Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2023.637.650

Research Article
Genome-Wide Systematic Characterization of the Yellow Stripe-Like Gene Family and Their Expression Profile in Rice

[1]Le Ngoc Diep, [1]Do Minh An, [1]Le Tran Tuyet Mai, [1]Le Hong Diep, [1]Nguyen Quang Huy, [1]Do Thi Phuc, [1]Ha Minh Ngoc, [1]Hoang Hai Yen, [2]Chu Duc Ha and [1]Le Quynh Mai

[1]Department of Biology, Faculty of Science, Vietnam National University, Thanh Xuan, Hanoi 100000, Vietnam
[2]Department of Agricultural Technology, Faculty of Engineering and Technology, Vietnam National University, Cau Giay, Hanoi 122300, Vietnam

Abstract

Background and Objective: The yellow stripe-like (YSL) proteins have certain roles in metal transportation and nutrient remobilization in storage tissue in plants. The objective of this current study was to comprehensively identify, annotate and characterize the YSL family in rice. **Materials and Methods:** In this study, a total of 18 members of the *OsYSL* gene family have been comprehensively identified in rice by various bioinformatics approaches. Particularly, the gene organization and chromosomal distribution of each member of the *OsYSL* gene family have been analyzed. Next, the physic-chemical properties and subcellular localization of the OsYSL proteins were estimated. The rice assembly, including genome, proteome and transcriptome databases was explored by various web-based tools and software. **Results:** The phylogenetic analysis indicated that the OsYSL family could be categorized into three distinct clades. The predicted *cis*-acting elements of 1.5 kb upstream regions of *OsYSL* genes showed that besides many promoter-related cis-elements (422), genes had many stress responsive cis-elements (127) and also many phytohormone regulation *cis*-elements (117). The expression profile of the *OsYSL* gene family indicated the variation in different developmental stages and demonstrated the function of these genes in Fe transport and response to environmental conditions. **Conclusion:** The analysis of expression and *cis*-elements in the promoter region of *OsYSL* genes could provide broad information for further functional characterization of *OsYSL* genes.

Key words: Yellow stripe-like, rice, bioinformatics, phylogenetic, *cis*-acting elements, expression profile

Citation: Diep, L.N., D.M. An, L.T.T. Mai, L.H. Diep and N.Q. Huy *et al*., 2023. Genome-wide systematic characterization of the yellow stripe-like gene family and their expression profile in rice. Asian J. Plant Sci., 22: 637-650.

Corresponding Author: Le Quynh Mai, Department of Biology, Faculty of Science, Vietnam National University, Thanh Xuan, Hanoi 100000, Vietnam

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Iron, an essential micronutrient, has been well-characterized to play a pivotal role in plant growth and development. As a micronutrient, iron is integral to various biological processes, like chlorophyll synthesis, respiration, photosynthesis and DNA synthesis[1-3]. Particularly, the appropriate concentration of iron contributes to the rich green hue of plants, an outcome linked to its function in generating chlorophyll, the pigment underpinning photosynthesis[4]. However, maintaining an equilibrium of iron levels is crucial, as both deficiency and excess can have detrimental impacts on plant health. Iron deficiency, often seen in soils with higher pH levels, can cause chlorosis, a condition characterized by yellow leaves with green veins due to hindered chlorophyll synthesis[2,4]. Conversely, excessive iron can lead to toxicity, manifesting as brown or bronze leaf spots and potentially disrupt the uptake of other essential nutrients[5]. Therefore, achieving a balanced iron concentration is a prerequisite for optimal plant growth and overall health.

Principally, plants maintain iron homeostasis primarily through two major strategies of iron uptake[6,7]. High concentrations of iron are concentrated in the rhizosphere but present in the oxidized form of iron(III) leading to low solubility[6]. The main difference between these two iron absorption strategies is the reduction of iron(III) to iron(II). Particularly, ATPase pumps H^+ to increase the acidity of the rhizosphere region to increase the solubility of iron(III), then Fe^{3+} is reduced to Fe^{2+} through the action of ferric reduction oxidase 2 and then Fe^{2+} is transported into the roots via iron-regulated transporter 1[8,9]. Another strategy is present in graminaceous plants, which have the ability to synthesize phytosiderophores in the roots and secrete the rhizosphere[10,11].

The *Yellow stripe-like* (YSL) proteins are oligopeptide transporters which have been considered as membrane-bound transporters[12]. Members of the YSL family were demonstrated to play a significant role in iron homeostasis in plants via long-distance transport of metal-nicotinamide. Briefly, the study of Murata *et al.*[13] predicted that, ZmYS1 and HvYS1 have 12 transmembrane domains, while the study of DiDonato *et al.*[12] and some previous studies using TMAP algorithm[14,15] predicted that YSL protein has 15 transmembrane domains. The YSL protein family is predicted to be divided into 4 subfamilies, in which subfamily I including ZmYS1, OsYSL15 and HvYS1 is involved in the uptake of iron from the soil by the Fe^{3+} -PS transport pathway. Subfamily II includes AtYSL4 and AtYSL6 residing in the tonoplast membrane and chloroplast outer membrane, along with OsYSL6 in the cytoplasm responsible for transporting metal chelates in the cell[16]. The ZmYS1 was first isolated from the maize genome, with the function of transporting the Fe(III)-PS complex from the outside to the roots. The maize ys1 mutant has reported an iron deficiency in the mutant form due to the inability to transport Fe^{3+} -PS. Under iron deficiency conditions, *ZmYS1* gene expression was found to be increased in roots and stems[16,17]. The ZmYS1 is found in the epidermal cells and mesophyll cells of the roots, which are involved in the function of intracellular iron absorption and transport. The ZmYS1 has been studied to participate in the transport of Fe(III)-DMA, Fe(III)-MA, Fe(II)-NA, Zn-DMA, Ni-NA and Cu-MA[18,19]. Although closely involved in the transport of metal chelates, proteins of the YS family are still expressed in non-graminaceous plants, which are incapable of synthesizing and utilizing PS. The YSL protein is required for long-distance transport of metals and NA-coupled chelates (e.g., zinc, copper, manganese, nickel, iron(II)...)[16]. In *Arabidopsis thaliana*, the YSL protein family is predicted to have eight genes involved in coding and the AtYSL proteins are involved in the transport of the Fe-NA or Fe-citrate complex[10]. Under iron-deficient conditions, the expression of *AtYSL1*, *AtYSL2* and *AtYSL3* genes was noted to be reduced[12,20,21]. The presence of AtYSL1 increased in the xylem parenchyma of leaves, pollen grains and siliques, while AtYSL3 was highest in senescent rosette leaves and cauline leaves[20,22]. The individual mutants *ysl1* and *ysl3* did not produce obvious symptoms, however, the co-mutant *ysl1ysl3* produced mutants with markedly reduced iron content in leaves, but concentrations of other metals Mn, Zn and Cu increased[20].

Rice (*Oryza sativa*) is one of the four main food sources for the people and promises to be one of the promising sources of micro minerals, specifically iron, to replace functional foods. Understanding the mechanisms of iron absorption, distribution and storage in rice is a prerequisite for studies to enhance iron content in rice grains. In this study, *in silico* methods were used to analyze and compare the genes encoding the YSL protein family in rice and *Arabidopsis thaliana*, thereby predicting the function and relationship of these iron transport proteins.

MATERIALS AND METHODS

Study area: The study was started from October, 2022 to August, 2023 in Plant Physiology Laboratory, Department of Plant Sciences, Faculty of Biology, University of Science, Vietnam National University in Hanoi, Vietnam.

***In silico* identification and structural analysis of the YSL family in rice:** The full-length protein sequence of well-characterized ZmYS1 collected from the UniProt database[23] was used as a query to search for similar sequences in rice. This sequence was aligned with the *O. sativa* v7.0 database in Phytozome v13[24] using the BlastP tool. All sequences with E-value ≤1E-10 were downloaded a set of data, like coding DNA sequences and promoter regions (1.5 kb upstream from the start codon site) as described by researchers[25-27]. Next, information on molecular weight (mW), calculated isoelectric point (pI), aliphatic index (AI) and grand average of hydropathicity (GRAVY) were determined using the ExPASY tool[28] as reported by researchers[25-27]. The subcellular localization of these proteins was predicted using the WoLF PSORT tool[29]. The conserved motifs of the OsYSL protein family were identified using MEME online tool[30]. The setting parameters for MEME include: Maximum number of search motifs, 15; minimum motif length, 6; Maximum motif length, 50. The physical distribution of the *OsYSL* genes on chromosomes was identified using giff3 files which were downloaded from the Phytozome v13 database and visualized using TBtool[31]. The TBtool was used to visualize the exon/intron structure and protein motifs of the OsYSL family[31].

Scanning *cis*-acting elements in the promoters of *YSL* genes in rice: Promoter regions of the *OsYSL* genes were used to search for *cis*-acting elements by using the PlantCARE tool[32] as described by La *et al.*[25]. Only elements located on the positive strand were collected for analysis. All data were then sorted by their function including biotic stress responses, *cis*-elements in developmental processes, *cis*-elements in phytohormone responsiveness, light-responsive *cis*-elements, promoter-related *cis*-elements and stress-responsive *cis*-elements.

Phylogenetic tree of YSL proteins in rice: All full-length protein sequences of YSL members in *O. sativa* and *A. thaliana* and several identified YSL sequences of other higher plant species in previous studies were used to construct the phylogenetic tree. These sequences were aligned using the Clustal X2 program[33]. The phylogenetic tree of related YSL families in different species was built using MEGA11[34] based on the aligned data. The Neighbor-Joining method was used with bootstraps 1000 and number of threads 3. The annotation of the phylogenetic tree was displayed using the iTOL v6 tool[35].

Expression profile analysis of *YSL* genes in rice: To further investigate the gene expression of the OsYSL family in rice, the expression profiles of 18 *OsYSL* genes in developmental stages were analyzed via microarray data (GSE6893). The comparison of the expression of this family under Fe excess condition and normal condition was also evaluated through high throughput sequencing data (GSE150103). Finally, the expression profiles of the *OsYSL* gene family in two indica cultivars that differ in terms of accumulated iron content in the seeds were collected and processed (GSE70093). The heatmaps based on log2(FPKM+1) were used to visualize the expression data built by TBTool.

RESULTS

Genome-wide survey of the *OsYSL* gene family in rice assembly: Based on the full-length sequence of well-known ZmYS1 in maize, a total of 18 homologous sequences in rice were found. The information about the *OsYSL* gene family in rice was provided in Table 1. Results showed that the gene lengths of the *OsYSL* genes ranged from 2794 to 6690 nucleotides (Table 1), while the exon/intron organizations of the *OsYSL* genes were indicated in Fig. 1. According to the annotation of the *OsYSL* genes, the position of each member of the OsYSL family on the chromosome was also determined (Fig. 2). Particularly, these 18 *OsYSL* genes were randomly distributed on chromosomes 1, 2, 4, 5 and 8 (Fig. 2). Among those, chromosome 4 contained the most members belonging to the OsYSL family (8 out of 18 genes), followed by chromosome 2 containing 5 (out of 18) genes, while the remaining three chromosomes contained only 1 or 2 (out of 18) genes (Fig. 2).

Table 1 also indicated some physic-chemical parameters of the OsYSL proteins. The length of predicted OsYSL proteins ranged from 423 to 727 amino acid residues and the mW values of these OsYSL proteins varied from 46353.56 to 78767.31 Da (Table 1). The pI scores of the OsYSL proteins were recorded to be between 4.99 (acidic) and 10.06 (base). Additionally, the GRAVY values of all members of the OsYSL proteins are positive (more than 0) (Table 1), suggesting that OsYSL proteins are polar. Our prediction showed that the OsYSL proteins are basically localized on the cell membrane.

Construction of the phylogenetic tree of the OsYSL family in rice: An unrooted phylogenetic tree was built based on the full-length amino acid sequences of 18 members of OsYSL and YSL proteins in *A. thaliana* and other higher plant species.

Table 1: Gene and protein information of OsYSL family

Gene name	LOC_ID	Transcript Name	Chromosome Name	Strand	Gene start (bp)	Gene end (bp)	Number of amino acids	Molecular weight (kDa)	Theoretical pI	Aliphatic index	Grand average of hydropathicity (GR)	Cellular localization
OsYSL1	LOC_Os01g13710	LOC_Os01g13710.1	Chr1	1	7672983	7676403	708	75242.41	8.47	90.59	0.442	Plas: 10, vacu: 2, E.R.: 2
OsYSL18	LOC_Os01g61390	LOC_Os01g61390.1	Chr1	-1	35501850	35505052	679	73819.09	9.35	97.08	0.426	Plas: 12, E.R.: 2
OsYSL7	LOC_Os02g02450	LOC_Os02g02450.1	Chr2	1	862272	865065	683	74762.2	9.21	95.39	0.511	Plas: 11, E.R.: 2, vacu: 1
OsYSL8	LOC_Os02g02460	LOC_Os02g02460.1	Chr2	-1	866007	869292	694	75632.58	9.14	97.26	0.505	Plas: 12, vacu: 1, E.R.: 1
OsYSL14	LOC_Os02g42220	LOC_Os02g42220.1	Chr2	1	25399803	25404333	727	78493.53	8.31	98.28	0.466	Plas: 6, vacu: 5, golg: 2, cyto: 1
OsYSL2	LOC_Os02g43370	LOC_Os02g43370.1	Chr2	-1	26170387	26174970	674	73342.87	9.03	95.86	0.416	Plas: 11, E.R.: 2, vacu: 1
OsYSL15	LOC_Os02g43410	LOC_Os02g43410.1	Chr2	-1	26200013	26204720	672	73149.73	9.2	99.46	0.412	Plas: 12, E.R.: 2
OsYSL6	LOC_Os04g32050	LOC_Os04g32050.1	Chr4	1	19220804	19225379	678	73321.91	7.37	102.70	0.587	Plas: 8, vacu: 3, E.R.: 3
OsYSL5	LOC_Os04g32060	LOC_Os04g32060.2	Chr4	1	19227268	19231880	423	46353.56	4.99	114.14	0.716	Plas: 10, E.R.: 3, vacu: 1
OsYSL13	LOC_Os04g44300	LOC_Os04g44300.1	Chr4	-1	26236216	26240651	724	78767.31	9.1	100.66	0.495	Plas: 9, vacu: 2, golg: 2, E.R.: 1
OsYSL12	LOC_Os04g44320	LOC_Os04g44320.1	Chr4	1	26247729	26252271	717	77691.92	8.96	98.80	0.512	Plas: 10, golg: 2, vacu: 1, E.R.: 1
OsYSL11	LOC_Os04g44330	LOC_Os04g44330.1	Chr4	1	26253371	26256915	712	77815.91	8.52	96.21	0.443	Plas: 9, vacu: 2, golg: 2, E.R.: 1
OsYSL9	LOC_Os04g45860	LOC_Os04g45860.1	Chr4	-1	27154339	27158799	657	70953.18	8.09	98.34	0.519	Plas: 9, vacu: 2, golg: 2, cyto: 1
OsYSL16	LOC_Os04g45900	LOC_Os04g45900.1	Chr4	-1	27183872	27187869	675	73807.79	9.3	99.72	0.441	Plas: 12, vacu: 1, E.R.: 1
OsYSL10	LOC_Os04g57840	LOC_Os04g57840.1	Chr4	-1	34440270	34444076	686	74654.78	9.06	99.50	0.48	Plas: 8, E.R.: 4, golg: 2
OsYSL3	LOC_Os05g16280	LOC_Os05g16280.1	Chr5	-1	9217013	9222943	640	70104.7	8.56	97.67	0.565	Plas: 12, vacu: 1, E.R.: 1
OsYSL4	LOC_Os05g16290	LOC_Os05g16290.1	Chr5	-1	9223732	9229546	686	75099.95	8.68	106.18	0.634	Plas: 8, vacu: 3, golg: 2, E.R.: 1
OsYSL17	LOC_Os08g17830	LOC_Os08g17830.1	Chr8	1	10938444	10945133	636	68292.2	10.06	99.72	0.513	Plas: 10, E.R.: 2, vacu: 1, golg: 1

plas: Plasma membrane, Vacu: Vacuole; E.R.: Endoplasmic reticulum, Golgi: Golgi apparatus and Cyto: Cytoplast

Fig. 1(a-b): Phylogenetic and gene structure analysis of OsYSL family in rice, (a) Conserved motifs of the OsYSL family and (b) Gene structures of *OsYSL* genes family

(a) Each motif is represented by a number in the colored box, black lines represent the non-conserved sequences and (b) Yellow box represents the exons, the black lines connecting two exons represent introns and the green box indicates untranslated regions

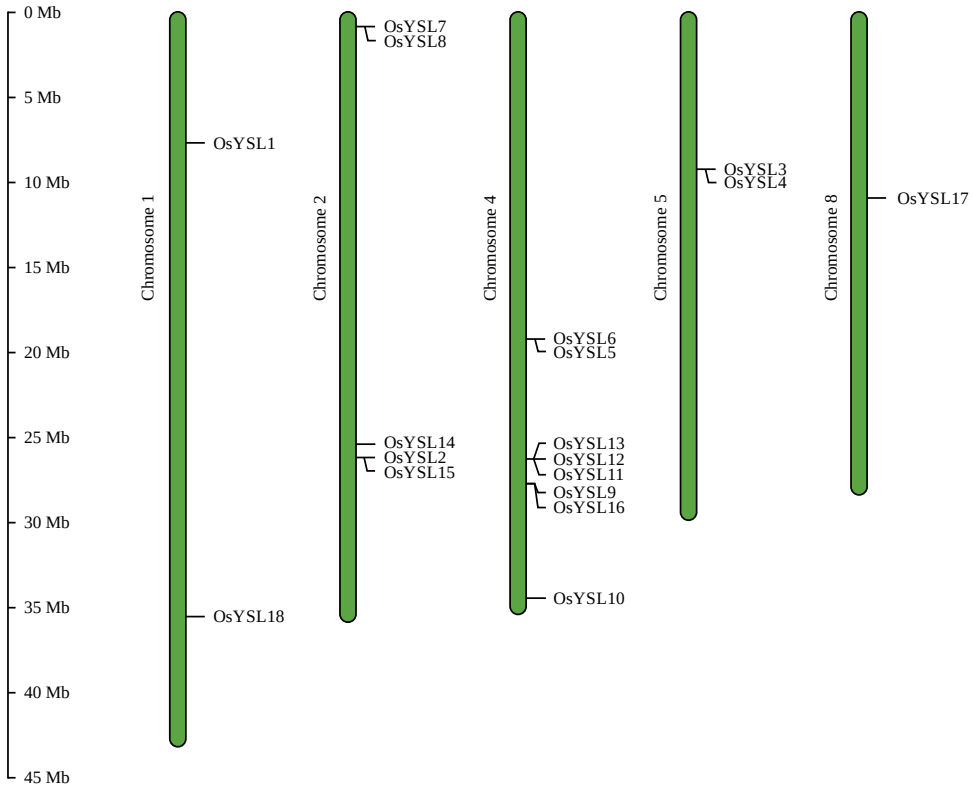

Fig. 2: Chromosome distribution of *OsYSL* gene family

Positions of the 18 *OsYSL* genes are identified in *Oryza sativa* L. genome. The chromosome number is on the left of each chromosome

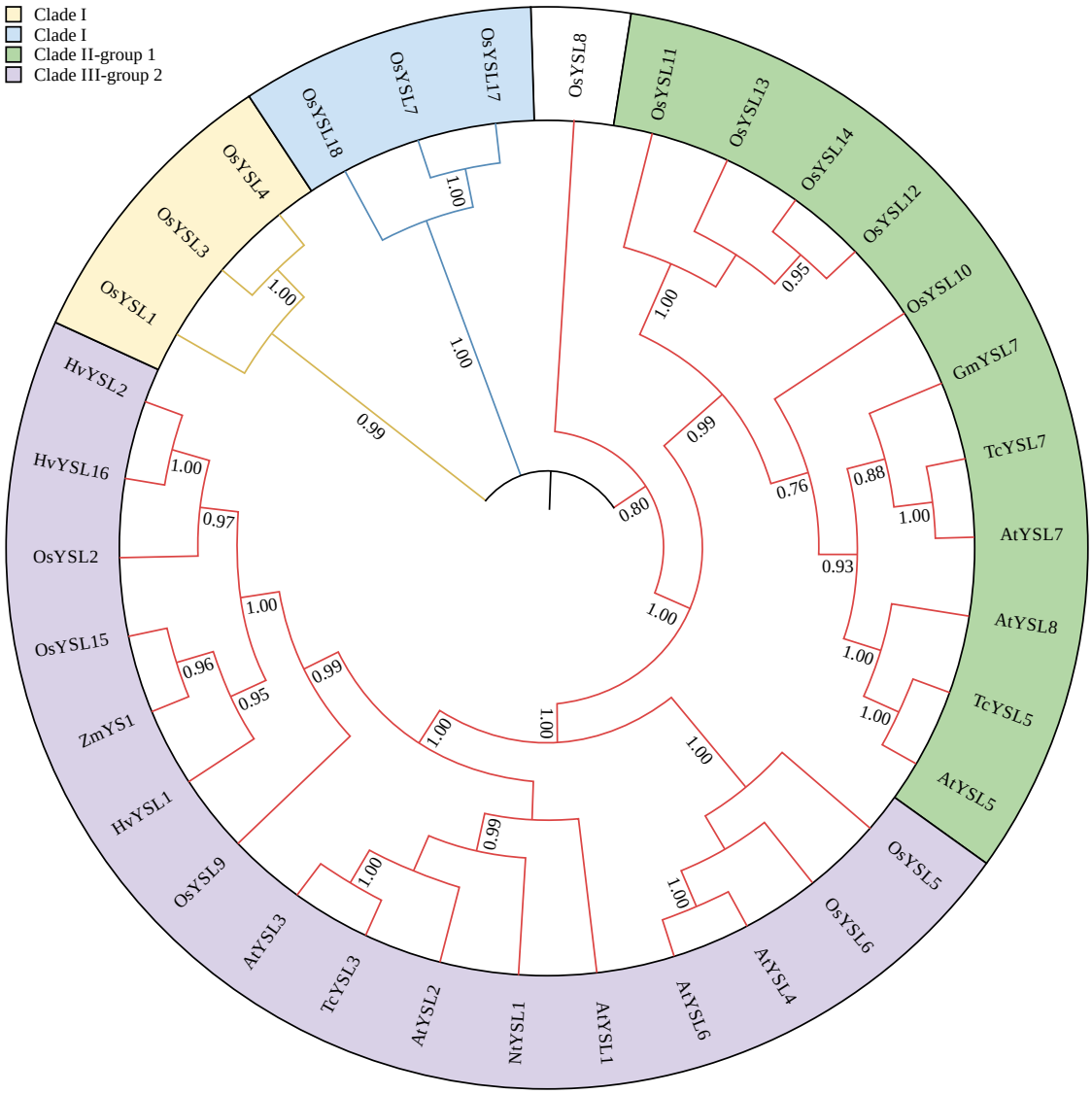

Fig. 3: Phylogenetic tree of the YSL proteins in plants

Phylogenetic tree was constructed using the neighbor-joining method with bootstraps 1000 and number of thread 3. The YSL proteins were classificated into 3 clades: Clade I, II and III. Clade III was then divided into two main groups (group 1 and 2) and an outgroup (OsYSL8)

As expected, the OsYSL family could be divided into 3 different clades, namely clade I, clade II and clade III (Fig. 3). Particularly, clade I contained three members of the OsYSL family, like OsYSL1, OsYSL3 and OsYSL4, while clade II had three members, including OsYSL7, OsYSL17 and OsYSL18 (Fig. 3). Clade III contained YSL sequences of other species divided into 2 sub-groups (sub-group 1 and 2) and an outgroup (OsYSL8) (Fig. 3). The MEME tool was used to predict the conserved motifs of OsYSL proteins and the results were shown in Table 2 and Fig. 1. Particularly, motifs 1, 2, 3, 4, 5, 7, 10, 11 and 14 are present in all members of the OsYSL family. Furthermore, members of a group tend to have the same motif structure. For example, group I is characterized by lacking motif 12 and motif 15.

Prediction of *cis*- acting elements in the promoter regions of *OsYSL* genes in rice: The predicted *cis*-acting elements in the upstream regions of *OsYSL* genes were shown in Table 3 and Table 3S (Supplementary). Briefly, a total of 25 *cis*-elements involved in biotic stress responses, 60 *cis*-elements in developmental processes, 117 *cis*-elements in phytohormone regulation, 75 light responsive *cis*-elements, 422 promoter-related *cis*-element and 127 stress responsive *cis*-elements were recorded in the 1.5 kb upstream regions

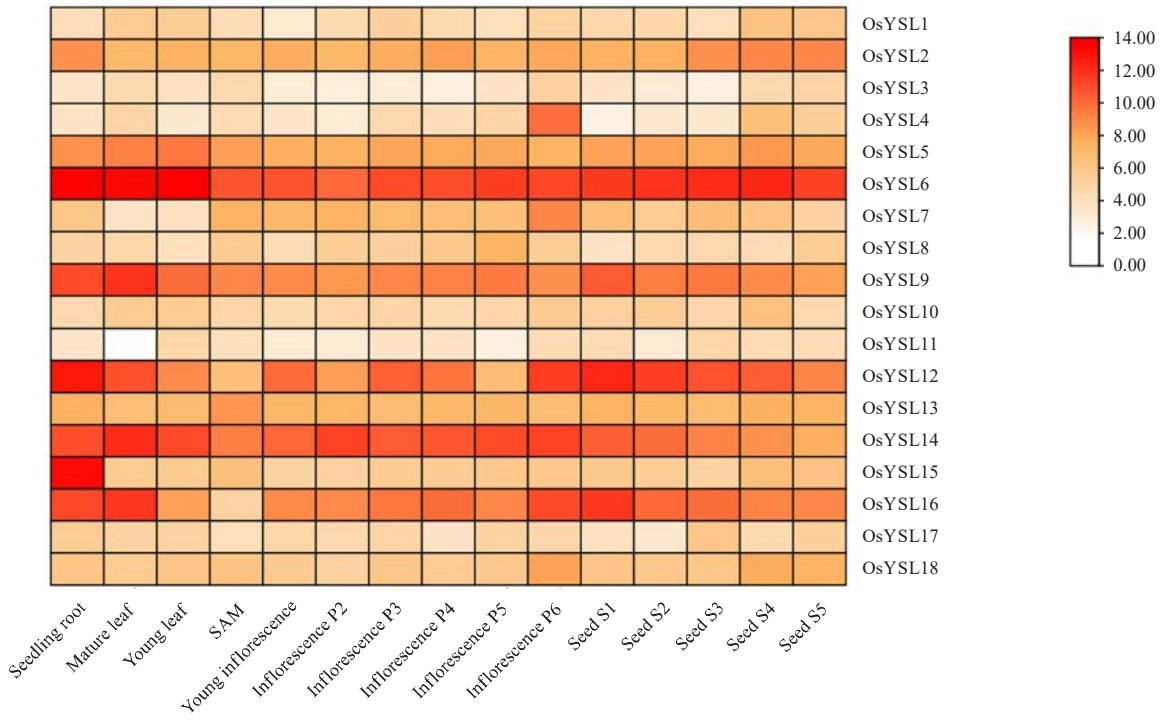

Fig. 4: Expression profiling of OsYSL family for reproductive development in rice

SAM: Shoot apical meristem and rachis meristem, up to 0.5 mm, P1: Floral transition and floral organ development, 0-3 cm, P2 and P3: Meiotic stage, 3-10 cm, P4: Young microspore stage, 10-15 cm, P5: Vacuolated pollen stage, 15-22 cm, P6: Mature pollen stage, 22-30 cm, S1: Early globular embryo, 0-2 dap, S2: Middle and late globular embryo, 3-4 dap, S3: Embryo morphogenesis, 5-10 dap, S4: Embryo maturation, 11-20 dap and S5: Dormancy and desiccation tolerance, 21-29 dap

Table 2: Specific conserved motifs identified by MEME among OsYSL proteins in rice

Motif	E-value	Sites	Width	Consensus sequences
1	8.1E-480	18	50	L[AV]ACG[VIL][MV][MK]S[IL]VST[AS][AS]DLM[QH]D[FL]KTG[YH][LM]TL[TA]SPR[SA]M[FL][VI][SG]Q[VA]IGTA[ML]GC[VI][IV][AN]P
2	5.0E-449	18	41	VPLRK[VI]M[IV][IV]DYKLT[YF]PSG[TS]ATA[HV]LIN[SG]FHTP[QEH]GAK[LQ]AKKQV
3	1.3E-441	18	43	GD[GN]CGFS[SQ]FPT[FL]GL[EK]A[YFW]K[NHR][RT]F[YF]FDFS[PAL]TYVG[VA]GMIC[PS][HY][IL][VI]N[FVL]S
4	7.0E-444	18	50	[IM]FPQ[LV][KR][WY]Y[HY][VI][AL][VI]AY[VL][VL]APVL[AG]FCN[AS]YGTGLTD[WM][NS][LM][AS][STY][TN]YGK[IL]A[IL]F[IV]F[AG][AS]W
5	5.7E-414	18	50	WE[RK][IV][DN][KR][KA][EKR]A[AE]L[FL][AGV]PAVASGLICGDG[IL]W[TS][LF]P[SQ][SA][LIV]L[AS]LAKVKPP[IM]CMKFL[SP][RG]
6	3.3E-356	17	41	[RQ]PFTRQENTV[IV]QTC[VA][VI][AS]CY[GTS][IL]AF[SG]GGFG[ST]Y[LI][LF][GA][ML][SN][EK][KTR][IT][AY][EK][LQ]
7	1.20E-231	18	29	G[AGS][IV][IL]SWG[IF][ML]WP[LY]I[ES][KT]KKG[DS]WYPA[DN]L[PS]E[SN]S
8	2.2E-321	16	50	VP[PS]WREQ[VL]T[VA]R[AG][MF][VA][VA][SA][AFV][LV]L[GS][VI][MV]FS[VF][IV]VMKLNLTTGI[IV]P[ST]LNVSA[GA]LL[GA]FF
9	8.20E-248	15	41	[PK]APYA[LI][IV][YF]RN[MI]AI[LI]GV[ED]G[FV]S[AS]LP[KR][HY]CL[TE]LC[YAV][GIAV][FA]F[ALV][AF]A[IV][ALI]I[ND]
10	2.00E-242	18	38	S[FY]D[DE][RK]RR[NT][EQ][VL]FL[KR]DQIP[ST][WT][VL]A[VY][ASG][GA]YV[VLA]L[AS]A[IV][SA][VIT][VAGI][AT][IV]PX
11	4.20E-227	18	34	[VY]SR[YF][IV]P[LS]P[MT][AG]MA[VI]PF[YFL][VIL]G[APS]YFAIDM[CF][IV]G[ST][LV][IV][LV]F[VAL]
12	9.80E-172	15	41	[LM][HK][GS][LI]QGY[KR][VS]FI[SCA][IV][AS][LMV]I[LM]GDGL[YF]N[FL][VL]K[VI][IL][GILV]RT[AIT]K[SAG][LFV][RI]N[RM][RS][RK][KR]
13	3.00E-147	16	29	[EP][GA]ND[PA]G[NS][VIY]K[EN]P[HSG][LI]G[WR]MI[GA]FLFL[VI]SF[VI]GL[FL]
14	2.80E-143	18	21	[TG][LF][FGL]K[YS]FG[GI]SF[FL]W[SG]FFQWF[YF][TS][GA]
15	1.20E-40	14	11	[VT]FWLFYKAF[DN]I

of the *OsYSL* genes (Table 3). Interestingly, the majority of promoter-related *cis*-element is CAAT-box (205 elements) and TATA-box (186 elements) and appear in all members of the *OsYSL* gene family Table 3S.

Expression profile of OsYSL in various tissues under different conditions: The expression patterns of the *OsYSL* genes varied during the developmental stages (Fig. 4). It can be seen that *OsYSL6* was highly expressed. The *OsYSL2* expression enhanced at Seed S3, Seed S4 and Seed S5 stages. The *OsYSL4* and *OsYSL7* expression enhanced significantly at the P6 stage. The *OsYSL9* expression increased remarkably in seedling root and mature leaf as well as early embryonic stage S1. The *OsYSL14* expression was decreased at the granulation stage. The *OsYSL15* was strongly expressed only in the root. The *OsYSL16* expression was increased at the root and young leaf stages as well as at the P6 and S1 stages.

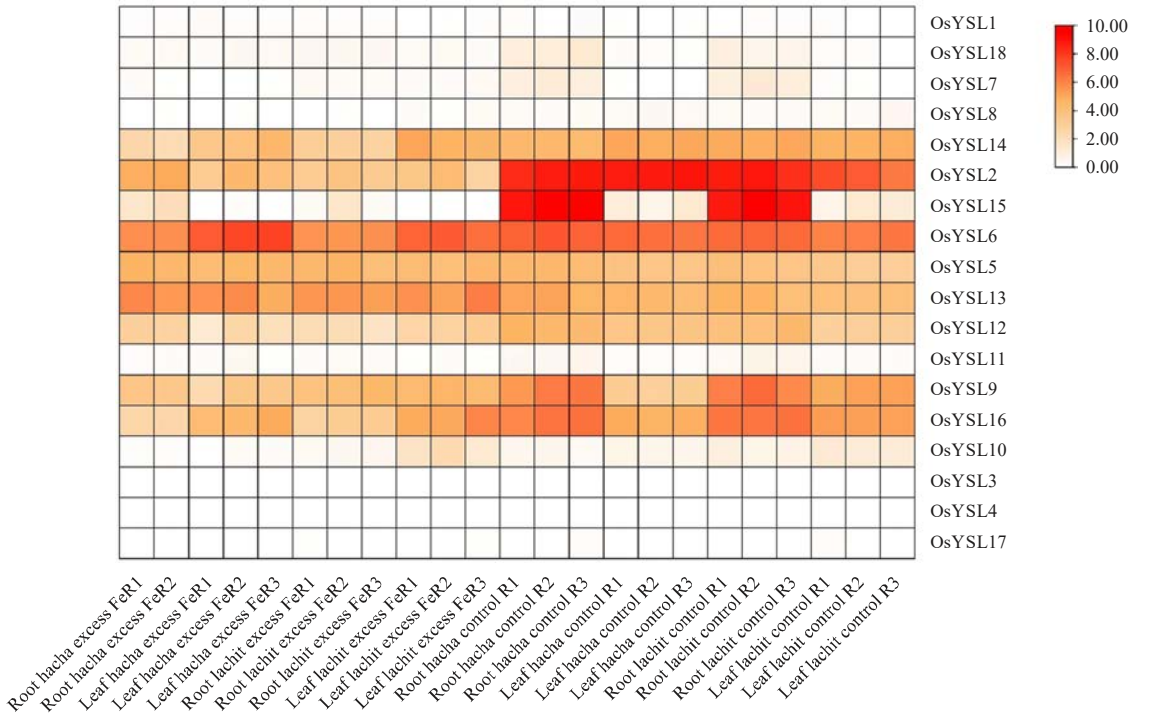

Fig. 5: Expression profiling of OsYSL family in high iron stress condition

Fe-tolerant lachit and Fe-susceptible hacha, were treated with normal (control) or excess iron concentrations (excess Fe) for 2 days and mRNA profiles were generated in triplicate

Table 3: Number of predicted *cis*-acting regulatory elements identified in the 1500 bp upstream region of OsYSL family

	cis-elements					
Gene name	Biotic stress responses	Developmental processes	Phytohormone regulation	Light responsive	Promoter-related	Stress responsive
OsYSL1	0	4	6	4	25	3
OsYSL2	1	4	3	3	21	4
OsYSL3	3	3	4	3	28	7
OsYSL4	4	4	6	4	26	5
OsYSL5	2	0	5	5	18	8
OsYSL6	3	3	15	9	23	7
OsYSL7	0	0	1	2	22	8
OsYSL8	1	4	8	5	29	5
OsYSL9	1	5	9	2	24	5
OsYSL10	0	5	18	7	7	10
OsYSL11	0	4	6	2	27	11
OsYSL12	1	5	2	4	22	5
OsYSL13	1	6	12	6	37	10
OsYSL14	1	4	4	5	36	4
OsYSL15	2	3	4	5	31	8
OsYSL16	1	2	3	2	21	12
OsYSL17	2	3	8	6	19	10
OsYSL18	2	1	3	1	6	5
Sum	25	60	117	75	422	127

Under the iron excess condition in the soil, changes in the expression of *OsYSL* genes are shown in Fig. 5. Under conditions of iron excess, there was a remarkable decrease in the *OsYSL2* expression in both roots and leaves of the two varieties. Meanwhile, the *OsYSL15* expression was reduced strongly in the roots under the condition of excess iron in the soil while in the leaves this decrease in expression was not significant.

Table 4: FPKM values of *OsYSL* genes in grain of 2 indica rice genotypes differing in grain iron concentration

Gene name	GENE_ID	FPKM_AFG1 (Sharbati_grain)	FPKM_AFG2 (Lalat_grain)	ln (fold_change)	p-value
OsYSL1	LOC_Os01g13710.1	0.0142741	0.0998689	2.80663	0.100141
OsYSL2	LOC_Os02g43370.2	10.8051	3.1684	-1.76989	0.00858291
OsYSL2	LOC_Os02g43370.1	10.8051	3.1684	-1.76989	0.00858291
OsYSL5	LOC_Os04g32060.2	0.23367	0.854914	1.87131	0.0738913
OsYSL5	LOC_Os04g32060.1	0.23367	0.854914	1.87131	0.0738913
OsYSL6	LOC_Os04g32050.1	0.826101	3.74226	2.17952	0.00724735
OsYSL6	LOC_Os04g32050.2	0.826101	3.74226	2.17952	0.00724735
OsYSL7	LOC_Os02g02450.1	0.0668346	0.392819	2.5552	0.029344
OsYSL9	LOC_Os04g45860.1	0.305884	1.28417	2.06978	0.0289065
OsYSL10	LOC_Os04g57840.1	0.0278182	0.194636	2.80667	0.0327287
OsYSL11	LOC_Os04g44330.1	0.0493292	0.258888	2.39181	0.0517487
OsYSL12	LOC_Os04g44320.1	0.0262555	0.110228	2.0698	0.128084
OsYSL13	LOC_Os04g44300.1	0.144507	0.472722	1.70986	0.120126
OsYSL13	LOC_Os04g44300.2	0.144507	0.472722	1.70986	0.120126
OsYSL14	LOC_Os02g42220.1	0.164632	0.407628	1.30801	0.22364
OsYSL16	LOC_Os04g45900.1	0.0373473	0.121958	1.7073	0.218005
OsYSL18	LOC_Os01g61390.1	0.0507579	0.142068	1.48488	0.248918

Table 5: FPKM values of *OsYSL* genes in root of 2 indica rice genotypes differing in grain iron concentration

Gene name	GENE_ID	FPKM_AFR1 (Sharbati_root)	FPKM_AFR2 (Lalat_root)	ln (fold_change)	p-value
OsYSL2	LOC_Os02g43370.2	6.34842	7.08922	0.159229	0.878416
OsYSL2	LOC_Os02g43370.1	6.34842	7.08922	0.159229	0.878416
OsYSL5	LOC_Os04g32060.2	3.52215	8.82082	1.32446	0.313439
OsYSL5	LOC_Os04g32060.1	3.52215	8.82082	1.32446	0.313439
OsYSL6	LOC_Os04g32050.1	9.84086	28.2679	1.52231	0.183547
OsYSL6	LOC_Os04g32050.2	9.84086	28.2679	1.52231	0.183547
OsYSL7	LOC_Os02g02450.1	0.430008	1.17771	1.45355	0.375683
OsYSL8	LOC_Os02g02460.1	0.802866	0.215093	-1.9002	0.311175
OsYSL9	LOC_Os04g45860.1	0.427833	3.8209	3.15879	0.0432343
OsYSL12	LOC_Os04g44320.1	47.7215	17.5755	-1.44108	0.0859457
OsYSL13	LOC_Os04g44300.1	5.73409	2.14011	-1.42188	0.331512
OsYSL13	LOC_Os04g44300.2	5.73409	2.14011	-1.42188	0.331512
OsYSL14	LOC_Os02g42220.1	2.03698	2.1344	0.0674035	0.963671

When comparing two varieties of indica rice with different grain iron content in brown rice samples, Sharbati (24.88 ppm of Fe) and Lalat (7.80 ppm of Fe) showed different expressions of genes related to the *OsYSL* gene family (Table 4 and 5). Notably, the seed samples of the high-iron variety Sharbati (FPKM = 10.8051) showed significantly higher expression of the *OsYSL2* gene compared with Lalat (FPKM = 3.1684) with a p-value approximately of 0.008. The expression of *OsYSL6* in grain was decreased in the Sharbati variety (FPKM = 0.826101) when compared with the Lalat variety (FPKM = 3.74226) (p-value = 0.00724735). In contrast, *OsYSL6* was higher expressed in Sharbati than in Lalat root samples.

DISCUSSION

The YSL proteins are known as metal transporter in plants[10,16]. Up till now, numerous members of the plant YSL families have been identified. For example, at least eight members of the YSL family were recorded in *A. thaliana*, while 19 and 67 YSL proteins have been found in *Brachypodium distachyon* and hexaploid wheat, respectively[12,36,37]. In this study, a total of 18 members of this family in rice were found that are similar to ZmYS1 in maize.

It is hypothesized that proteins in the same branch might share a similar function. For example, two similar YSL proteins, like HvYSL1 from barley and OsYSL15 from rice were previously demonstrated to act as a transporter of the Fe(III)-PS complex[38,39]. Similarly, a previous study indicated the function of HvYSL2 from barley in the transport of various metal complexes including Fe(III), Zn(II), Ni(II), Cu(II), Mn(II) or Co(II)[40]. In the subgroup with HvYSL2 from barley, OsYSL16 from rice also was shown a role in the transport of Fe(III) and Cu (II)[41,42].

The *cis*-acting elements play an important role in regulating gene expression in response to environmental conditions. As shown in Table 3S, the promoter regions of the *OsYSL* genes contained various types of *cis*-elements involved in biotic stress responses, developmental processes, phytohormone regulation, light response, promoter and stress

response, which could significantly support the function in the regulation of *OsYSL* expression of these motifs in flexible response to various conditions. The predicted *cis*-acting element data presents a large number of TATA box and CAAT box motifs, which are the binding sites of the transcription factors. Previously, the TATA box was involved in the frequencies of EST collections suggesting its function in gene expression regulation[43], while the CAAT box's function in gene expression[44,45]. Changes in CAAT box elements affect the promoter activity response to environmental factors significantly.

Expression profile analysis of the *OsYSL* gene family at different developmental stages revealed their role specific to each stage of plant development. The microarray data showed a higher expression of *OsYSL2* in the seed stage. When comparing two tropical *Indica* rice genotypes differing in grain iron concentration, the data also shows that OsYSL2 is higher expressed in Sharbati (high Fe concentration) than Lalat (low Fe concentration). This result was confirmed by a previous study[46]. Particularly, OsYSL2 functions in iron accumulation in grain[46]. The function of OsYSL2 in response to iron-deficient stress is also mentioned in this research therein *OsYSL2* expression was increased remarkably in Fe-deficient leaves[46]. Under the iron excess conditions, the decrease of the *OsYSL2* expression in both root and leaf samples was detected in our data indicating that this protein is involved in response to iron stress. In the seedling root samples, there was a strong expression of *OsYSL15*. In the analysis of OsYSL in Fe excess conditions, *OsYSL15* is down-regulated significantly in root samples but did not change their expression in leaf samples when compared with normal conditions. In the study of Inoue *et al.*[39], *OsYSL15* strongly expressed in the iron-deficiency root epidermis. So in different Fe conditions, *OsYSL15* was demonstrated having the role in the iron uptake in roots. The *OsYSL6* in the expression profile is also remarkable data. It can be seen that this gene is strongly expressed in all developmental stages in rice but the understanding of *OsYSL6* function is still unclear. Sasaki *et al.*[47] revealed the role of this gene in the detoxification of excess manganese when comparing the knockout line of OsYSL6 and the wild-type line. Also this study indicated that OsYSL6 is not involved in Fe transport in rice. Table 4 and 5 showed that, *OsYSL6* has higher expression in Lalat variety (low Fe concentration) when compared with the Sharbati variety (high Fe concentration). In root samples, *OsYSL6* was higher expressed in Sharbati (high Fe concentration) than in Lalat (low Fe concentration). More research is still needed to clarify the function of this gene in metal transport in rice.

Thus, this study analyzed the similarities and differences between 18 OsYSL transporters, compared with the YSL transporters in other plants. Thereby, they were classificated and the function of them was predicted. The analysis of *cis*-acting elements and expression of genes encoding these proteins implicated the roles of these transporters during growth and development, as well as during stress response in rice.

CONCLUSION

In this study, a comprehensive analysis of the *OsYSL* gene family in rice was performed. The structural information of the OsYSL was shown including the intron/exon distribution, the chromosomal position, the conserved motifs and the subcellular localization. The phylogenetic tree of the OsYSL family with other species YSL was constructed and classified into 3 main clades. The *cis*-acting elements of 1.5 kb upstream regions of *OsYSL* genes were also predicted and sorted by their functions including 25 *cis*-elements involved in biotic stress responses, 60 *cis*-elements in developmental processes, 117 *cis*-elements in phytohormone regulation, 75 light responsive *cis*-elements, 422 promoter-related *cis*-element and 127 stress responsive *cis*-elements. The expression of the *OsYSL* gene family varies at different developmental stages. The data of expression involved in Fe concentration also demonstrates the function of these genes in Fe transport and response to environmental conditions.

SIGNIFICANCE STATEMENT

In the study, a comprehensive analysis of the OsYSL family in rice was performed. The structural information of the OsYSL was shown including the intron/exon distribution, the chromosomal position, the conserved motifs and the subcellular localization. The phylogenetic tree of the OsYSL family with other species YSL was constructed and classified into 3 main clades. The *cis*-acting elements of 1.5 kb upstream regions of *OsYSL* genes were also predicted and sorted by their functions. The expression of the *OsYSL* gene family varies at different developmental stages. The data of expression involved in Fe concentration also demonstrates the function of these genes in Fe transport and response to environmental conditions. Current study could provide broad information for further functional characterization of *OsYSL* genes.

ACKNOWLEDGMENT

This research was carried out within the framework of the project "Research on micronutrient biofortification in rice using biotechnology", code: NDT/CN/21/24 funded by Vietnam Ministry of Science and Technology.

REFERENCES

1. Kobayashi, T., T. Nozoye and N.K. Nishizawa, 2019. Iron transport and its regulation in plants. Free Radical Biol. Med., 133: 11-20.
2. Li, J., X. Cao, X. Jia, L. Liu, H. Cao, W. Qin and M. Li, 2021. Iron deficiency leads to chlorosis through impacting chlorophyll synthesis and nitrogen metabolism in *Areca catechu* L. Front. Plant Sci., Vol. 12. 10.3389/fpls.2021.710093.
3. Morrissey, J. and M.L. Guerinot, 2009. Iron uptake and transport in plants: The good, the bad, and the ionome. Chem. Rev., 109: 4553-4567.
4. Kroh, G.E. and M. Pilon, 2020. Regulation of iron homeostasis and use in chloroplasts. Int. J. Mol. Sci., Vol. 21. 10.3390/ijms21093395.
5. Li, W. and P. Lan, 2017. The understanding of the plant iron deficiency responses in strategy I plants and the role of ethylene in this process by omic approaches. Front. Plant Sci., Vol. 8. 10.3389/fpls.2017.00040.
6. Connorton, J.M., J. Balk and J. Rodríguez-Celma, 2017. Iron homeostasis in plants-A brief overview. Metallomics, 9: 813-823.
7. Marschner, H., V. Romheld and M. Kissel, 1986. Different strategies in higher plants in mobilization and uptake of iron. J. Plant Nutr., 9: 695-713.
8. Eide, D., M. Broderius, J. Fett and M.L. Guerinot, 1996. A novel iron-regulated metal transporter from plants identified by functional expression in yeast. Proc. Natl. Acad. Sci. U.S.A., 93: 5624-5628.
9. Yi, Y. and M.L. Guerinot, 1996. Genetic evidence that induction of root Fe(III) chelate reductase activity is necessary for iron uptake under iron deficiency. Plant J., 10: 835-844.
10. Curie, C., Z. Panaviene, C. Loulergue, S.L. Dellaporta, J.F. Briat and E.L. Walker, 2001. Maize *Yellow stripe1* encodes a membrane protein directly involved in Fe(III) uptake. Nature, 409: 346-349.
11. Tagaki, S., K. Nomoto and T. Takemoto, 1984. Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. J. Plant Nutr., 7: 469-477.
12. DiDonato, Jr. R.J., L.A. Roberts, T. Sanderson, R.B. Eisley and E.L. Walker, 2004. *Arabidopsis Yellow Stripe-Like2 (YSL2)*: A metal-regulated gene encoding a plasma membrane transporter of nicotianamine-metal complexes. Plant J., 39: 403-414.
13. Murata, Y., E. Harada, K. Sugase, K. Namba and M. Horikawa *et al*., 2008. Specific transporter for iron(III): Phytosiderophore complex involved in iron uptake by barley roots. Pure Appl. Chem., 80: 2689-2697.
14. Persson, B. and P. Argos, 1994. Prediction of transmembrane segments in proteins utilising multiple sequence alignments. J. Mol. Biol., 237: 182-192.
15. Argos, P. and B. Persson, 1996. Topology prediction of membrane proteins. Protein Sci., 5: 363-371.
16. Curie, C., G. Cassin, D. Couch, F. Divol and K. Higuchi *et al*., 2009. Metal movement within the plant: Contribution of nicotianamine and yellow stripe 1-like transporters. Ann. Bot., 103: 1-11.
17. Roberts, L.A., A.J. Pierson, Z. Panaviene and E.L. Walker, 2004. Yellow stripe1. Expanded roles for the maize iron-phytosiderophore transporter. Plant Physiol., 135: 112-120.
18. Murata, Y., J.F. Ma, N. Yamaji, D. Ueno, K. Nomoto and T. Iwashita, 2006. A specific transporter for iron(III)-Phytosiderophore in barley roots. Plant J., 46: 563-572.
19. Schaaf, G., U. Ludewig, B.E. Erenoglu, S. Mori, T. Kitahara and N. von Wiren, 2004. ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. J. Biol. Chem., 279: 9091-9096.
20. Jean, M.L., A. Schikora, S. Mari, J.F. Briat and C. Curie, 2005. A loss-of-function mutation in *AtYSL1* reveals its role in iron and nicotianamine seed loading. Plant J., 44: 769-782.
21. Schaaf, G., A. Schikora, J. Häberle, G. Vert and U. Ludewig *et al*., 2005. A putative function for the *Arabidopsis* Fe-phytosiderophore transporter homolog AtYSL2 in Fe and Zn homeostasis. Plant Cell Physiol., 46: 762-774.
22. Waters, B.M., H.H. Chu, R.J. DiDonato, L.A. Roberts and R.B. Eisley *et al*., 2006. Mutations in arabidopsis *Yellow stripe-like1* and *Yellow stripe-like3* reveal their roles in metal ion homeostasis and loading of metal ions in seeds. Plant Physiol., 141: 1446-1458.
23. Apweiler, R., A. Bairoch, C.H. Wu, W.C. Barker and B. Boeckmann *et al*., 2004. UniProt: The universal protein knowledgebase. Nucleic Acids Res., 32: D115-D119.
24. Goodstein, D.M., S. Shu, R. Howson, R. Neupane and R.D. Hayes *et al*., 2012. Phytozome: A comparative platform for green plant genomics. Nucleic Acids Res., 40: D1178-D1186.
25. La, H.V., H.D. Chu, C,D. Tran, K.H. Nguyen and Q.T.N. Le *et al*., 2022. Insights into the gene and protein structures of the *CaSWEET* family members in chickpea (*Cicer arietinum*), and their gene expression patterns in different organs under various stress and abscisic acid treatments. Gene, Vol. 819. 10.1016/j.gene.2022.146210.
26. Le, T.M., T.T.H. Tran, X.Q. Vu, H.D. Chu and T.C. Pham, *et al*., 2022. Genome-wide identification and analysis of genes encoding putative heat shock protein 70 in papaya (*Carica papaya*). Pak. J. Biol. Sci., 25: 468-475.
27. La, H.V., H.D. Chu, Q.T. Ha, T.T.H. Tran and H.V. Tong, *et al*., 2022. *SWEET* gene family in sugar beet (*Beta vulgaris*): Genome-wide survey, phylogeny and expression analysis. Pak. J. Biol. Sci., 25: 387-395.

28. Gasteiger, E., A. Gattiker, C. Hoogland, I. Ivanyi, R.D. Appel and A. Bairoch, 2003. ExPASy: The proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res., 31: 3784-3788.
29. Horton, P., K.J. Park, T. Obayashi, N. Fujita, H. Harada, C.J. Adams-Collier and K. Nakai, 2007. WoLF PSORT: Protein localization predictor. Nucleic Acids Res., 35: W585-W587.
30. Bailey, T.L., J. Johnson, C.E. Grant and W.S. Noble, 2015. The MEME Suite. Nucleic Acids Res., 43: W39-W49.
31. Chen, C., H. Chen, Y. Zhang, H.R. Thomas and M.H. Frank *et al*., 2020. TBtools: An integrative toolkit developed for interactive analyses of big biological data. Mol. Plant, 13: 1194-1202.
32. Lescot, M., P. Dehais, G. Thijs, K. Marchal and Y. Moreau *et al*., 2002. PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. Nucleic Acids Res., 30: 325-327.
33. Larking, M.A., G. Blackshields, N.P. Brown, R. Chenna and G.A. McGettigan *et al*., 2007. Clustal W and clustal X version 2.0. Bioinformatics, 23: 2947-2948.
34. Tamura, K., G. Stecher and S. Kumar, 2021. MEGA11: Molecular evolutionary genetics analysis version 11. Mol. Biol. Evol., 38: 3022-3027.
35. Letunic, I. and P. Bork, 2021. Interactive tree of life (iTOL) v5: An online tool for phylogenetic tree display and annotation. Nucleic Acids Res., 49: W293-W296.
36. Kumar, A., G. Kaur, P. Goel, K.K. Bhati, M. Kaur, V. Shukla and A.K. Pandey, 2019. Genome-wide analysis of oligopeptide transporters and detailed characterization of yellow stripe transporter genes in hexaploid wheat. Funct. Integr. Genomics, 19: 75-90.
37. Yordem, B.K., S.S. Conte, J.F. Ma, K. Yokosho, K.A. Vasques, S.N. Gopalsamy and E.L. Walker, 2011. *Brachypodium distachyon* as a new model system for understanding iron homeostasis in grasses: Phylogenetic and expression analysis of Yellow Stripe-Like (YSL) transporters. Ann. Bot., 108: 821-833.
38. Harada, E., K. Sugase, K. Namba, T. Iwashita and Y. Murata, 2007. Structural element responsible for the Fe(III)-phytosiderophore specific transport by HvYS1 transporter in barley. FEBS Lett., 581: 4298-4302.
39. Inoue, H., T. Kobayashi, T. Nozoye, M. Takahashi and Y. Kakei *et al*., 2009. 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., 284: 3470-3479.
40. Araki, R., J. Murata and Y. Murata, 2011. A novel barley yellow stripe 1-like transporter (HvYSL2) localized to the root endodermis transports metal-phytosiderophore complexes. Plant Cell Physiol., 52: 1931-1940.
41. Kakei, Y., Y. Ishimaru, T. Kobayashi, T. Yamakawa, H. Nakanishi and N.K. Nishizawa, 2012. OsYSL16 plays a role in the allocation of iron. Plant Mol. Biol., 79: 583-594.
42. Zhang, C., W. Lu, Y. Yang, Z. Shen, J.F. Ma and L. Zheng, 2018. OsYSL16 is required for preferential Cu distribution to floral organs in rice. Plant Cell Physiol., 59: 2039-2051.
43. Molina, C. and E. Grotewold, 2005. Genome wide analysis of *Arabidopsis* core promoters. BMC Genomics, Vol. 6. 10.1186/1471-2164-6-25.
44. Dai, Z., K. An, G.E. Edward and G. An, 1999. Functional role of CAAT box element of the nopaline synthase (*nos*) promoter. J. Plant Biol., 42: 181-185.
45. Shaw, C.H., G.H. Carter, M.D. Watson and C.H. Shaw, 1984. A functional map of the nopaline synthase promoter. Nucleic Acids Res., 12: 7831-7846.
46. Koike, S., H. Inoue, D. Mizuno, M. Takahashi, H. Nakanishi, S. Mori and N.K. Nishizawa, 2004. OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. Plant J., 39: 415-424.
47. Sasaki, A., N. Yamaji, J. Xia and J.F. Ma, 2011. OsYSL6 is involved in the detoxification of excess manganese in rice. Plant Physiol., 157: 1832-1840.

Table 3S: Predicted cis-acting regulatory elements identified in the 1500 bp upstream region of OsYSL family

cis-elements	*OsYSL1*	*OsYSL2*	*OsYSL3*	*OsYSL4*	*OsYSL5*	*OsYSL6*	*OsYSL7*	*OsYSL8*	*OsYSL9*	*OsYSL10*	*OsYSL11*	*OsYSL12*	*OsYSL13*	*OsYSL14*	*OsYSL15*	*OsYSL16*	*OsYSL17*	*OsYSL18*	Sum
Biotic stress responses																			
Box S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
W box	0	0	1	0	2	0	0	0	0	0	0	0	1	0	1	1	0	0	6
WRE3	0	1	2	3	0	3	0	0	1	0	0	1	0	1	0	0	1	1	14
WUN-motif	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	1	4
***cis*-elements in developmental processes**																			
as-1	1	1	2	0	0	2	0	2	4	3	1	0	1	2	1	1	2	1	24
CARE	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
CAT-box	1	0	0	0	0	1	0	1	0	0	0	2	2	1	1	0	0	0	9
CCGTCC-box	0	0	1	0	0	0	0	1	1	0	2	1	1	1	0	1	0	0	9
circadian	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2
dOCT	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
E2Fb	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
GCN4_motif	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
motif I	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
MSA-like	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
NON	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
NON-box	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
O2-site	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3
re2f-1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
RY-element	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	2
***cis*-elements in phytohormone regulation**																			
ABRE	2	1	0	2	2	5	1	1	1	8	3	0	2	1	0	1	0	0	30
ABRE3a	1	1	0	0	0	2	0	0	0	3	0	0	1	0	0	0	0	0	8
ABRE4	0	0	1	0	0	1	0	1	1	0	0	0	0	0	1	0	1	0	6
AT~ABRE	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	2
AuxRR-core	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	3
CGTCA-motif	1	0	1	1	2	3	0	1	1	2	0	0	5	0	2	0	1	0	20
ERE	0	0	0	3	1	0	0	0	1	0	1	0	0	0	0	0	2	0	8
GARE-motif	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2
P-box	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SARE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
TATC-box	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
TCA	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2
TCA-element	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	1	0	4
TGA-box	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
TGACG-motif	1	1	2	0	0	2	0	2	4	3	1	0	1	2	1	1	2	1	24
TGA-element	1	0	0	0	0	1	0	0	0	1	0	2	0	0	0	0	0	0	5

Table 3S: Continue

cis-elements	*OsYSL1*	*OsYSL2*	*OsYSL3*	*OsYSL4*	*OsYSL5*	*OsYSL6*	*OsYSL7*	*OsYSL8*	*OsYSL9*	*OsYSL10*	*OsYSL11*	*OsYSL12*	*OsYSL13*	*OsYSL14*	*OsYSL15*	*OsYSL16*	*OsYSL17*	*OsYSL18*	Sum
Light responsive *cis*-elements																			
ACE	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AE-box	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	3
ATCT-motif	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	2
Box 4	0	0	0	1	0	1	1	2	1	0	0	1	0	2	0	0	3	0	12
Box II	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
chs-Unit 1 m1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
GATA-motif	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2
G-box	2	1	0	1	2	5	0	1	0	5	0	1	3	1	2	2	2	0	28
GT1-motif	1	1	1	0	2	1	1	0	0	0	0	1	0	1	2	0	0	1	12
GTGGC-motif	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
I-box	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	2
LAMP-element	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
MRE	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Sp1	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	5
TCCC-motif	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
TCT-motif	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2
Promoter-related *cis*-element																			
A-box	0	0	1	0	0	0	0	1	1	0	2	1	1	1	0	1	0	0	9
AT~TATA-box	2	0	2	2	1	2	2	2	0	0	0	2	2	0	3	0	0	0	20
AT-rich element	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2
CAAT-box	11	12	13	8	9	8	10	20	5	5	15	11	20	17	13	14	9	5	205
TATA-box	12	9	12	15	8	13	10	6	18	2	10	8	14	17	15	6	10	1	186
Stress responsive *cis*-elements																			
ARE	1	0	0	0	1	1	1	0	2	1	2	1	1	0	0	0	4	0	15
CCAAT-box	0	1	0	0	0	0	0	0	1	1	0	1	2	0	1	1	1	0	9
DRE core	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	2
GC-motif	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	2	0	0	5
LTR	0	0	0	1	0	0	0	0	0	0	2	0	0	1	1	0	0	0	5
MBS	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	1	0	1	6
Myb	1	1	1	1	1	4	4	4	0	2	2	2	2	1	3	3	4	2	38
MYB recognition site	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Myb-binding site	0	0	1	0	0	0	0	1	0	1	2	0	0	1	0	0	0	0	6
MYC	1	0	2	1	2	1	2	0	0	4	1	1	5	1	1	1	0	0	23
STRE	0	2	2	1	1	0	0	0	0	1	1	0	0	0	0	3	1	2	14
TC-rich repeats	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	3