Genome-wide identification and characterization of lncRNAs responding to iron deficiency in *Oryza sativa*

Shoudong Wang
zhejiang university
Shuo Sun
zhejiang university
Huixia Shou (huixia@zju.edu.cn)
Zhejiang University

Research article

Keywords: lncRNAs, iron deficiency, rice, Strand-specific RNA sequencing, RT-qPCR

Posted Date: March 17th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-17428/v1

Abstract

Background: Iron (Fe) plays a vital role in various cellular processes in plants, including chlorophyll biosynthesis, photosynthesis and respiration. Fe deficiency directly affects crop growth and development, ultimately resulting in reduced crop yield and quality. Long non-coding RNAs (lncRNAs) have recently been demonstrated to play critical regulatory roles in a multitude of pathways across numerous species. However, systematic screening of lncRNAs responding to Fe deficiency in plants has not been reported. In this work, genome-wide identification and characterization of lncRNAs responsive to Fe deficiency were performed by strand-specific RNA sequencing in rice.

Results: In total, 6477 lncRNAs were identified. In Fe-deficient conditions, 47 lncRNAs were up-regulated and 33 lncRNAs were down-regulated in shoots, while 89 lncRNAs were up-regulated and 32 lncRNAs were down-regulated in roots compared to normal conditions. The lncRNAs that were differentially expressed under Fe-sufficient and -deficient conditions appear to be clustered in the genome. Among five monocotyledon species, 88 lncRNAs were conserved, but only 3 lncRNAs responded to Fe deficiency in shoots. Two lncRNAs were identified as putative target mimics of miRNAs that respond to Fe deficiency.

Conclusions: Our results provide evidence that lncRNAs are involved in Fe-deficiency signaling pathway in plants, providing a new pathway for further investigation into Fe-regulatory and response mechanisms.

Background

Iron (Fe) is an essential micronutrient for plants, but is often limited due to low availability in the soil [1]. To overcome Fe deficiency, plants have evolved two strategies to optimize Fe acquisition and uptake, i.e., the reduction strategy (Strategy I) for non-gramineous plants and the chelation strategy (Strategy II) for gramineous plants [2, 3]. Rice, which is adapted to growing in the paddy field where the reduced form of Fe is available, utilizes both strategy I and strategy II Fe-uptake systems [4–6]. A large number of genes are known for their involvement in Fe uptake and homeostasis, including those that encode transcription factors for regulating expression of downstream Fe-responsive genes, enzymes for synthesis of phytosiderophores (MAs), and transporters of MA-Fe(III) or Fe(II) in rice [2, 4, 7–14]. However, regulation of Fe-responses by long noncoding RNAs (lncRNAs) has not been reported.

LncRNAs are transcripts of more than 200 nucleotides in length but without coding potential [15] that have recently gained widespread attention. LncRNAs plays roles in numerous crucial biological processes across many species by regulating expression of mRNAs at epigenetic, transcriptional, post-transcriptional, translational and post-translational levels [16–18]. LncRNAs are classified as sense, antisense, intronic, and intergenic according to their position in relation to neighboring coding genes [16, 19]. In plants, lncRNAs were reported to be involved in development and stress responses [20, 21]. For instance, the lncRNAs COLDAIR (Cold-Assisted Intronic noncoding RNA) and COOLAIR (Cold-Induced Long Antisense Intragenic RNA) are both located in the FLC gene, which regulates flowering time. COLDAIR and COOLAIR regulate expression of FLC at the epigenetic level by interacting with an

evolutionarily conserved repressive complex PRC2 (Polycomb Repressive Complex 2) [22–24]. Another lncRNA, the 1,236-nucleotide long LDMAR (Long-day-specific male-fertility–associated RNA), regulates photoperiod-sensitive male sterility (PSMS) in rice [25]. A number of lncRNAs have been reported to regulate phosphate homeostasis. IPS1 (Induced by phosphate starvation 1) reduces phosphorus acquisition by inhibiting the activity of miR399, through the target mimicry mechanism [26]. The cis-natural antisense RNA (cis-NATPHO1;2), transcribed from OsPHO1;2, was found to be a translational enhancer of its sense gene (OsPHO1;2) [27]. In yeast, prt (pho1-repressing transcript), generated from the promoter region of the pho1 gene, regulates expression of pho1 responding to different phosphate levels [28]. These studies show a complicated network involving lncRNAs that regulate phosphate homeostasis. In contrast, there are no reported lncRNAs responding to Fe deficiency. In this study, the transcriptome of rice was surveyed to systematically identify and characterize any lncRNAs that respond to Fe deficiency.

Results

Genome-wide identification of lncRNAs

To systematically identify and characterize lncRNAs in rice, ssRNA sequencing was performed on shoot and root samples from rice seedlings grown in Fe-sufficient and -deficient conditions. After 10 days of Fe-deficient growth, rice plants showed significant chlorosis and lower chlorophyll content in the young leaves (Fig. 1A and 1B). The expression of typic Fe-deficiency responsive genes, such as the iron-related bHLH transcription factor 2 (IRO2), nicotianamine synthases 1 and 2 (NAS1/2), Fe(III)-DMA transporters (YSL15/16) and Iron-Regulated Transporter 1 (IRT1), were significantly increased (Fig. 1C), indicating that the rice seedlings is under iron deficiency condition and at the sampling time.

The pipeline for lncRNA identification and characterization is shown in Figure S1 (see methods). Using this pipeline, the approximately 700 million 150-bp pair-end reads were assembled into 31947 transcripts using Cufflinks. The Coding Potential Calculator (CPC) was used to evaluate the protein-coding potential of the transcripts to distinguish protein coding transcripts and lncRNAs. Transcripts more than 200 bp in length with CPC scores < 0 were defined as lncRNAs, the remaining transcripts were classified as protein-coding transcripts (mRNAs). Using this method, 25470 mRNAs and 6477 lncRNAs were identified.

Fe-deficiency responsive lncRNAs and mRNAs in rice shoot and root

To identify the lncRNAs and mRNAs that are differentially expressed in response to Fe deficiency, the normalized expression levels (in fragments per kilobase of exon per million fragments mapped, FPKM) of lncRNAs or mRNAs were compared between the Fe-deficient and Fe-sufficient treatments (Figure S2). In shoots, 80 differently expressed lncRNAs were identified (Log2 (fold change) ≥ 1 or ≤−1, probability > 0.8). Among them, 47 lncRNAs were up-regulated and 33 lncRNAs were down-regulated (Figure S2A and S3A; Table S2A). In roots, 89 lncRNAs were up-regulated and 32 lncRNAs were down-regulated under Fe deficiency (Fig. S2B and S3A; Table S2B). In addition, 394 and 841 mRNAs were differentially expressed

in either roots or shoots due to Fe deficiency, respectively (Log2 (fold change) ≥ 1 or ≤−1, probability > 0.8). In shoots, 240 mRNAs were up-regulated and 154 were down-regulated (Figure S2C and S3B; and Table S2C), while in roots, 536 mRNAs were up-regulated and 305 mRNAs were down-regulated (Figure S2D and S3B; Table S2D).

The differentially expressed lncRNAs and mRNAs were used to generate a heat map (Fig. 2). Classes I and III contained lncRNA and mRNA transcripts that were expressed significantly higher in Fe-sufficient than in Fe-deficient conditions in either roots (Class I) or shoots (Class III), respectively. In contrast, transcripts in Classes and had higher expression in roots or shoots under Fe-deficient conditions, respectively. Transcripts in Class were more highly expressed in both shoots and roots under Fe-deficient conditions. Among the five groups, Class , the transcripts induced under Fe-deficient roots, contained the largest number of both lncRNAs (Fig. 2A) and mRNAs (Fig. 2B). In total, 171 lncRNAs and 1001 mRNAs were differentially expressed under different Fe supply conditions (Fig. 2; Table S3).

Verification of lncRNAs responding to Fe deficiency using quantitative RT-PCR

Quantitative RT-PCR (qRT-PCR) was performed to verify the accuracy of the RNA-seq data for the lncRNAs. Nine intergenic lncRNAs were picked for the verification. Expression of the Class IV lncRNAs XLOC_006153 and XLOC_028199 were induced in shoots but not detected in roots regardless of the Fe supply status. The Class II lncRNAs XLOC_052823 and XLOC_007199 were up-regulated by Fe deficiency in the roots. The remaining 5 lncRNAs belonged to Class b, which were induced upon Fe deficiency in both shoots and roots (Fig. 3). Thus, qRT-PCR results were consistent with the RNA-Seq results.

Distribution of lncRNAs and mRNAs in the rice genome

Based on their relative position to protein-coding genes, lncRNAs can be classified into three types. Intergenic lncRNAs have no overlap with any protein-coding sequences, while sense lncRNAs and anti-sense lncRNAs overlap with one or more exons of another transcript on the same or opposite strand, respectively [20]. Among the 6477 lncRNAs identified in this work, 3730 (57%) were intergenic lncRNAs, 1696 were cis-lncRNAs, and 1051 were antisense lncRNAs (Figure S4).

Recent studies have shown that lncRNAs regulate the expression of genes via either cis- or trans-acting modes based on their genomic proximity to protein-coding genes [29]. To determine the modes of action of the lncRNAs in response to Fe deficiency, the genomic locations of the Fe-related lncRNAs and mRNAs mapped to each chromosome of the rice genome (Fig. 4). No significant genomic proximities were found between the Fe-responsive mRNAs and lncRNAs. Fe-related lncRNAs showed a higher degree of clustering in the genome than did the protein coding transcripts.

The conservation of lncRNAs

To investigate the conservation of the lncRNAs in the Oryza genus, putative lncRNAs were aligned with lncRNAs from 8 species of Oryza (O. barthii, O. brachyantha, O. glaberrima, O. glumipatula, O. meridionalis, O. nivara, O. punctata and O. rufipogon). Most of the lncRNAs were highly conserved among the Oryza species. In total, 1662 lncRNAs were detected in more than 7 species, while 860 lncRNAs were conserved in all 8 species (Fig. 5A).

The 860 conserved lncRNAs were aligned with lncRNAs from the monocots Zea mays, Hordeum vulgare, Sorghum bicolor, and Triticum aestivum. Among these 5 grass species, 88 of the lncRNAs were conserved (Fig. 5B; Table S4). However, only 3 of these conserved lncRNAs showed significant response to Fe deficiency (Fig. 5C). The results demonstrated that the lncRNAs were weakly conserved between Oryza and other gramineous plants.

Prediction of potential miRNA precursors and miRNA target mimics

miRNAs regulate key aspects of development, cell signaling, and responses to various biotic and abiotic stresses via binding to specific complementary transcripts, including protein coding or non-coding sequences, resulting in the degradation or translational repression of the target. LncRNA have been shown to function as precursors of miRNA in many studies [18, 30]. In this study, 23 lncRNAs were identified to be precursors of 29 known miRNAs in rice (Table S5). TCONS_00034827 was predicted to be the precursor of four miRNAs (osa-miR1428b, osa-miR1428c, osa-miR1428d, osa-miR1428e). TCONS_00000537 was predicted to generate two miRNAs (osa-miR156b and osa-miR156c). The results indicated the lncRNAs are quite complex and versatile and that at least some Fe-responsive lncRNAs may function through miRNAs.

In addition to generating miRNAs, lncRNAs are also targets of miRNAs. In this case, lncRNAs function as target mimicry with the sequestered transcript known as an endogenous target mimic (eTM) to inhibit miRNA activity [26]. In order to further verify whether the target mimicry mechanism is involved in Fe regulation in rice, any potential interactions between the Fe-responsive lncRNAs and known Fe-related microRNAs were investigated. Two endogenous target mimics (eTMs), osa-eTM159 and osa-eTM408, were identified. The lncRNAs, XLOC_012715 and XLOC_054182, were predicted to bind the miR159 and miR408, respectively (Fig. 6). The results demonstrated that target mimicry might be a part of the regulation of Fe uptake.

Discussion And Conclusions

LncRNAs play important roles in a wide range of biological processes, including development, stress responses, and plant nutrition. In this work, lncRNAs that respond to Fe deficiency in rice roots and shoots were identified. Results generated in this study promote our understanding of how rice plants respond to Fe deficiency.

LncRNAs arise from intergenic, intronic, or coding regions in the sense and antisense directions with lower expression level than mRNAs. Thus, identification of lncRNAs requires the use of an ssRNA-seq strategy. In this study, 6477 lncRNAs were identified and characterized. Differentially expressed lncRNAs and mRNAs were identified by comparing the expression level between + Fe and –Fe conditions. The expression patterns divided them into five classes (Fig. 2). Class □, which comprised the molecules up-regulated in the rice root, had the greatest number of transcripts. Among the differentially expressed lncRNAs and mRNAs, we found that the number of lncRNAs and mRNAs responding to Fe deficiency had a similar trend, with more lncRNAs and mRNAs up-regulated under Fe deficiency in both shoots and roots. Moreover, a greater number of lncRNAs and mRNAs were detected in roots in Fe-deficient condition. LncRNA orthologs in different species of Oryza and five grass plants were also found, despite limited sequence similarity. Just 3 lncRNAs that were involved in response to Fe limitation were identified as conserved across the five gramineous plants (Fig. 5).

LncRNAs have been shown to regulate phosphate homeostasis in plants by a novel mechanism called target mimicry. Two endogenous lncRNA target mimics (eTMs) were identified in rice, namely osa-eTM159 and osa-eTM408, which target the miRNAs osa-miR159 and osa-miR408, both of which are known to be related to Fe deficiency (Fig. 6). We speculated that these two lncRNAs might function as target mimics to regulate Fe homeostasis in rice.

Our study provides an insight into the potential functions and regulatory interactions of mRNA and lncRNA molecules when rice plants are grown in Fe-deficient conditions. We believe our study would be an initial and reference for understanding the function of the lncRNAs in regulating iron homeostasis in Oryza sativa.

Methods

Plant growth condition

Oryza sativa L. cv. Nipponbare (Nip) was used in this study as the wild type (WT) [14]. The WT seeds were germinated in the dark for 3 days, and then placed on a net floating on a solution with or without iron (1.43 mM NH_4NO_3, 1.0 mM $CaCl_2$, 0.32 mM NaH_2PO_4, 1.64 mM $MgSO_4$, 0.51 mM K_2SO_4, 0.13 ∝M $CuSO_4$, 9.0 ∝M $MnCl_2$, 0.08 ∝M $(NH_4)_6Mo_7O_{24}$, 0.02 ∝M H_3BO_3, 0.15 ∝M $ZnSO_4$, 0.25 mM Na_2SiO_3 and 0 or 125 ∝M EDTA-Fe(□), pH 5.5–5.6). The nutrient solution was exchanged every 3 days. Rice plants were grown in a growth chamber at 30 ℃ during the day and 22 ℃ at night.

Strand-specific RNA library construction and sequencing

Shoots and roots were separately collected from seedlings grown hydroponically for 10 days after germination with or without Fe, and frozen in the liquid nitrogen immediately. Three biological replicates were used for each sample. Total RNA was extracted from these tissues using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Strand-specific RNA (ssRNA) library construction and RNA sequencing were performed by the Beijing Genomics Institute in Shenzhen (BGI,

Shenzhen, China). To construct the ssRNA library, the rRNA was removed with Ribo-Zero Gold rRNA Removal Kit (Epicentre, Madison, WI, USA) from the pooled RNA. The RNA was fragmented into 200–500 nts in length using fragmentation buffer. After synthesis of first-strand and second-strand cDNA, adapters were added to both sides of the short fragments. The second strand was degraded by Uracil-N-Glycosylase. The resulted single strand was PCR amplified and then sequenced by Illumina HiSeq PE151.

Identification and characterization pipeline of lncRNAs

The raw data obtained by Illumina sequencing was filtered into clean data by removing the adaptor, low quality reads and rRNA-containing reads with SOAPnuke and SOAP [31]. The dataset was aligned to the rice genome (Rice Genome Annotation Project) using the improved TopHat v 2.0 [32]. Cufflinks was used to reconstruct the transcripts. After filtering the background noise transcripts, the final expression data was produced [33]. Transcripts shorter than 200 bp were discarded. For the remaining sequences, the transcript coding potential values were predicted by the Coding Potential Calculator (CPC) [34]. Each transcript with a CPC score < 0 were considered long non-coding RNAs. mRNA transcripts (CPC scores > 0) were also identified from the transcriptome in this work.

Validation of several lncRNAs using RT-qPCR

Tissues were collected from shoots and roots grown with or without iron for 10 days. Total RNA was isolated using TRIzol (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized from total RNA using a cDNA Synthesis Kit (TIANGEN), and RT-qPCR was performed on a LightCycler480 machine (Roche) with SYBR Green Supermix (CWBIO). ACTIN mRNA was used as the internal control for sample normalization. Means ± SD were calculated by three biological repeats. The RT-qPCR primers (synthesized by TSINGKE) are shown in Table S1.

Conserved lncRNAs in eight species of Oryza

To analyze the sequence conservation of lncRNAs, all the lncRNAs identified in this work were used as the query dataset and searched against the lncRNA transcripts collected in CANTATAdb (http://cantata.amu.edu.pl/) and the genome sequences downloaded from NCBI (https://www.ncbi.nlm.nih.gov/) for 8 species belonging to the Oryza genus (O. barthii, O. brachyantha, O. glaberrima, O. glumipatula, O. meridionalis, O. nivara, O. punctata, and O. rufipogon). The BLASTn cutoff threshold for significant hits was an E-value < 1e-5, coverage > 40% and identity > 50% for the matched regions.

Analysis of conserved lncRNAs with miRNAs responding to iron deficiency

miRNAs responding to iron deficiency in rice were collected from previous studies [35, 36]. Targets of miRNAs were predicted using the Web-based tool psRNATarget with the default settings [37]. eTMs (endogenous target mimicries) were predicted according to the pipeline in a previous study [38].

Abbreviations

LncRNA
Long non-coding RNA
ssRNA
Strand-specific RNA
CPC
Coding Potential Calculator
FPKM
Fragments per kilobase of exon per million fragments mapped
eTM
Endogenous target mimic

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Competing interests

The authors declare that they have no competing interests.

Funding

The sampling, sequencing and data analysis in this work was supported by the Ministry of Science and Technology of China (2016YFD0100703), the National Natural Science Foundation of China (31401934, 31572189 and 31771689) and the Ministry of Education (B14027) to HS.

Author Contributions

SW and HS conceived the project, designed the experiments, and wrote the manuscript. SW and SS performed experiments and analysis. All authors have read and approved the manuscript.

Acknowledgements

We thank the Beijing Genome Institute (Shenzhen, China) for carrying out the ssRNA-Seq analysis.

Author' information

State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, P. R. China.

References

1. Guerinot, M. L. and Yi, Y. Iron: Nutritious, noxious, and not readily available. Plant Physiol. 1994;104(3):815-820.
2. Kobayashi, T., Nakanishi Itai, R., and Nishizawa, N.K. (2014). Iron deficiency responses in rice roots. Rice (N Y). 2014;7(1):
3. Kobayashi, T., and Nishizawa, N.K. (2012). Iron uptake, translocation, and regulation in higher plants. Annu Rev Plant Biol. 2012;63:131-152.
4. Bughio, N., Yamaguchi, H., Nishizawa, N.K., Nakanishi, H., and Mori, S. Cloning an iron-regulated metal transporter from rice. J Exp Bot. 2002;53(374):1677-1682.
5. Ishimaru, Y., Suzuki, M., Tsukamoto, T., Suzuki, K., Nakazono, M., Kobayashi, T., et al. Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}. Plant J. 2006;45(3):335-346.
6. Cheng, L., Wang, F., Shou, H., Huang, F., Zheng, L., He, F., et al. Mutation in nicotianamine aminotransferase stimulated the Fe(II) acquisition system and led to iron accumulation in rice. Plant Physiol. 2007;145(4):1647-1657.
7. Inoue, H., Higuchi, K., Takahashi, M., Nakanishi, H., Mori, S., and Nishizawa, N.K. Three rice nicotianamine synthase genes, *OsNAS1*, *OsNAS2*, and *OsNAS3* are expressed in cells involved in long-distance transport of iron and differentially regulated by iron. Plant J. 2003;36(3):366-381.
8. Ogo, Y., Itai, R.N., Nakanishi, H., Inoue, H., Kobayashi, T., Suzuki, M., et al. Isolation and characterization of IRO2, a novel iron-regulated bHLH transcription factor in graminaceous plants. J Exp Bot. 2006;57(11):2867-2878.
9. Kobayashi, T., Ogo, Y., Itai, R.N., Nakanishi, H., Takahashi, M., Mori, S., et al. The transcription factor IDEF1 regulates the response to and tolerance of iron deficiency in plants. Proc Natl Acad Sci USA. 2007;104(48):19150-19155.
10. Ogo, Y., Kobayashi, T., Nakanishi Itai, R., Nakanishi, H., Kakei, Y., Takahashi, M., et al. A novel NAC transcription factor, IDEF2, that recognizes the iron deficiency-responsive element 2 regulates the genes involved in iron homeostasis in plants. J Biol Chem. 2008;283(19):13407-13417.
11. Inoue, H., Kobayashi, T., Nozoye, T., Takahashi, M., Kakei, Y., Suzuki, K., 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(6):3470-3479.
12. Zheng, L., Ying, Y., Wang, L., Wang, F., Whelan, J., and Shou, H. Identification of a novel iron regulated basic helix-loop-helix protein involved in Fe homeostasis in *Oryza sativa*. BMC Plant Biol. 2010;10:
13. Wang, L., Ying, Y., Narsai, R., Ye, L., Zheng, L., Tian, J., et al. Identification of OsbHLH133 as a regulator of iron distribution between roots and shoots in *Oryza sativa*. Plant Cell Environ. 2013;36(1):224-236.

14. Wang, S., Li, L., Ying, Y., Wang, J., Shao, J. F., Yamaji, N., et al. A transcription factor OsbHLH156 regulates Strategy II iron acquisition through localizing IRO2 to the nucleus in rice. New Phytol. 2019;225(3):1247-1260.
15. Consortium, E.P., Birney, E., Stamatoyannopoulos, J.A., Dutta, A., Guigo, R., Gingeras, T.R., et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. 2007;447(7146):799-816.
16. Ponting, C.P., Oliver, P.L., and Reik, W. Evolution and functions of long noncoding RNAs. 2009;136(4):629-641.
17. Rinn, J.L., and Chang, H.Y. Genome regulation by long noncoding RNAs. Annu Rev Biochem. 2012;81:145-166.
18. Dykes, I.M., and Emanueli, C. Transcriptional and post-transcriptional gene regulation by long non-coding RNA. Genom Proteom Bioinf. 2017;15(3):177-186.
19. Lu, Z., Xia, X., Jiang, B., Ma, K., Zhu, L., Wang, L., et al. Identification and characterization of novel lncRNAs in *Arabidopsis thaliana*. Biochem Biophys Res Commun. 2017;488(2):348-354.
20. Chekanova, J.A. Long non-coding RNAs and their functions in plants. Curr Opin Plant Biol. 2015;27:207-216.
21. Yamada, M. Functions of long intergenic non-coding (linc) RNAs in plants. J Plant Res. 2017;130(1):67-73.
22. Swiezewski, S., Liu, F., Magusin, A., and Dean, C. Cold-induced silencing by long antisense transcripts of an *Arabidopsis* Polycomb target. 2009; 462(7274):799-802.
23. Heo, J.B., and Sung, S. Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. Science. 2011;331(6013):76-79.
24. Sun, Q., Csorba, T., Skourti-Stathaki, K., Proudfoot, N.J., and Dean, C. R-loop stabilization represses antisense transcription at the *Arabidopsis* FLC locus. Science. 2013;340(6132):619-621.
25. Ding, J., Lu, Q., Ouyang, Y., Mao, H., Zhang, P., Yao, J., et al. A long noncoding RNA regulates photoperiod-sensitive male sterility, an essential component of hybrid rice. Proc Natl Acad Sci USA. 2012;109(7):2654-2659.
26. Franco-Zorrilla, J.M., Valli, A., Todesco, M., Mateos, I., Puga, M.I., Rubio-Somoza, I., et al. Target mimicry provides a new mechanism for regulation of microRNA activity. Nat Genet. 2007;39(8):1033-1037.
27. Jabnoune, M., Secco, D., Lecampion, C., Robaglia, C., Shu, Q., and Poirier, Y. A rice cis-natural antisense RNA acts as a translational enhancer for its cognate mRNA and contributes to phosphate homeostasis and plant fitness. Plant Cell. 2013;25(10):4166-4182.
28. Shah, S., Wittmann, S., Kilchert, C., and Vasiljeva, L. LncRNA recruits RNAi and the exosome to dynamically regulate pho1 expression in response to phosphate levels in fission yeast. Genes Dev. 2014;28(3):231-244.

29. Elkouris, M., Kouroupi, G., Vourvoukelis, A., Papagiannakis, N., Kaltezioti, V., Matsas, R., et al. Long non-coding RNAs associated with neurodegeneration-linked genes are reduced in parkinson's disease patients. Front Cell Neurosci. 2019;13: 58.
30. Liu, X., Hao, L., Li, D., Zhu, L., Hu, S., et al. Long non-coding RNAs and their biological roles in plants. Genom Proteomi Bioinf. 2015;13(3):137-147.
31. Li, R., Yu, C., Li, Y., Lam, T.W., Yiu, S.M., Kristiansen, K., et al. SOAP2: an improved ultrafast tool for short read alignment. 2009;25(15): 1966-1967.
32. Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., and Salzberg, S.L. (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 2013;14(4):
33. Trapnell, C., Williams, B.A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M.J., et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol. 2010;28(5):511-515.
34. Kong, L., Zhang, Y., Ye, Z.Q., Liu, X.Q., Zhao, S.Q., Wei, L., et al. CPC: assess the protein-coding potential of transcripts using sequence features and support vector machine. Nucleic Acids Res. 2007;35:W345-349.
35. Mendoza-Soto, A.B., Sanchez, F., and Hernandez, G. MicroRNAs as regulators in plant metal toxicity response. Front Plant Sci. 2012;3:
36. Paul, S., Gayen, D., Datta, S.K., and Datta, K. Analysis of high iron rice lines reveals new miRNAs that target iron transporters in roots. J Exp Bot. 2016;67(19):5811-5824.
37. Dai, X., and Zhao, P.X. psRNATarget: a plant small RNA target analysis server. Nucleic Acids Res. 2011;39:W155-159.
38. Ye, C.Y., Xu, H., Shen, E., Liu, Y., Wang, Y., Shen, Y., et al. Genome-wide identification of non-coding RNAs interacted with microRNAs in soybean. Front Plant Sci. 2014;5:

Supplementary Information

Figure S1. Identification and characterization of lncRNAs.

Figure S2. Identification of differentially expressed lncRNAs and mRNAs.

Figure S3. Total number of differentially expressed lncRNAs and mRNAs.

Figure S4. Location, of lncRNAs relative to the nearest protein-coding genes.

Table S1. Primers used in this study for validation of lncRNAs.

Table S2. All differentially expressed lncRNAs and mRNAs in shoot and root. LncRNAs significantly up- or down-regulated by Fe deficiency in shoot (A) and root (B). mRNAs significantly up- or down-regulated by Fe deficiency in shoot (C) and root (D).

Table S3. All differentially expressed mRNAs and lncRNAs.

Table S4. List of 88 conserved lncRNAs among 5 grass species.

Table S5. List of lncRNAs identified as precursors of known miRNAs in rice.

Figures

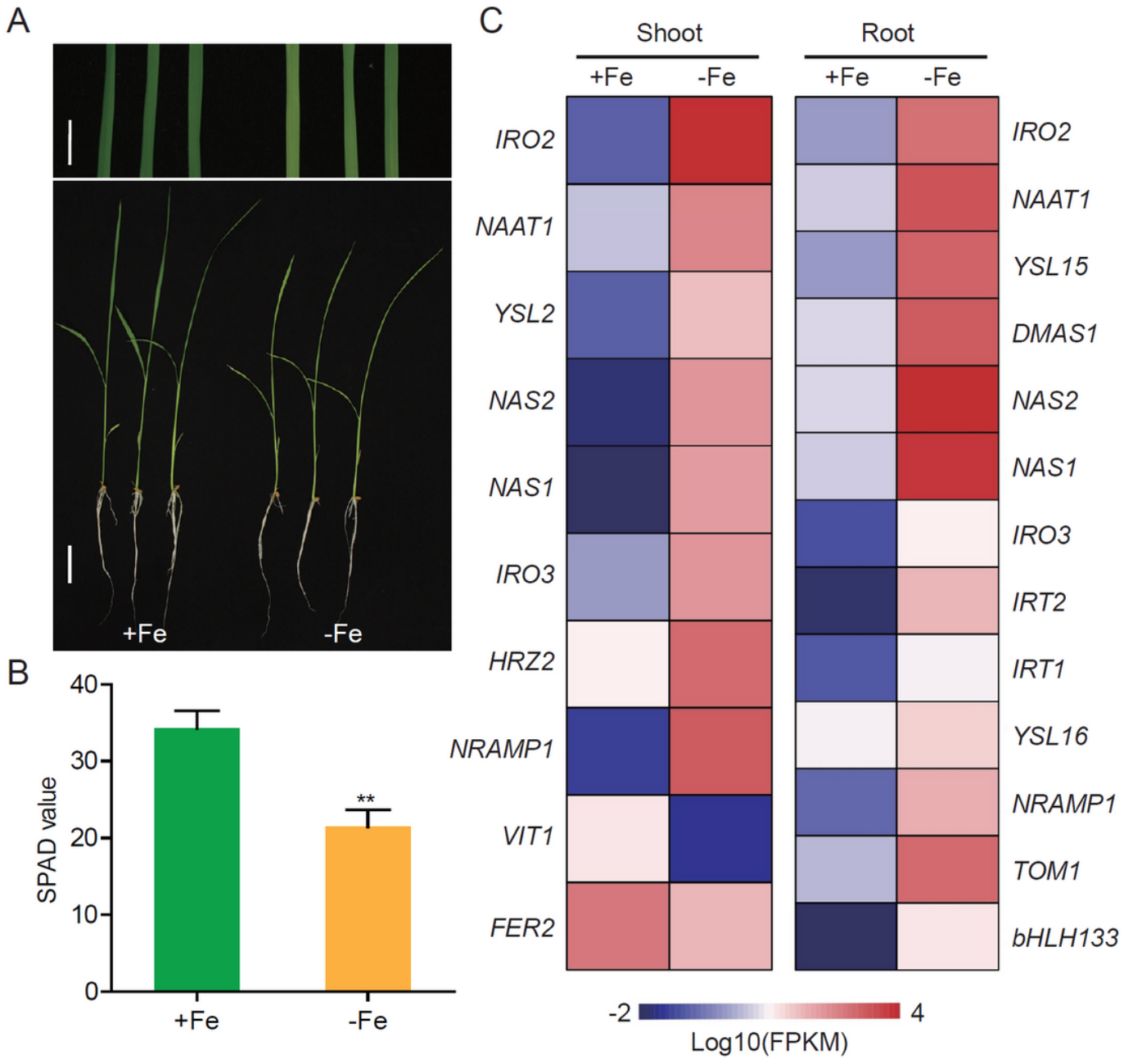

Figure 1

Physiological responses of rice seedlings to iron deficiency and expression pattern of Fe-related marker genes in RNA-seq. (A) Phenotypes of Nipponbare grown with 125 μM EDTA-Fe(II) (+Fe) or no Fe (-Fe) for 10 days. (Upper bar represents 1 cm, lower bar represents 3 cm). (B) SPAD values representing chlorophyll content of leaves. Data represent means ± SD, n = 4; **P < 0.01, one-way ANOVA followed by Tukey test. (C) Heatmap of Fe-related marker genes in rice shoot and root under iron deficiency.

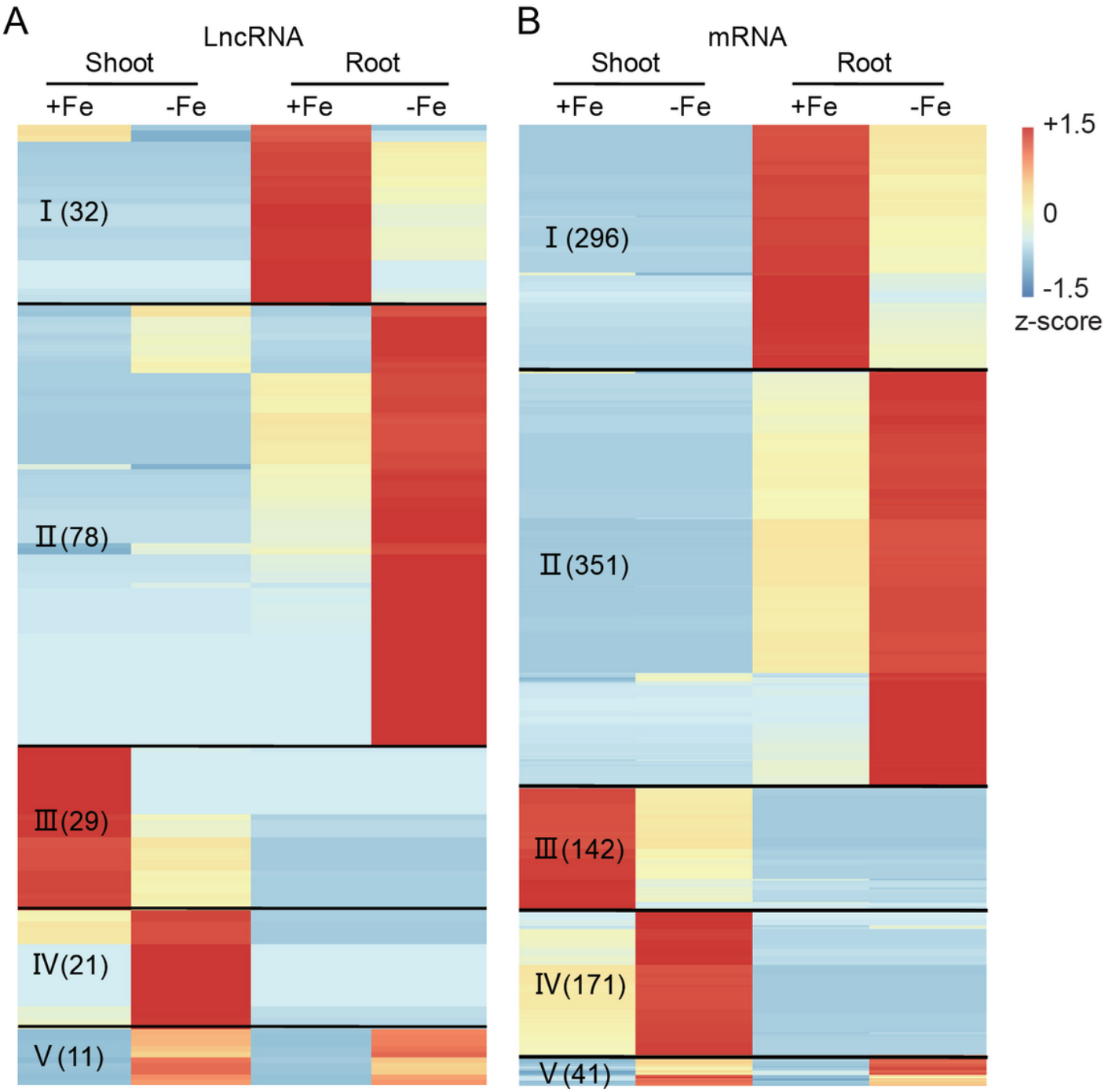

Figure 2

Heatmap of differentially expressed lncRNAs and mRNAs under ±Fe condition in both shoot and root. The lncRNAs (A) and mRNAs (B) identified as differentially expressed by the Volcano plots (Figure S2)

were used to make the heatmap. lncRNAs and mRNAs were divided the into five classes based on their expression patterns. Class , highly expressed in root and down-regulated under Fe deficiency. , up-regulated under Fe deficiency in root. , highly expressed in shoot and down-regulated under Fe deficiency. , up-regulated under Fe deficiency in shoot. , up-regulated both in shoot and root. The number of molecules in each class are listed in parentheses.

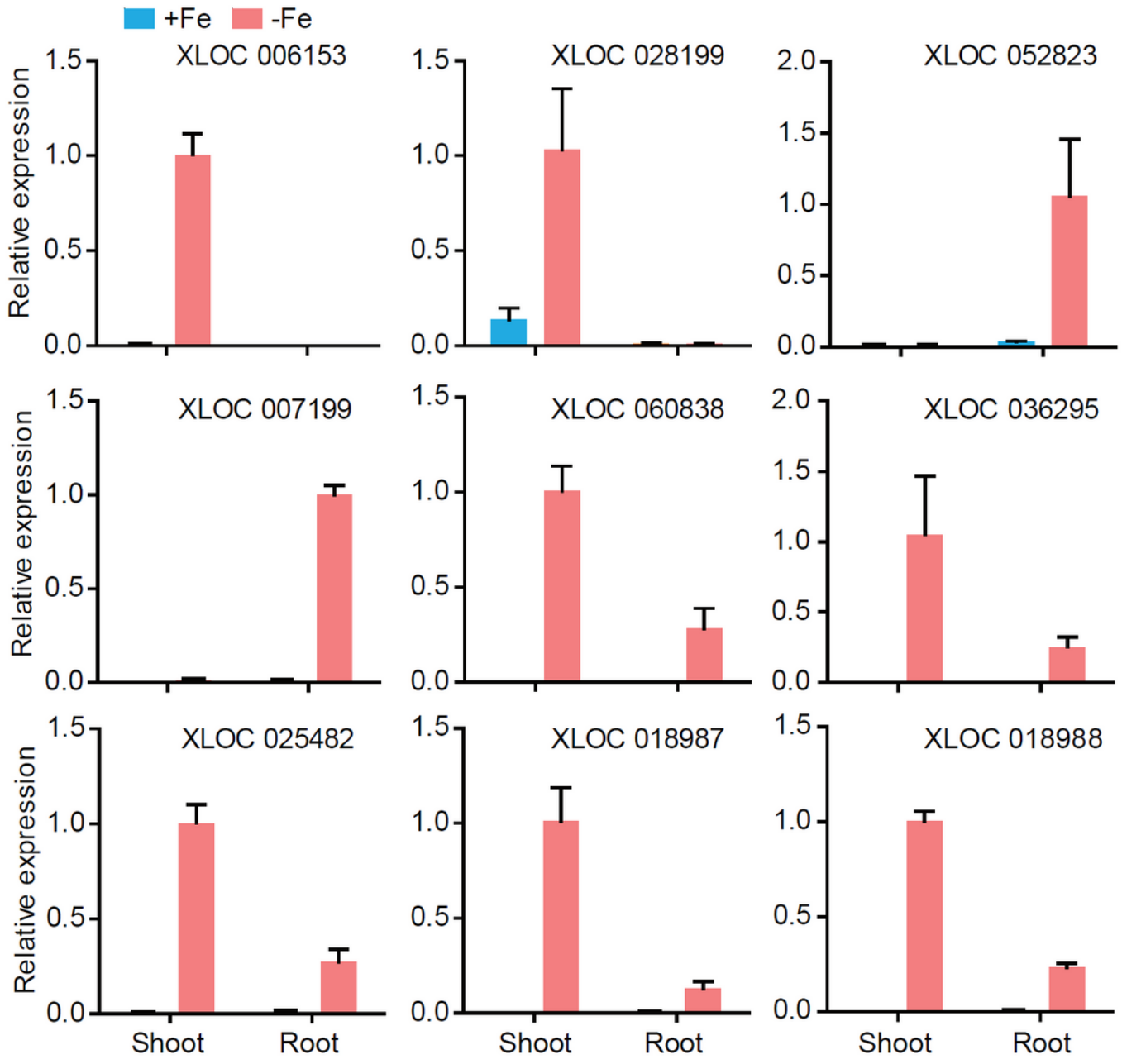

Figure 3

qRT-PCR validation of lncRNAs responding to Fe deficiency. Nine lncRNAs were chosen. Actin was used as a reference gene, Means ± SD were determined from three biological repeats.

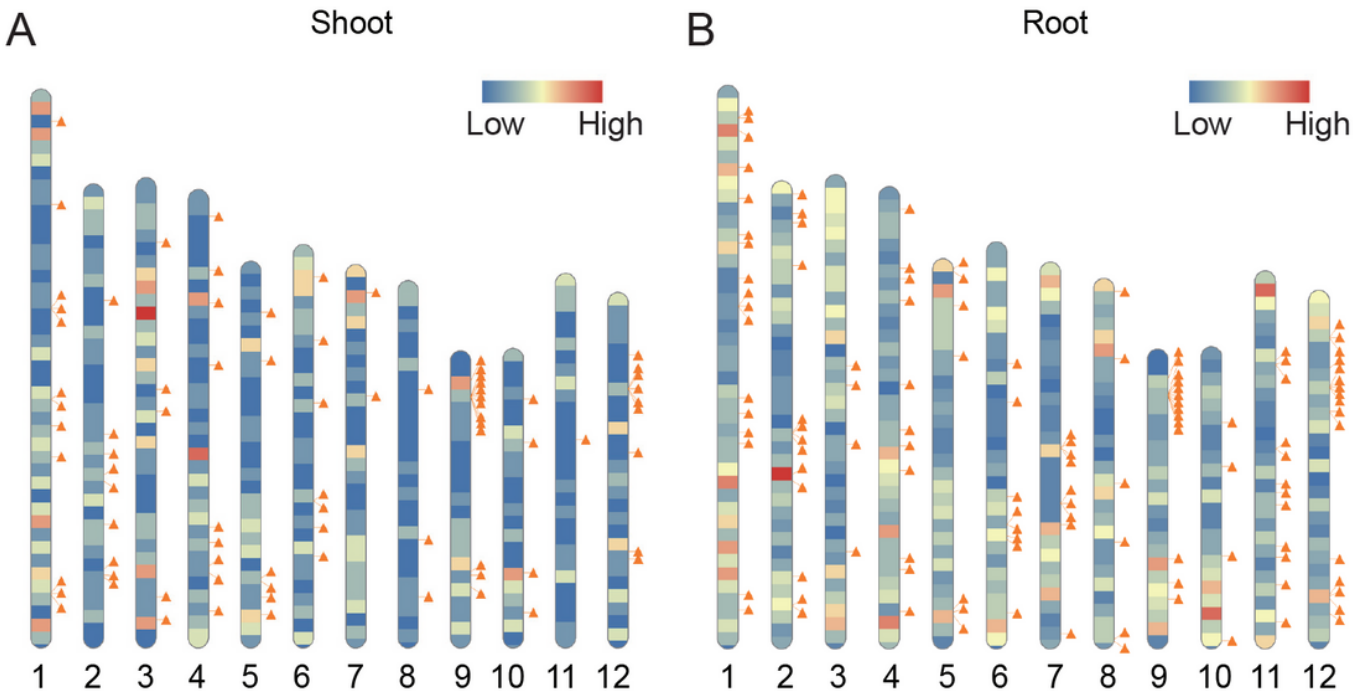

Figure 4

Distribution of differentially expressed mRNAs and lncRNAs on the 12 rice chromosomes. The transcripts that were differentially expressed in shoots (A) and roots (B) are mapped to the chromosomes separately for clarity. Different colors represent different densities of mRNAs on the chromosome, with red color denoting a high density and blue a low density. Each triangle represents one lncRNA identified in this study.

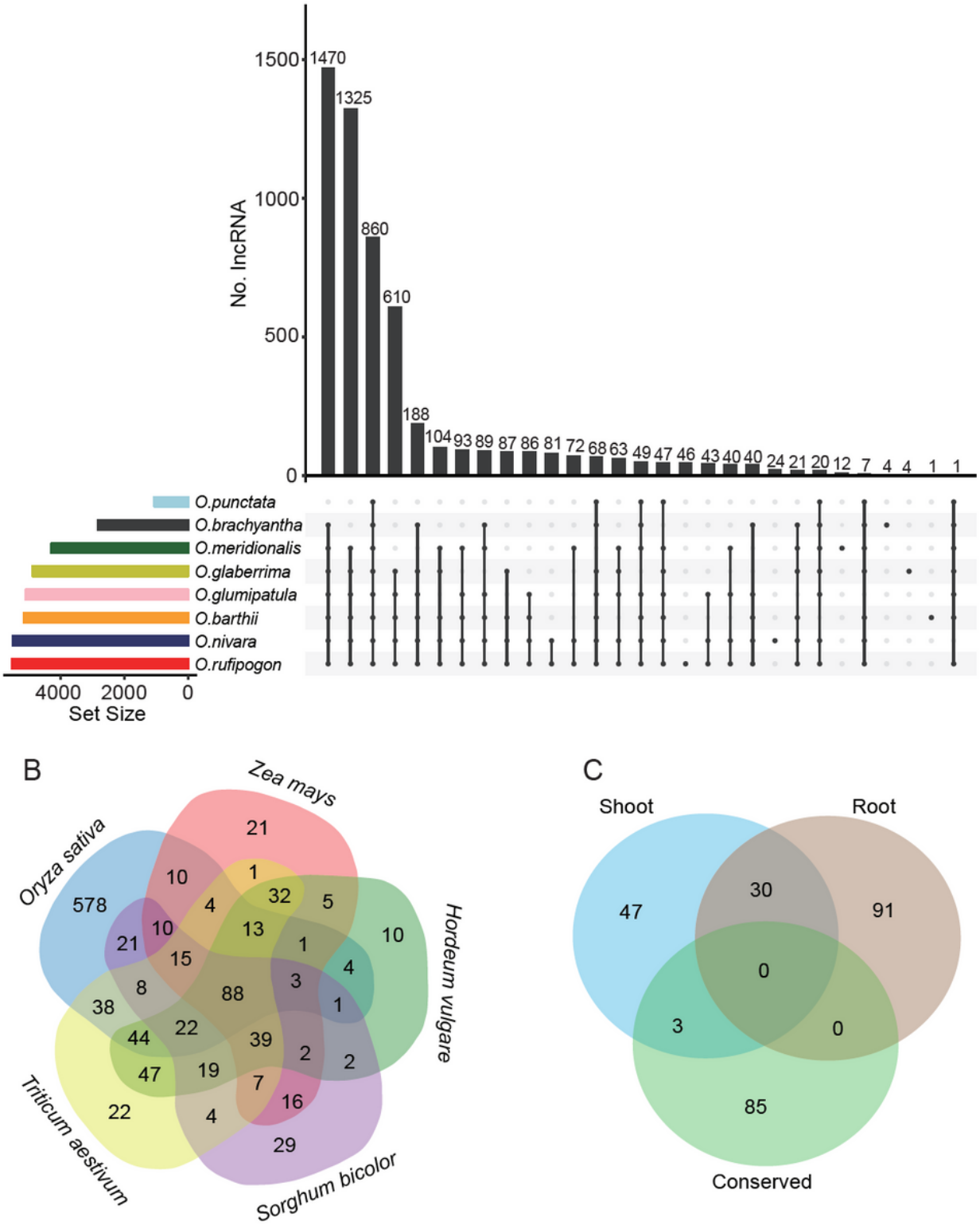

Figure 5

Conservation of differentially expressed lncRNAs. (A) Conservation of differentially expressed lncRNAs in Oryza genus. (B) Conservation of differentially expressed lncRNAs in five Monocotyledons (Oryza sativa, Zea mays, Triticum aestivum, Hordeum vulgare, and Sorghum bicolor). (C) Comparison of conserved lncRNAs identified in (B) in shoot and (C) in root.

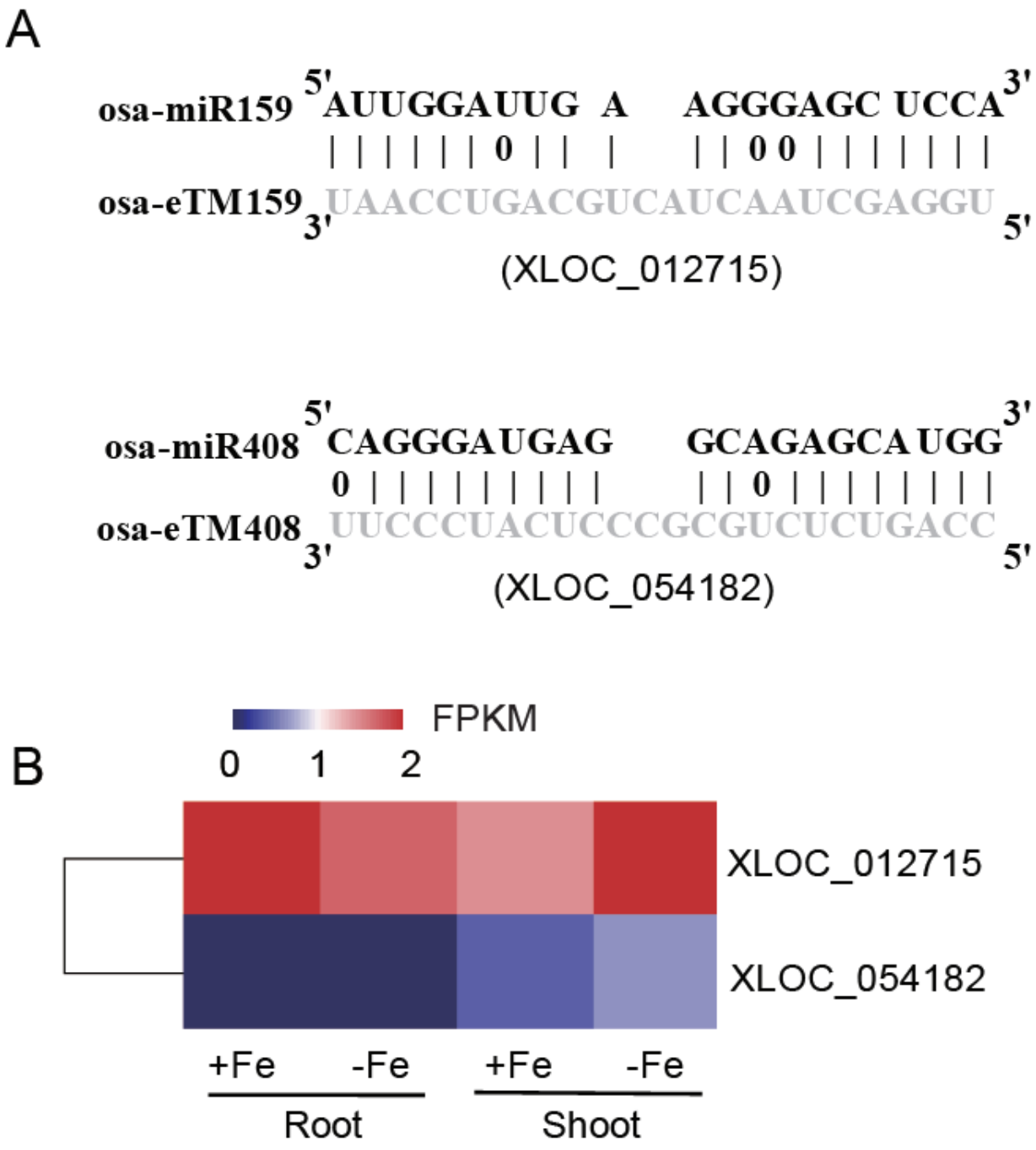

Figure 6

Two endogenous target mimics (eTMs), osa-eTM159 and osa-eTM408 respond to iron deficiency in rice. (A) The predicted base-pairing pattern between osa-miR159 and osa-miR408 and their eTMs. Base pairing between the miRNA and its lncRNA target mimic are shown, in which a vertical line means a Watson-Crick pair, two dots represent a G-U pair, and 0 means a mismatch. (B) The expression levels of osa-eTM159 and osa-eTM408 in the ssRNA-seq.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS4.xls
- SupplementaryFigs.pdf
- TableS3.xlsx
- TableS2.xlsx
- TableS5.xlsx