	Bradi3g50260.1.p	OPT	Plasma Membrane	7	676	73.7	9.26	31.9	12
8	Bradi3g50263.1.p	OPT	Plasma Membrane	7	682	74.9	9	35.8	14
9	Bradi3g50267.2.p	OPT	Plasma Membrane	7	770	84.8	9.4	35	14
10	Bradi5g16190.1.p	OPT	Plasma Membrane	6	707	76.7	8.5	34.5	12
11	Bradi5g17220.2.p	OPT	Plasma Membrane	9	664	72.0	8.2	31	13
12	Bradi5g17230.2.p	OPT	Plasma Membrane	23	704	76.3	9.2	31.8	14
13	Bradi5g25987.1.p	OPT	Plasma Membrane	6	687	74.9	8.77	33.9	14
14	Brara.F02656.1.p	OPT	Plasma Membrane	6	666	73.2	9	29.4	14
15	Cagra.2460s0005.1.p	OPT	Plasma Membrane	8	672	73.6	9	33	14
16	Ciclev10030844m	OPT	Plasma Membrane	6	704	77.5	8.89	38.9	14
17	DCAR_030390	OPT	Plasma Membrane	7	714	78.6	8.3	32.8	14
18	Eucgr.K02316.2.p	OPT	Plasma Membrane		555	61.1	9	31	9
19	Eucgr.K02318.1.p	OPT	Plasma Membrane	8	707	78.4	9	32.5	14
20	Eucgr.K02319.1.p	OPT	Plasma Membrane	13	714	78.2	9	33.6	12
21	evm.model.supercontig_108.9	OPT	Plasma Membrane	6	693	76.0	8.9	31	14
22	evm.model.supercontig_3.127	OPT	Plasma Membrane	7	678	74.4	9.2	30.1	12
23	Glyma.09G164500.1.p	OPT	Plasma Membrane	6	703	77.6	9	29.5	14
24	Glyma.16G212900.1.p	OPT	Plasma Membrane	6	702	77.6	8.9	29.4	14
25	Gorai.004G262100.1	OPT	Plasma Membrane	6	697	76.8	8.7	30.3	14
26	Gorai.013G070800.2	OPT	Plasma Membrane	12	559	61.4	9	36.4	11
27	GRMZM2G004440_P01	OPT	Plasma Membrane	6	679	73.8	9	33.9	13
28	GRMZM2G018148_P01	OPT	Plasma Membrane	3	684	75.2	8.9	37.3	12
29	GRMZM2G026391_P01	OPT	Plasma Membrane	15	672	72.9	9	29.6	14

30	Lus10013323	OPT	Plasma Membrane	7	725	79.5	8.6	39.43	12
31	Lus10034177	OPT	Plasma Membrane	6	717	78.8	8.72	34.5	12
32	Lus10043408	OPT	Plasma Membrane	6	717	78.8	8.8	33.7	14
33	Manes.04G061100.1.p	OPT	Plasma Membrane	6	687	75.4	8.8	32.4	12
34	Manes.05G182000.1.p	OPT	Plasma Membrane	6	714	79.3	8.5	32.4	12
35	Manes.05G192800.1.p	OPT	Plasma Membrane	8	664	72.7	9	30.3	14
36	Medtr3g092090.2	OPT	Plasma Membrane	16	664	72.8	8.7	32.1	14
37	Medtr5g091600.1	OPT	Plasma Membrane	5	682	74.4	8.9	31.6	12
38	Phvul.004G138900.1.p	OPT	Plasma Membrane	6	701	77.2	8.84	28.5	12
39	Potri.017G150500.2	OPT	Plasma Membrane	17	680	74.8	8.59	30	12
40	Potri.017G151200.1	OPT	Plasma Membrane	6	698	76.9	8.9	30.39	12
41	Potri.017G151800.1	OPT	Plasma Membrane	6	698	76.8	8.8	28.5	12
42	Seita.1G118000.1.p	OPT	Plasma Membrane	6	678	74.4	9.25	38.8	14
43	Seita.1G118200.1.p	OPT	Plasma Membrane	2	686	75.3	8.95	36.5	12
44	Seita.1G254100.1.p	OPT	Plasma Membrane	7	687	74.4	8.9	31.6	14
45	Seita.1G254200.1.p	OPT	Plasma Membrane	7	623	67.3	8.8	36.4	13
46	Sobic.003G343300.1.p	OPT	Plasma Membrane	6	680	74.1	9.27	33	12
47	Sobic.004G011800.1.p	OPT	Plasma Membrane	4	685	75.7	8.7	38.7	15
48	Sobic.004G011900.1.p	OPT	Plasma Membrane	4	684	75.0	8.9	37.6	11
49	Sobic.006G164300.2.p	OPT	Plasma Membrane	15	677	73.6	9.1	27.76	14
50	Solyc03g031920.2.1	OPT	Plasma Membrane	6	696	76.8	9.25	32.2	14
51	Solyc02g081570.2.1	OPT	Plasma Membrane	6	694	76.2	8.9	36.1	12

4. CONCLUSION

The present study is focused on identification of homologues of OsYSL2, OsYSL15 and OsYSL18 Fe transporters in 21 different plant species. The study found a total of 51 putative Fe transporter proteins which could be characterized with 555 to770 amino acids sequence length with 61 to 84.8 kDa molecular weight and pI of 8.2 to 9.4 having basic nature with 9 to15 TMD with an average of 13 TMDs having oligopeptide transporter (OPT) domain and localized in plasma membrane. Therefore, Identification of putative Fe transporter homologues in various plant species and their analysis at different structural levels has led us to better understand the Fe transporters. Hence, the results of this study will become essential theoretical basis to further elucidate Fe transporters in different plants, as well as they will make significant contributions to the future studies for developing genetically engineered and biofortified plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ravet K, Touraine B, Boucherez J, Briat JF, Gaymard F, Cellier F. Ferritins control interaction between iorn homeostasis and oxidative. The Plant Journal. 2009;57(3): 400-412.
2. Briat JF, Lobr´eaux S. Iron transport and storage in plants. Trends Plant Sci. 1997;2:187-193.
3. Halliwell B, Gutteridge JM. Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. Arch Biochem Biophys. 1986;246(2):501–514.
4. Briat JF, Fobis-Loisy I, Grignon N, Lobr´eaux S, Pascal N, Savino G, et al. Cellular and molecular aspects of iron metabolism in plants. Biology of the Cell. 1995;84:69-81.
5. Kobayashi T, Nishizawa NK. Iron uptake, translocation and regulation in higher plants. Annu Rev Plant Biol. 2012;63:131-152.
6. Vert G, Grotz N, Dédaldéchamp F, Gaymard F, Guerinot ML, Briat JF, Curie C. An Arabidopsis transporter essential for iron uptake from the soil and for plant growth. Plant Cell Online. 2002;14:1223-1233.
7. Romheld V, Marschner H. Evidence for a specific uptake system for iron phytosiderophore in roots of grasses. Plant Physiol. 1986;80:175-180.
8. Bashir K, Nishizawa NK. Deoxymugineic acid synthase; a gene important for Fe-acquisition and homeostasis. Plant Signal Behavior. 2006;1(6):290–2.
9. Ma JF, Taketa S, Chang YC, Takeda K, Matsumoto H. Biosynthesis of phytosiderophores in several Triticeae species with different genomes. J Exp. Bot.1999;50:723–726.
10. Takahashi M, Yamaguchi H, Nakanishi H, Shioiri T, Nishizawa NK, Mori S. Cloning two genes for nicotianamine aminotransferase, a critical enzyme in iron acquisition (Strategy II) in graminaceous plants. Plant Physiol. 1999;121:947–956.
11. Inoue H, Kobayashi T, Nozoye T, Takahashi M, Kakei Y et al. Rice OsYSL15 is an iron-regulated iron(III)-deoxymugineic acid transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings. J Biol. Chem. 2009;284: 3470–79.
12. Thomine S, Vert G. Iron transport in plants: better be safe than sorry. Curr Opin Plant Biol. 2013;16:322-327.
13. Koike S, Inoue H, Mizuno D, Takahashi M, Nakanishi H et al. OsYSL2 is a rice metalnicotianamine transporter that is regulated by iron and expressed in the phloem Plant J. 2004;39:415–424.
14. Ishimaru Y, Masuda H, Bashir K, Inoue H, Tsukamoto T et al., Rice metal-nicotianamine transporter, OsYSL2, is required for the long-distance transport of iron and manganese. Plant J. 2009;62:379–390.
15. Lubkowitz M. The oligopeptide transporters: A small gene family with a diverse group of substrates and Functions. Molecular Plant. 2011;4(3):407–415.
16. Yen MR, Tseng YH, Saier Jr MH. Maize yellow stripe1, an ironphytosiderophore uptake transporter is a member of the oligopeptide transporter (OPT) family. Microbiology. 2001;147:2881–2883.
17. Guerinot ML. The ZIP family of metal transporters. Biochim Biophys Acta. 2000;1465(1-2):190–198.
18. Lanquar V, Lelièvre F, Bolte S, Hames C, Alcon C, Neumann D, et al. Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron. EMBO. 2005;24:4041–4051.

19. Thomine S, Leli`evre F, Debarbieux E, Schroeder JI, Barbier-Brygoo H. AtNRAMP3, a multispecific vacuolar metal transporter involved in plant responses to iron deficiency. Plant J. 2003;34:685–95.
20. Romiti M, Entrez nucleotide and entrez protein FAQs. Gene. 2010;1(270)
21. Goodstein D, Shu S, Howson R, Neupane R, Hayes RD, Fazo J et al. Phytozome: A comparative platform for green plant genomics. Nucleic Acids Research. 2012;40:D1178–D1186.
22. Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD et al. identification and analysis tools on the ExPASy server. The proteomics protocols handbook. Humana, Louisville. 2005;571–607.
23. Yu CS, Chen YC, Lu CH, Hwang JK. Prediction of protein subcellular localization. Proteins. 2006;64:643–651.
24. Shennan Lu et al., CDD/SPARCLE: The conserved domain database in 2020, Nucleic Acids Res. 2020;48(D1):265-268.
25. Krogh A, Larsson B, von Heijne G, Sonnhammer ELL. Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. J Mol Biol. 2001;305:567-580
26. Timothy L, Mikael B, Buske FA, Frith M, Grant CE, Clementi L et al. Meme suite: Tools for motif discovery and searching. Nucleic Acids Res. 2009;37:202–208.
27. Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. Cytoscape 2.8: New features for data integration and network visualization. Bioinformatics. 2011;27:431–432.
28. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A et al. STRING v9. 1: Protein-protein interaction networks with increased coverage and integration. Nucleic Acids. 2013;41.
29. Persson B, Argos P. Prediction of transmembrane segments in proteins utilising multiple sequence alignments. J Mol Biol. 1994;237:182– 192.
30. Persson B, Argos P. Topology prediction of membrane proteins. Protein Sci. 1996;5:363– 371.
31. Lee S, Chiecko JC, Kim S, Walker EL, Lee Y, Guerinot ML. Disruption of OsYSL15 Leads to Iron Inefficiency in Rice Plants. Plant Physiol. 2009;150:786.
32. Shanmugam V, Jing-Chi Lo, Kuo-Chen Yeh. Control of Zn uptake in Arabidopsis halleri: a balance between Zn and Fe. Front Plant Sci. 2013;4:281.
33. Yokosho K, Yamaji N, Ma JF. OsFRDL1 expressed in nodes is required for distribution of iron to grains in rice. J Exp. Bot. 2016;67(18):5485–5494.
34. Stacey MG, Patel A, McClain WE, Mathieu M, Remley M, Rogers EE et al. The arabidopsis AtOPT3 protein functions in metal homeostasis and movement of iron to developing seed. Plant Physiol. 2008;146:589-601.
35. Zheng L, Huang F, Narsai R et al., Physiological and transcriptome analysis of iron and phosphorus interaction in rice seedlings. Plant Physiol. 2009;151:262-27.
36. Vasconcelos MW, Li GW, Lubkowitz MA, Grusak MA. Characterization of the PT clade of oligopeptide transporters in rice. Plant Genome. 2008;1:77-88.
37. Masuda H, Usuda K, Kobayashi T, Ishimaru Y, Kakei Y et al., Overexpression of the barley nicotianamine synthase gene HvNAS1 increases iron and zinc concentrations in rice grains. Rice. 2009;2:155–166.
38. Ogo Y, Itai RN, Kobayashi T, Aung MS, Nakanishi H, Nishizawa NK. OsIRO2 is responsible for iron utilization in rice and improves growth and yield in calcareous soil. Plant Mol Biol. 2011;75:593–605.
39. Kobayashi T, Nakayama Y, Itai RN, Nakanishi H, Yoshihara T et al. Identification of novel cisacting elements, IDE1 and IDE2 of the barley IDS2 gene promoter conferring iron deficiency-inducible, root-specific expression in heterogeneous tobacco plants. Plant J. 2003;36:780–793.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62826