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Identification of drought stress-responsive long non-coding RNAs (lncRNAs) in root tip region of rice (*Oryza sativa*)

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Original paper

Abstract: Drought severely affects global rice production. Recent evidence highlights the critical role of long non-coding RNAs (lncRNAs) in the response to abiotic stress. Our research identified drought stress-induced lncRNAs in the root tip region of rice using transcriptome sequencing analysis on drought-stressed and control conditions in the sensitive rice genotype (IR64). We identified 358 differentially expressed lncRNAs (DElncRNA), with over 60% located in intergenic regions. Our results demonstrated that DElncRNAs can directly or indirectly regulate 710 and 7535 mRNAs in cis and trans, respectively. Additionally, the target genes of DElncRNAs were involved in drought resistance, lateral root growth, and auxin transport. We also identified 24 conserved sequence motifs in the upstream regions of DElncRNAs and differentially expressed mRNAs (DEmRNAs) motifs. Functional analysis revealed their involvement in the regulation of transcription, translation, and the transmembrane receptor protein tyrosine kinase signaling pathway. Finally, we constructed a network of DElncRNAs and DEmRNAs. Our functional analysis of the top 10 hub lncRNAs in the network demonstrated their involvement in growth processes, cellular responses to stimuli, and signaling pathways. These results offer a comprehensive perspective on potentially functional lncRNAs and provide insight into the molecular mechanisms underlying drought resistance in rice root tip.

Keywords: drought stress, rice, transcriptome, root tip, lncRNAs.

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Introduction

Rice (*Oryza sativa* L.) is one of the most important crops in the world and the main source of food for billions of people (Sun et al., 2013). Asian cultivated rice (*Oryza sativa* L.) is one of the most important cultivated species that provides almost 20% of the calories consumed by humans (Smith, 1995). Unpredictable climate changes have caused aggravation of all types of stress, including drought stress, and this, in turn, leads to a decrease in the yield of cultivated rice (Lafitte et al., 2004). Leaves and roots are organs that coordinate the defense mechanism to respond to abiotic stresses (Kaashyap et al., 2018; Nadarajah and Kumar, 2019). It has been shown that the root system can change under stress leading to an increase in yield (Lynch et al., 2014). Due to its spreading and dense root system, rice is very sensitive to drought stress. Therefore, root length density and root diameter determine the development rate of the rice root system (Yoshida and Hasegawa, 1982). Drought tolerance is a complex trait that involves the regulation of several physiological and biochemical processes, including stomatal compaction (Ishimaru et al., 2001), leaf rolling (Yue et al., 2006), osmotic regulation (Peleg et al., 2009), and root system development (Barbez et al., 2017) at different developmental stages. Different mechanisms and a wide range of genes are also involved in response to drought stress. lncRNAs, eukaryotic RNAs with a length of more than 200 nucleotides, are part of the plant's defense mechanism, which, in addition to responding to abiotic stresses, play a vital role in various processes such as growth and development, seed yield, male sterility, and seed and leaf morphology (Caixia et al., 2020). In recent years, studies have identified a number of stress-related lncRNAs in plants. The identification of lncRNAs involved in drought stress can provide important help in identifying regulatory gene networks and improving drought stress tolerance in drought-sensitive rice cultivars. It has been reported that lncRNAs derived from transposon elements are responsible for the response to abiotic stresses in rice (Brunkard and Baker, 2018). Also, some lncRNAs have been discovered in rice in response to heat stress, cold stress, heavy metals, nitrogen and phosphorus treatments, and abscisic acid (Chen et al., 2018; Tang et al., 2019). On the other hand, the types of lncRNAs involved in the response to drought stress have been identified in different rice varieties, which play a vital role in the mechanism of improving tolerance against that stress (Xu et al., 2016; Li et al., 2019). In addition, research has shown that lncRNAs can act as traps for miRNAs targeting mRNAs involved in the response to drought stress and thereby protect mRNA translation. Also, as precursors of miRNA or siRNA, they can play a role in tolerance to biotic and abiotic stresses in rice (Nejat and Mantri, 2018). In the present study, we performed a transcriptome analysis to identify lncRNAs that participate in response to drought stress in the root tip region.

Materials and Methods

Plant materials and experimental design

IR64 genotype seeds were obtained from the International Rice Research Institute (IRRI). The seeds were surface disinfected using 5.25% sodium hypochlorite for 30 minutes followed by 70% ethanol for one minute. Then, the sterilized seeds were placed on moist filter paper for germination. The seven-day-old seedlings were transferred to a container containing Yoshida's liquid culture medium (Yoshida, 1976) for two weeks at a temperature of 27-25 degrees Celsius and a humidity of 60-70%. They were in light for a duration of 8 hours and 16 hours in dark with a light intensity of 500. They were placed up to 1000 par. After that, the seedlings were transferred to 3 × 25 × 40 cm boxes containing a mixture of clay, cocopeat, and sand in a ratio of 1:1:2, and each box contained two seedlings.

The seedlings were kept in the growth conditions mentioned above until stress was applied. Applying water stress (interruption of irrigation) was done on one hundred 35-day-old plants as a treatment, which reached 25-35% field capacity in 14 days. After the stress, sampling was done from the root tips of the stressed and control seedlings. The samples were collected and quickly frozen in liquid nitrogen. The roots tip from forty stressed seedlings were individually collected as the samples for whole transcriptome sequencing, with two biological repetitions.

RNA extraction and preparation

Total RNA was extracted from a pool of root tip samples from each condition using Invitrogen TRIZol reagent (Thermo Fisher Scientific, Waltham, MA, USA) (Xiao et al., 2011). RNA quantity and quality were determined using Nanodrop (NP80 NanoPhotometer, IMPLEN, Munich, Germany) and 1% agarose gel, respectively.

Sequence data analysis

RNA sequencing was performed based on Illumina HiSeq 2500 technique at the Beijing Genomics Institute (BGI, China). FastQC tool (version 0.11.9) was used to evaluate the quality of raw data reads. After pre-processing (remove adapter sequences and low-quality reads) clean reads were mapped to the *Oryza sativa Japonica* (IRGSP- 1.0) reference genome using hiSAT2 software. StringTie (version 2.1.5) under default settings was used to assemble the mapped reads.

lncRNA identification

To identify lncRNAs, transcripts of less than 200 nucleotides and transcripts with significant homology to known rice genes were removed. In addition, remaining transcripts were BlastX Pfam and UniProt databases, and transcripts with known protein domains were excluded. Finally, the coding potential of the transcripts was evaluated using CPC2 (Coding Potential Calculator) and CNCI (Coding-Noncoding Index) software and transcripts with a score less than zero were considered as potential lncRNA. According to the location of lncRNAs on the chromosome, they were divided into 4 overlapping, antisense, intergenic and intronic groups. The DESeq2 R package was used to identify differentially expressed long non-coding RNA (DElncRNA) between normal and stress conditions with a $|\text{fold change}| \geq 1$ and an adjusted p-value < 0.01.

Prediction of target genes of lncRNAs

Potential target genes of lncRNAs were divided into cis and trans based on their location. The genes located within 10 kb upstream and downstream of lncRNAs were predicted as the cis-targets. In addition, RIblast Fukunaga and Fukunaga and Hamada (2017) was used to predict the trans-targets mRNAs based on a cutoff of hybridization energy < −30 kcal/mol.

Enrichment analysis

Gene ontology enrichment analysis of target genes of lncRNAs was performed using agriGo version 2.0 (http://systembiology.cau.edu.cn/agriGOv2/). GO terms with the FDR value ≤ 0.01 were considered as the statistical significance. In addition, the DAVID tool was employed to perform pathway analysis. Pathways with an adjusted p-value < 0.05 were only considered as enriched.

Cis-elements analysis

The 1 kbp upstream flanking regions of DElncRNA and DEmRNA were extracted from Ensembl Plants (http://plants.ensembl.org). MEME (meme.nbcr.net/meme/intro.html) (Bailey et al., 2009) was used to discover conserved motifs on the sequences with its default parameters and threshold E-value of <1e-4. We used Tomtom v 5.0.1 tool (http://meme-suite.org/tools/tomtom) (Gupta et al., 2007) to eliminate redundant motifs and define known CRE based on plant JASPAR database (Khan et al., 2018) with a threshold E-value cut-off of 0.05. Subsequently, GoMo tool (http://meme-suite.org/tools/gomo) was applied to identify possible links of motifs with gene ontology terms (Buske et al., 2010).

Identification of lncRNAs as a potential target of miRNAs

The known miRNA sequences of *Oryza sativa* were downloaded from the miRBase database. The psRNATarget was utilized for the identification of the interaction relationship between lncRNAs and miRNAs with the default parameters and maximum expectation = 2.

lncRNA-mRNA network

Co-expression regulatory network was constructed based on calculation of correlation between DElncRNA and DEmRNA. Pearson correlations were determined by Hmisc R package and were filtered with $r > |0.7|$ and $P < 0.01$ thresholds. The network was visualized by Cytoscape (version 3.9.1.).

Results

Identification of differentially expressed lncRNAs

Total RNA from root tip in IR64 genotype under control and drought stress conditions were sequenced using the Illumina technique. By

removing reads containing adapter, reads containing ploy-N and low-quality reads from raw data, clean reads were eventually obtained (Supplementary Table S1). All assembled transcripts with a length of more than 200 nucleotides that do not have any indication of coding potential, including open reading frames (ORF) length of fewer than 300 nucleotides, were kept and CNC2 and CNCI packages and the Pfam database were used to further evaluate the remaining transcripts.

Finally, 1815 lncRNAs were identified, of which 358 were DElncRNAs with |FC|>1 and adjusted p-value<0.05 obtained from the IR64-Z1 sample (Supplementary Table S2). Figure 1 shows the distribution and frequency of DElncRNAs on rice chromosomes, where DElncRNAs are located on all 12 rice chromosomes. Among them, chromosome 1 had the highest number of lncRNAs (53) and chromosome 12 had the lowest number (only 18) (Figure 1A). Examining the expression levels of DElncRNAs involved in the response to drought stress by different types in rice showed that in general 204 and 154 DElncRNAs had increased and decreased expression, respectively. According to the relative position of lncRNAs compared to protein-coding genes, lncRNAs were divided into four groups: intergenic lncRNAs (long intergenic noncoding RNAs), intronic lncRNAs (derived from the Intron regions of protein-coding genes), overlapping lncRNAs, and antisense lncRNAs (overlapping with protein-coding genes on the opposite strand) (Lee, 2012). Examining the expression levels of DElncRNAs involved in the response to drought stress by different types in rice shown in Figure 1B. Overall, within this study, the Long intergenic non-coding RNAs (lincRNA) group accounted for the highest proportion, representing 69.6% of the total.

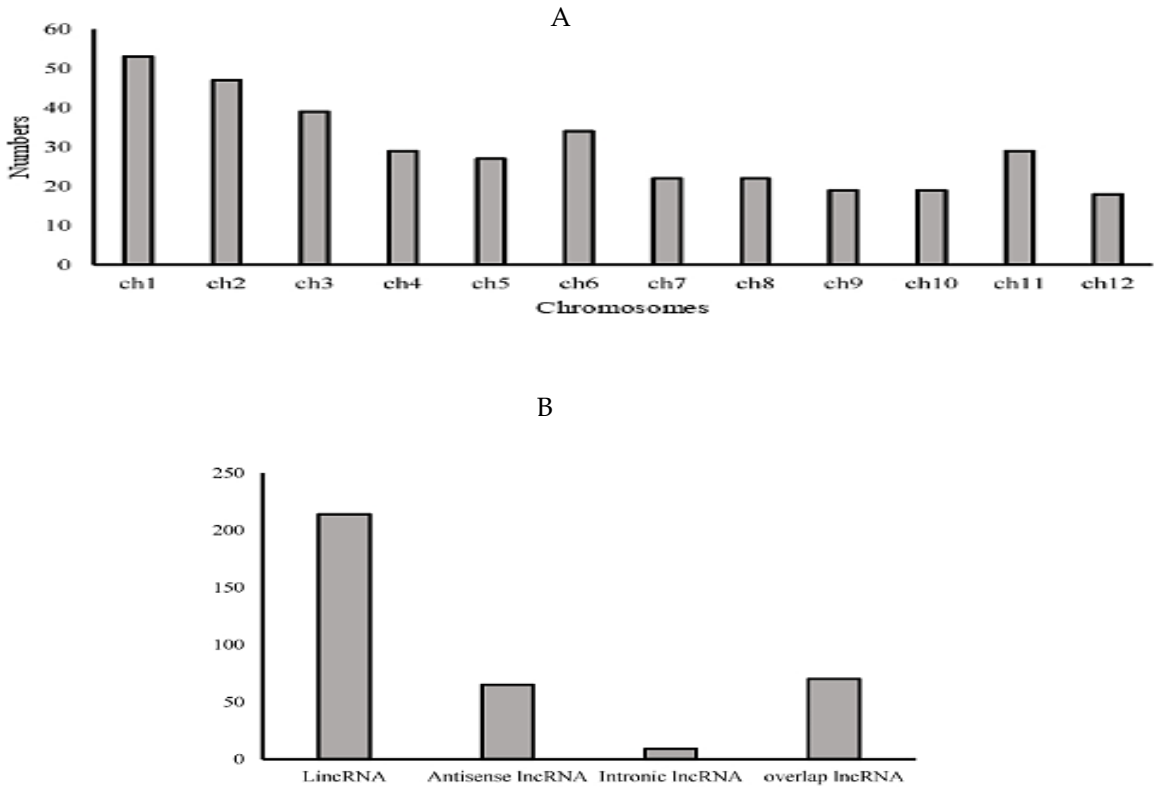


Figure 1. (A) Distribution of DElncRNAs on rice chromosomes. lncRNAs were distributed on all 12 rice chromosomes, with the highest number on chromosome 1 (53) and the lowest number on chromosome 12 (18). (B) Classification of rice lncRNAs based on genomic location. lncRNAs were divided into four categories according to their relative position compared to protein coding genes (LincRNA, Antisense RNA, Intronic RNA, Overlap lncRNA).

Target genes of DElncRNAs

In the current investigation, we examined the regulatory connections between lncRNAs and mRNAs within the root tip region of the IR64 variety. Specifically, we identified a total of 338 differentially expressed lncRNAs (DElncRNAs) that regulate 710 differentially expressed mRNAs (DEmRNAs) in a cis-regulatory manner, as indicated by their co-location within 10 kb upstream and downstream regions. Additionally, we found that 351 DElncRNAs are associated with 7535 DEmRNAs in a trans-regulatory state, established based on a minimum free energy of base pairing ($\Delta G < -30$ kcals mol-1) (Figure 2).

Functional annotation of the DElnRNAs

Gene ontology (GO) enrichment analysis of the predicted target genes of the lncRNAs showed that a total of 43 biological processes, 17 molecular functions and 38 cell components were significantly altered in response to salt treatment (Supplementary Table S3). The findings indicated that within the biological process group, the translation term featured 126 genes. In the molecular function category, the term structural molecule activity showcased an impressive 101 enriched genes. Meanwhile, within the cellular component category, the term intrinsic to membrane exhibited enrichment with 136 genes, and the ribonucleoprotein complex term featured 108 genes, demonstrating the highest rich factors. (Figure 3). Moreover, terms such as response to abiotic stimuli (GO:0009628), signal transduction (GO:0007165) and secondary metabolic process (GO:0006720) are significant in biological process (BP) and terms such as ribosome (GO:0005840) and plasma membrane (GO:0005886) are significant in cellular components (CC). Finally, in molecular functions (MF), the receptor activity term (GO:0004872) was significant (S6).

Cis-regulatory element analysis

To discover the conserved motifs and consensus cis-regulatory elements (CREs) in the promoters of DEmRNAs and DElncRNAs, we applied the MEME tool and identified 24 motifs with lengths ranging from 20 to 50 aa (Table 1). We also compared the identified motifs with known motifs in the JASPAR CORE 2022 plants database. We found that sixteen of the motifs were matched to the known motifs related to various TFs, including AP2/EREBP, CH3, C2H2 zinc finger factors, BBR/BPC, basic helix-loop-helix factors (bHLH), and other C4 zinc finger-type factors (Supplementary Table S4). GO term analysis for motifs revealed that motifs are involved in the regulation of transcription (GO:0006355), translation (GO:0006412), transmembrane receptor protein tyrosine kinase signaling pathway (GO:0007169) (Table 1, Supplementary Table S4, S5).

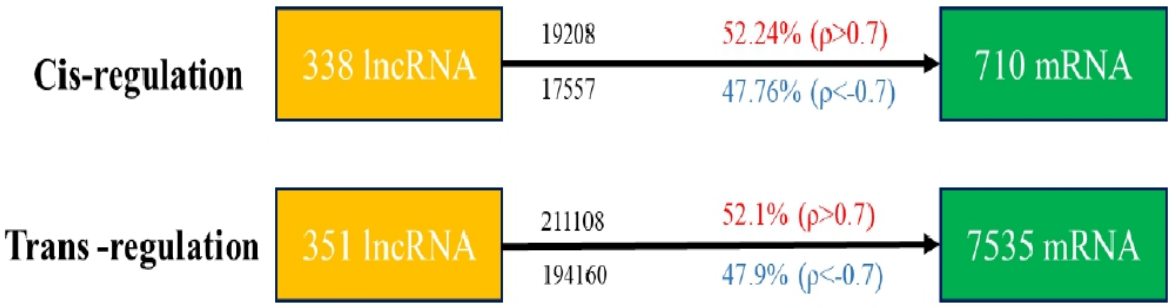


Figure 2. Summary of regulatory relationships between lncRNAs and mRNAs. The number of different combinations between lncRNAs and their target genes is marked in black. The red and blue values indicate the percentage of lncRNAs that show a positive and negative correlation with their target mRNAs, respectively. ρ is Pearson's correlation coefficient.

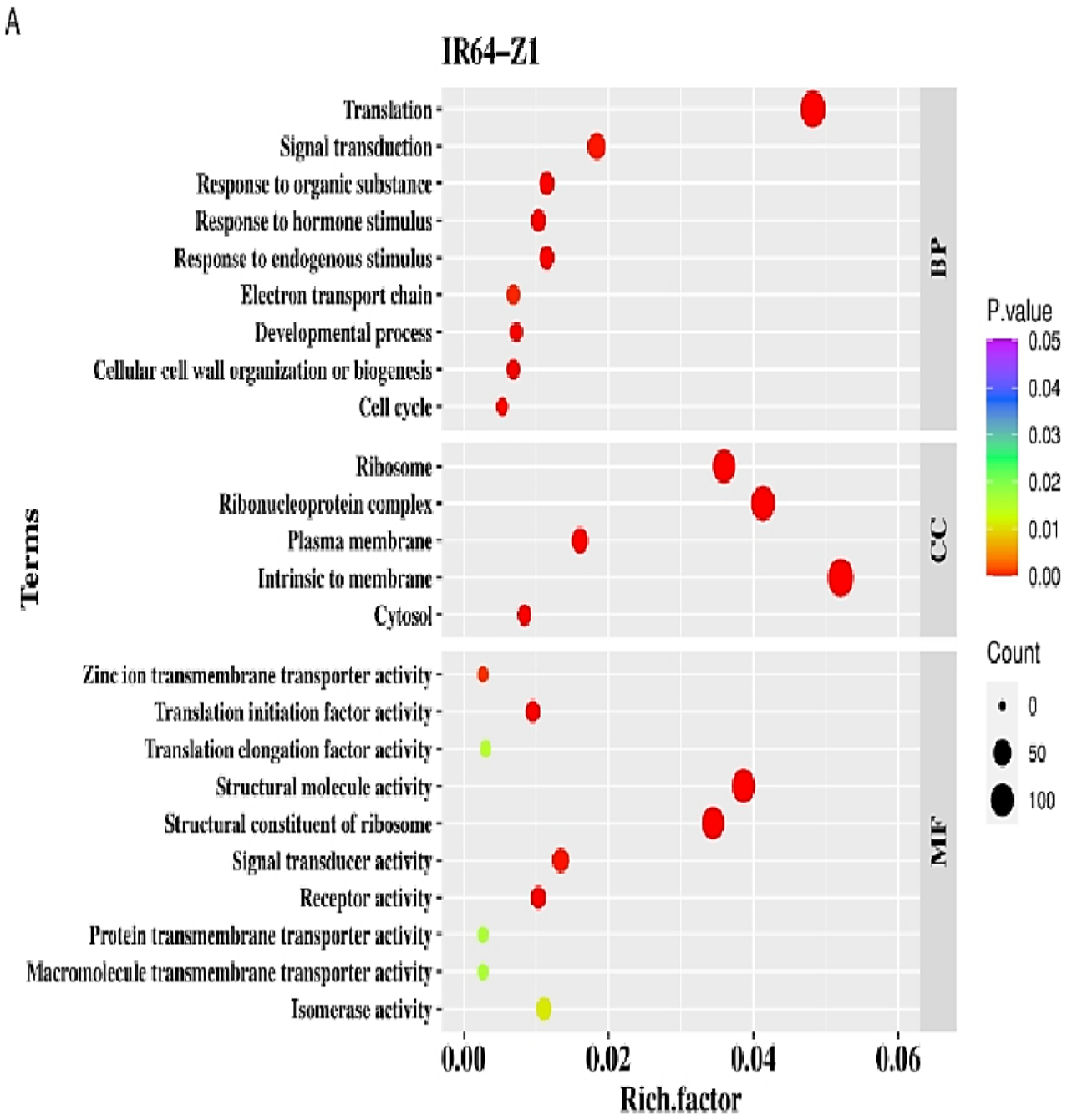
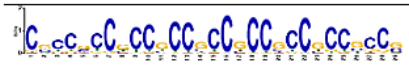
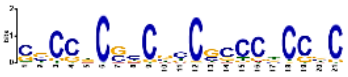
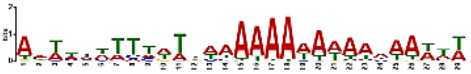
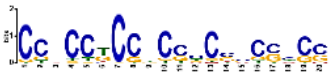


Figure 3. GO enrichment analysis of target mRNAs of DElncRNAs including biological process (BP), cellular components (CC) and molecular function (MF) categories.

Table 1. The conserved motifs found in promoter of DEmRNAs by the MEME analysis.

logo	E-value	Width	Best match in JASPAR	Significant GO term identified by GOMO
	6.1e-426	29	MA1257.1 (ERF9)	
	7.1e-249	21	MA2022.1 (LOB)	BP regulation of transcription, DNA-dependent
	6.6e-150	21	MA1404.1 (BPC1)	BP regulation of transcription
	1.4e-118	21	MA1818.1 (Zm00001d052229)	BP translation
	1.3e-134	29	MA1267.1 (DOF5.8)	
	4.1e-058	50	MA1812.1 (ASR1)	BP translation
	1.6e-055	29	MA1267.1 (DOF5.8)	
	1.3e-050	20	MA1240.1 (ERF10)	
	5.0e-033	21	MA1416.1 (RAMOSA1)	BP regulation of transcription
	9.7e-032	21	MA1063.1 (TCP19)	BP translation

Identification of lncRNAs as potential targets of miRNAs

In this study, a total of 32 miRNAs were found, all of which belonged to 14 conserved families (Supplementary Table S6). Among the detected miRNAs, the osa-miR439, osa-miR818 and osa-miR821families comprised the highest frequency with 9, 5, and 3 members, respectively. In this study, miRNAs from miR393 and miR439 and etc. families were identified.

Construction of lncRNA-mRNA network

The edge between nodes represents the interaction between these biomolecules (Shannon et al., 2003). The lncRNA-mRNA regulatory pairs were further integrated based on the common mRNA of lncRNA-mRNA co-expression interaction pairs, followed by visualization of the lncRNA-mRNA regulatory network using Cytoscape, an open-source bioinformatics software. As shown in Figure 4, the lncRNA-mRNA regulatory network is contained. We have recognized 10 pivotal lncRNAs (Os01g0768200, Os02g0686400, Os07g0203300, Os02g0229800, Os02g0216300, Os06g0692050, Os05g0138300, Os06g0308300, Os08g0553450, Os04g0474300) as central hub genes within the lncRNA-mRNA network. The target genes of these top 10 lncRNAs were analyzed ontologically in order to understand the potential function of lnRNAs during drought stress. The functional analysis revealed that they were involved in developmental process (GO:0032502), cellular response to stimulus (GO:0051716), and signaling pathway (GO:0023033).

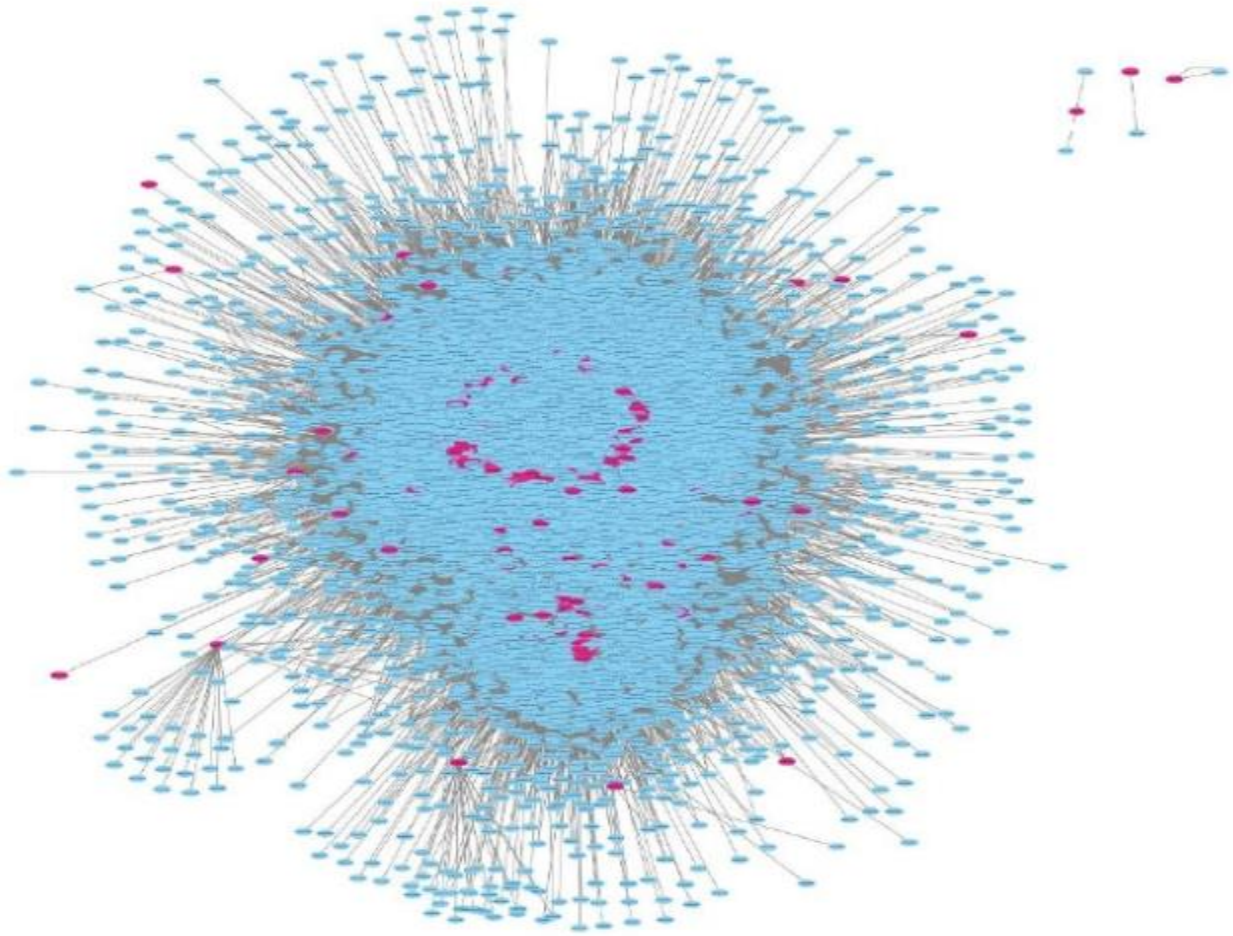


Figure 4. lncRNA-mRNA regulator network. Pink and blue circles indicate lncRNAs and mRNAs, respectively.

Discussion

Rice constitutes a crucial crop with regards to food security. However, the crop's susceptibility to yield loss due to drought stress is a notable concern. In 1985, the cultivar (lowland-*indica*) IR64 was developed by IRRI and released in the Philippines. In addition to high yield, early maturity and high disease resistance, it has excellent baking quality that matches the best available varieties. These concessions have led to its rapid expansion and cultivation in more than 10 million people in the two decades since its publication. Because of its success as a variety, it has been extensively studiedf for scientific use and is well characterized genetically. Continued basic studies on IR64 and related varieties should contribute to understanding the complex genetic control of yield and other traits of interest to rice farmers and consumers (Mackill and Khush, 2018). A dearth of knowledge exists regarding drought-responsive lncRNAs in rice. lncRNAs are a recently discovered class of molecules that serve important functions in a wide range of biological processes, including growth regulation and stress response. However, the precise mechanisms involved in these processes remain largely unknown. In the case of plants, recent evidence from various species inIicates that lncRNAs are expressed in response to multiple stresses, such as salI in *Gossypium hirsutum* (Deng et al., 2018) and *Medicago truncatula* (Wang et al., 2015), as well as heat and drought in *Brassica juncea* (Bhatia

et al., 2020). To further investigate the role of lncRNAs in response to drought stress, we conducted a study involving the IR64 cultivar, which has a shallow rooting system. The meristem region was sampled in order to gain a better understanding of the function of lncRNAs in response to drought. Since the tip includes the meristem and elongated regions where cells divide, grow and elongate and is one of the most important root factors in determining traits and adaptive strategies to water stress (Bizet et al., 2015). We selected the root tip region for further studies on root lncRNA strategy as a result of water deficit. The current study aimed to identify drought stress-responsive lncRNAs present in the root system architecture (RSA) as vital growth trait that performs a pivotal role in plant adaptation and productivity in environments constrained by water availability.

Here, a total of 1815 lncRNAs were detected the majority of them were lincRNAs. Previous studies have shown that, among lncRNAs, lincRNAs accounted for the largest number, which were observed in rice (76%) and maize (93%), respectively (Li et al., 2014; Zhang et al., 2014). Espinosa (2017) showed that lincRNAs can act as local enhancers to activate the transcription of their chromosomal neighboring genes, which emphasizes the possible important functions of lncRNAs in or nearby. In the results of research conducted on IR64 and Pokkali cultivars under salt stress, it has been shown that lncRNAs are distributed on all 12 chromosomes, and the lncRNAs that respond to salinity among the 12 chromosomes are unequal in both genetics. They have not followed a specific process similar to this research (Tiwari et al., 2023). The finding in this study further substantiate this conclusion. It is interesting that in this study, chromosomes 1, 2, and 3 have the number of lncRNA respectively. In a research conducted on the identification and functional analysis of lncRNAs on seed senescence, most lncRNAs were distributed on chromosomes 1, 2, and 3, and mRNAs and lncRNAs had a similar distribution in these chromosomes (Zhang et al., 2022). A total of 358 lncRNA genes were identified that showed differential expression in the stress response in the root tip which suggests that these lncRNAs may have a key role in the response to stress rice.

lncRNAs act by influencing gene expression and affect protein-coding genes through cis-regulation of neighboring genes and trans-regulation of distant genes (Rossetto et al., 2013). The functional enrichment analysis revealed that the potential target genes influenced by the differentially expressed lncRNAs (DElncRNAs) were primarily associated with responses to abiotic stimuli, reactions to chemical stimuli, and reactions to hormonal stimuli (Supplementary S6). Additionally, there was an enrichment of terms related to secondary metabolic processes and the transduction of hormonal signals. Moreover, an increased quantity of secondary metabolites, such as phenylpropanoids, is often observed in response to challenging environmental conditions. In many plants, endogenous phenolic compounds play a pivotal role in regulating drought tolerance mechanisms (Akula and Ravishankar, 2011). Drought stress triggers downstream pathways involving phytohormone regulation and their associated signaling pathways, consequently initiating the synthesis of diverse protective secondary metabolites. These secondary metabolites are instrumental in conferring tolerance to a wide range of stressors, including both abiotic and biotic stresses (Yadav et al., 2021).

We identified potential sequence motifs and TFs binding sites in DElncRNAs and DEmRNAs promoters. We detected 16 motifs related to the TF families such as AP2/EREBP, CH3, C2H2 zinc finger factors, BBR/BPC, basic helix-loop-helix factors (bHLH), and other C4 zinc finger-type factors. Multiple reports have indicated that all these transcription factors play a significant role in response to stress. The AP2/EREBP genes hold varied responsibilities in both developmental processes and stress-related responses in plants (Sakuma et al., 2002). In times of drought, the AP2/EREBP transcription factor regulates a multitude of target genes by binding to the cis-element (A/GCCGAC) located in the promoter region of the candidate genes (Sharoni et al., 2012). C2H2 zinc finger proteins are part of a relatively large family of transcriptional regulators in plants. Recent research has shown that C2H2 zinc finger proteins serve as crucial transcriptional regulators

in the plant's response to a broad spectrum of stress conditions such as extreme temperatures, salinity, drought, oxidative stress, excessive light, and silique shattering(Wang et al., 2019). Furthermore, these proteins can enhance drought resistance in rice plants by regulating the levels of ROS-scavenging activities, proline, H_2O_2, and other cellular components (Wang et al., 2019). The bHLH proteins are among the many transcription factors present in all eukaryotic organisms, involved in a wide range of regulatory processes (Jin et al., 2014). Overexpression of the bHLH12 gene endows plants with drought, salt, and osmotic stress resistance, whereas the bhlh122 mutant plants showed heightened sensitivity to these stressors when compared to the wild type plants. Additionally, bHLH122 regulates the expression of stress-related genes directly (Liu et al., 2014).

The lncRNAs act as target mimics of miRNAs that can affect regulation of gene expression. We identified the targets of lncRNAs on miRNAs using psRNATarget server. A total of 32 miRNAs were identified, with the majority of them belonging to the osa-miR439, osa-miR818, and osa-miR821 families. Studies have demonstrated that osa-miR821 is up-regulated in the roots of plants subjected to salt stress(Sanan-Mishra et al., 2009), while miR439 has been associated with H_2O_2 accumulation (Junhua et al., 2021). Furthermore, research has indicated that miRNA393 is involved in the heat stress response in wheat (Ragupathy et al., 2016).

We constructed a co-expression lncRNA-mRNA network to assess regulatory mechanism of lncRNAs in root tip region. Based on results, 10 hub genes, including Os01g0768200, Os02g0686400, Os07g0203300, Os02g0229800, Os02g0216300, Os06g0692050, Os05g0138300, Os06g0308300, Os08g0553450, and Os04g0474300 were identified. The function of these genes was significantly associated with developmental process, cellular response to stimulus and signaling pathway. One of the hub genes, Os02g0686400, demonstrated the maximum expression increase under drought conditions compared to control conditions. These hub genes have important targets, including the OsPIN9 gene (Os01g0802700), which has been reported to respond significantly to abiotic stress in the roots of rice seedlings (Xu et al., 2022). OsPIN9 potentially regulates abiotic stress and hormone signaling to balance plant growth and various exogenous stimuli by directing auxin flux. Another target gene is IAA13 (Os03g0742900), a candidate gene for salinity resistance. Auxin/IAA proteins are short-lived and regulate cell division and elongation to direct plant growth and development (Jung et al., 2015; Singh and Jain, 2015).

Conclusion

In the current investigation, the transcriptome analysis of the IR64 genotype with shallow roots led to the identification of a number of DE-lncRNAs, which are presumed to participate in the drought stress response of rice. The functional analysis of these lncRNAs exhibited a specific enrichment for signal transduction pathways and hormonal stimuli responses. Additionally, our findings revealed the relationship of DE-lncRNAs with several transcription factor families, including AP2/EREBP and bHLH, as well as with miRNAs such as osa-miR439, osa-miR818, and osa-miR821. Specifically, our results highlight the crucial roles played by lncRNAs, including Os01g0768200 and Os02g0686400, in the regulatory networks involved in the drought response of the root tip region. This study provides novel insights into the involvement of lncRNAs in the response to drought stress and enhances our understanding of their functions in the root tip region of rice.

Supplementary Materials

The Supplementary Material for this article can be found online at: https://www.jpmb-gabit.ir/article_709081.html

Supplementary Table S1. Transcriptome sequencing statistics.

Supplementary Table S2. List of DElncRNAs identified associated with drought stress response in IR64-Z1.

Supplementary Table S3. GO term enriched.

Supplementary Table S4. List of known motifs by Tomtom search against the JASPAR database.

Supplementary Table S5. Significant GO term enriched by GOMO analysis.

Supplementary Table S6. List of lncRNAs as potential target of miRNAs.

Author Contributions

Conceptualization, A.N. and S.E.T.; Methodology, S.E.T. and A.T.; software, S.E.T.; formal analysis, S.E.T. and A.T.; Investigation, A.N., S.E.T., A.A. and M.R.G.; Data curation, S.E.T.; Writing – original draft, S.E.T. and A.T.; Visualization, S.E.T.; Writing – review & editing, A.N., A.T., A.A. and M.R.G. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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شناسایی RNAهای طولانی غیر کدکننده (lncRNAs) پاسخگو به تنش خشکی در ناحیه نوک ریشه برنج

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چکیده: خشکسالی تولید جهانی برنج را به شدت تحت تاثیر قرار می دهد. شواهد اخیر، نقش کلیدی که RNAهای طولانی غیرکدکننده (lncRNAs) در پاسخ به استرس غیرزیستی ایفا می‌کنند، را نشان می‌دهند. در این مطالعه، با استفاده از آنالیز توالی‌یابی رونوشت‌های lncRNAهای ناشی از تنش خشکی در ناحیه نوک ریشه برنج روی نهال‌های ژنوتیپ حساس برنج (IR64) تحت شرایط خشکی و کنترل شناسایی شد. در مجموع ۳۵۸ lncRNA با بیان متفاوت (DElncRNA) شناسایی شد که بیش از ۶۰ درصد آنها در مناطق بین ژنی بودند. نتایج نشان داد که DElncRNAها می‌توانند به طور مستقیم یا غیرمستقیم ۷۱۰ و ۷۵۳۵ mRNA تنظیم کنند. علاوه بر این، DElncRNA بر روی ژن‌های هدف مرتبط با ژن‌های مقاومت به خشکی، رشد ریشه جانبی و ژن‌های مؤثر بر انتقال اکسین اثر داشته‌اند. همچنین ۲۴ موتیف حفاظت‌شده در نواحی بالادست DElncRNAها و mRNAها با بیان متفاوت (DEmRNA) شناسایی گردید. تجزیه و تحلیل عملکردی این موتیف‌ها نشان دهنده دخالت آن‌ها در تنظیم رونویسی، ترجمه و مسیر سیگنالینگ پروتئین گیرنده غشایی تیروزین کیناز بود. با استفاده از آنالیز شبکه DElncRNA و DEmRNA، ۱۰ lncRNA به عنوان ژن‌های مرکزی شناسایی شدند. تجزیه و تحلیل عملکردی نشان داد که این ژن‌ها در فرآیندهای رشد، پاسخ‌های سلولی به محرک‌ها و مسیرهای سیگنال‌دهی دخالت دارند. این نتایج دیدگاه جامعی را در مورد lncRNAهای بالقوه کاربردی و بینشی در مورد مکانیسم‌های مولکولی نهفته در مقاومت به خشکی در ناحیه نوک ریشه برنج ارائه می‌دهد.

کلمات کلیدی: تنش خشکی، برنج، رونوشت، نوک ریشه، lncRNAs.

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