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High throughput deep sequencing elucidates the important role of lncRNAs in Foxtail millet response to herbicides

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ABSTRACT

Long non-coding RNAs (lncRNAs) play an important function in plant growth and development as well as response to stresses. However, little information was known in foxtail millet; no study was reported on lncRNAs in plant response to herbicide treatment. In this study, by using deep sequencing and advanced bioinformatic analysis, a total of 2547 lncRNAs were identified, including 787 known and 1760 novel lncRNAs. These lncRNAs are distributed across all 9 chromosomes, and the majority were located in the intergenic region with 1–2 exons. These lncRNAs were differentially expressed between different genotypes under different herbicide treatments. lncRNAs regulate plant growth and development as well as response to herbicide treatments through targeting protein-coding genes that directly relate to chemical metabolism and defense system. Multiple potential target genes and lncRNA-mRNA-miRNA gene networks were discovered. These results elucidate the potential roles of lncRNAs in plant response to herbicides.

1. Introduction

Foxtail millet (*Setaria italica*), originated in China, is an important food and feed crop. Foxtail millet owns many elite traits, including high resistance to abiotic stresses; thus it is widely grown in arid and semi-arid regions in the world, particularly at Asia and Africa [1]. Due to its small genome size, lower repetitive DNA, inbreeding nature and short life cycle, foxtail millet has been becoming an ideal model monocot plant for genetic and genomic studies [2]. The published complete genome of foxtail millet is enhancing the research of molecular biology and functional genomics in foxtail millet [3–5].

Weeds are one of the most serious constraints on the yield of most crops in the world, which compete with crops for light, water, nutrients, and space. Controlling weeds requires a lot of labor, equipment and chemicals [6,7]. Currently, the most effective way to control weeds is to spray herbicides in the fields [8]. Imidazolinones are commonly used herbicides in the field, which are widely used in the field of alfalfa, peanuts, imidazolinone-tolerant maize, oilseed rape, rice, wheat, and sunflowers. Imidazolinone herbicides are highly active with broad-spectrum; they are not only used to control annual gramineous grasses and broadleaf weeds, but also perennial weeds [9,10]. Imazamox, imazapic, and imazaquin are a couple of commonly used imidazolinone herbicides. Imidazolinone herbicides control weeds by inhibiting acetohydroxyacid synthase activity (AHAS, EC 2.2.1.6), also known as acetolactate synthase (ALS), which is responsible for the biosynthesis of branched-chain amino acids in plants [11]. The imidazolinone herbicides are also effectively used for weed control in foxtail millet fields. However, the response of different cultivars to imidazolinone herbicide varies greatly; in many case, imidazolinone herbicides also affect the growth of sensitive cultivars.

Non-coding RNAs (ncRNAs) are an extensive class of RNAs, which do not encode proteins. Although they were considered as genome noise previously, more and more evidences recently show that they play an important regulate function of gene expression in plant development as well as response to different environmental biotic and abiotic stresses [12–16]. According to their length, ncRNAs can be classified into small ncRNAs (miRNAs, siRNAs, and piRNAs) and long ncRNAs (lncRNAs) [17]. In the last decade, many studies have demonstrated that small ncRNAs, particularly miRNAs, are an important gene regulator regulates gene expression through translational repression or the degradation of target mRNAs [18–20].

Long non-coding RNAs (lncRNAs) are transcripts with more than

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200 nucleotides but don't encode proteins [21,22]. The first lncRNAs were reported in mice [23]. As the rapid development and application of the next-generation sequencing technology, it becomes capable to identify and characterize these lncRNAs from short read strand-specific paired-end RNA sequencing data. Thus, an increasing number of studies about lncRNAs have been performed in plants, including maize [24,25], rice [26,27], *Arabidopsis thaliana* [28], wheat [29,30], barley [31], peach [32], Populus [33], cotton [34], and soybean [35]. These lncRNAs are widely involved in diverse biological processes, including gene silencing, responses to environmental stimulus, RNA alternative splicing, translational control and chromatin modification through regulation of target gene expression by cis- or trans-action [28,36–39]. For example, deep-sequencing was used to identify lncRNAs involving in rubber biosynthesis in *Eucommia ulmoides* [40], and to detect 16,551 novel lncRNAs associated with multiple dehydration stresses in switchgrass [41]. Chen and collogues [26] identified 144 differentially expressed lncRNAs affecting roots development at an early stage in rice response to cadmium stress [26]. Wang et al. [16] identified an *Arabidopsis* noncoding RNA (HID1), as a factor promoting photomorphogenesis in continuous red light [16]. However, no study has been reported that if lncRNAs is involved in plant response to herbicide exposure, one common chemicals used in all the field and there is no report on lncRNAs in foxtail millet.

In this study, strand-specific paired-end deep sequencing technology and bioinformatic analysis were employed to identify the lncRNAs associated with plant response to herbicides treatment using both resistant and sensitive foxtail millet cultivars. A total of 2547 lncRNAs were identified. The expression patterns of lncRNAs were profiled and their targets were identified according to co-expression and genomic co-location. Functional analysis of the target genes of differentially expressed lncRNAs provide insights into the potential roles of lncRNAs in response to herbicide stress. These results will help to understand the molecular mechanism on plant response to herbicide treatment and provide fundamental knowledge for crop improvement for breeding new cultivars with high tolerance to herbicides.

2. Materials and methods

2.1. Plant materials and treatments

Seeds of both foxtail millet (*Setaria italica* L.) herbicide resistant cultivar 5058 (R) and herbicide sensitive cultivar Yugu 18 (S) were collected from the Institute of Foxtail Millet, Anyang Academy of Agriculture Sciences, Henan, China. Yugu 18 is one parents of 5058 (R) that were bred by continuous self-crossing after Yugu18 was crossed with a germline with herbicide resistance. During summertime, the seeds were planted at the experimental filed station at Anyang Institute of Technology with traditional agronomic practices. After 15 days of seed germination, the seedlings were treated evenly by spraying with herbicide imazapic (BASF SE, Germany) at 300 ml ha^{-1} as suggested by the manufactory's recommendation. The untreated plants were served as control, in which the same amount of water was applied in the field. The first fully-opened young leaves were collected from both control and treated plants at 0 h (control) and 48 h of treatment. The samples were immediately frozen in liquid nitrogen and then stored at −80 °C for total RNA extraction. Each treatment was sampled for three time as three biological replications at each time point for each cultivar.

2.2. RNA extraction, cDNA library construction and sequencing

Total RNAs were extracted from young leaf tissues of R and S plants using RNeasy mini kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol, respectively. The purity, concentration and integrity of RNAs were assessed using the NanoPhotometer® Spectrophotometer (IMPLEN, CA, USA) and Qubit® RNA Assay Kit in Qubit® 2.0 Flurometer (Life Technologies, CA, USA).

A total of 15 μg RNA for each sample was used to make sequencing library. Each sequencing library was prepared by NEB Next® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer's instruction; the index codes were added to label the sequences of each sample. Briefly, mRNA was first purified from total RNAs using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in NEB Next First Strand Synthesis Reaction Buffer (5×). By using random hexamer primers and M-MuLV Reverse Transcriptase, first strand cDNA was synthesized followed by synthesizing the 2nd strand of cDNAs. After adenylation of 3′ ends of DNA fragments, NEB Next Adaptor with hairpin loop structure were ligated to prepare for hybridization. To select cDNA fragments with right size, the AMPure XP system (Beckman Coulter, Beverly, USA) was employed to purify the library fragments. Following, 3 μl USER Enzyme (NEB, USA) was added into the size-selected, adaptor-ligated cDNA at 37 °C for 15 min. Finally, by using Phusion High-Fidelity DNA polymerase, PCR was performed with the Universal PCR primers and Index (X) Primer. PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system.

The sequencing was performed by a commercial company, named HonorTech (Beijing, China). According to the manufacturer's instructions, the index-coded samples was clustered on a cBot Cluster Generation System using HiSeq 4000 PE Cluster Kit (Illumia). After the clustering, the libraries were sequenced on an Illumina Hiseq 4000 platform and 150 bp paired-end reads were generated. The sequencing data were deposited to National Center for Biotechnology Information (NCBI) (BioProject accessions: PRJNA590609; BioSample accessions: SAMN13335985, SAMN13335986, SAMN13335989, and SAMN13335990).

2.3. LncRNA identification pipeline

The cutadapt (v.1.6) program was employed to remove poor quality sequences and trim the adaptor sequences from the raw sequence data [42]. The clean sequencing data for each sample were aligned to reference genome (GCF_000263155.2_Setaria_italica_v2.0_genomic.fna) combined with known gene annotation (GCF_000263155.2_Setaria_italica_v2.0_genomic.fmt.gtf) [3] by using improved version STAR software (v.2.5). Cufflinks (v2.2.1) [43] with the parameter "–library-type fr-firststrand" was used to reconstruct transcripts for each sample, then StringTie (v1.2.4) [44] was used to merge transcripts of samples. For quantification, the normalize expression value 'TPM' (transcript per Million mapped reads) was calculated by RSEM software (v1.2.9) [45]. The detailed pipeline for identifying lncRNAs were listed in Fig. 1.

In order to identify high-confident lncRNAs, transcripts with length longer than 200 bp were retained. Coding Potential Calculator (CPC, version 0.9r2), Coding-Non-Coding Index (CNCI, version v2), and Coding-Potential Assessment Tool (CPAT version 1.2.2) were employed to assess the coding probability of transcripts. Transcripts with coding potential predicted by any of the three tools previously described were filtered out, and those without coding potential were retained. The remaining transcripts were further filtered to exclude those that have significant alignment with housekeeping RNAs ($p < 1.0E-10$), including ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), and signal recognition particles. The remaining transcripts were the identified lncRNAs and used for further analysis.

2.4. Identification of differentially expressed lncRNAs and mRNAs

The R language (v3.2.1) with edgeR package was used to identify the differential expression lncRNAs and mRNAs [46]. LogFC = log2 was used to calculate the fold change between the the control and the treatment groups. Genes in two groups, whose $|logFC| > 1$ and p value < 0.05, were assigned as deferentially expressed.

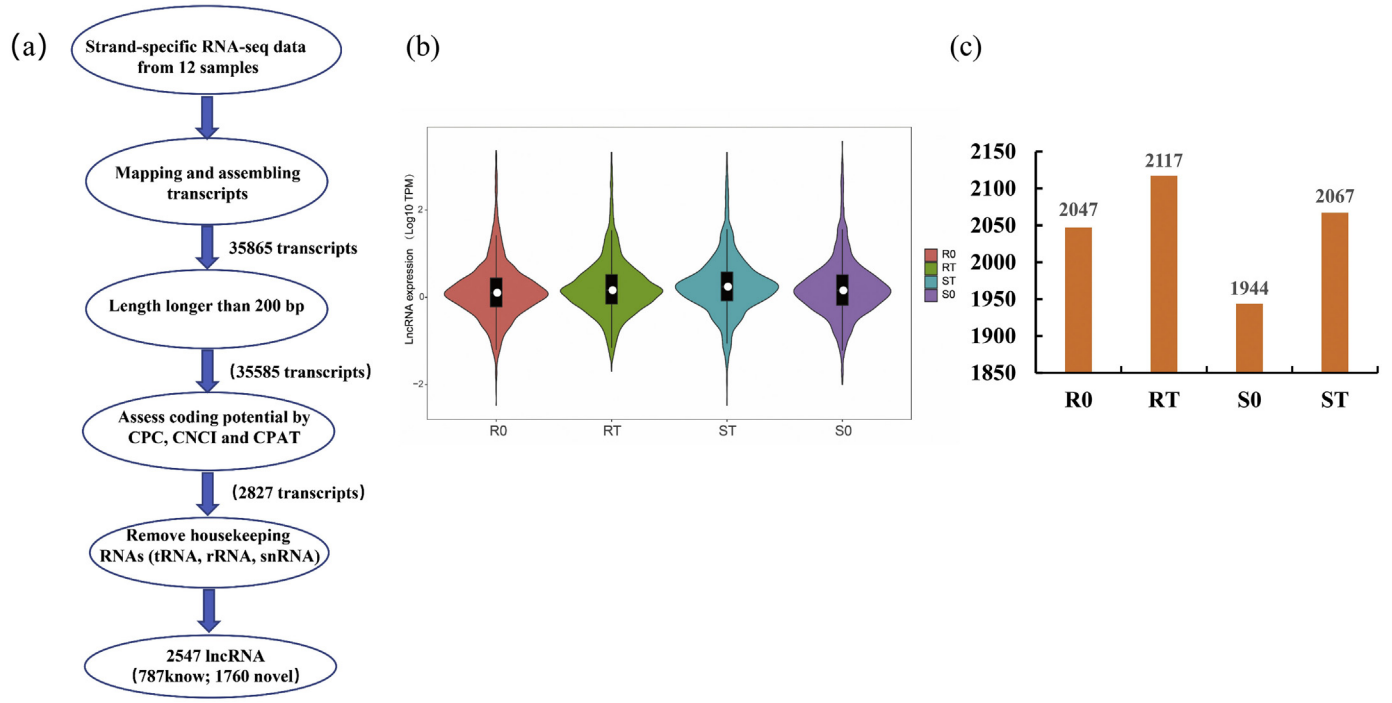

Fig. 1. Pipeline for the identification and expression of lncRNAs in foxtail millet using high throughput deep sequencing. (A) Pipeline for the identification of foxtail millet lncRNAs. (B) Expression density of lncRNAs. (C) Numbers of expressed lncRNAs in each cultivar under different herbicide treatment.

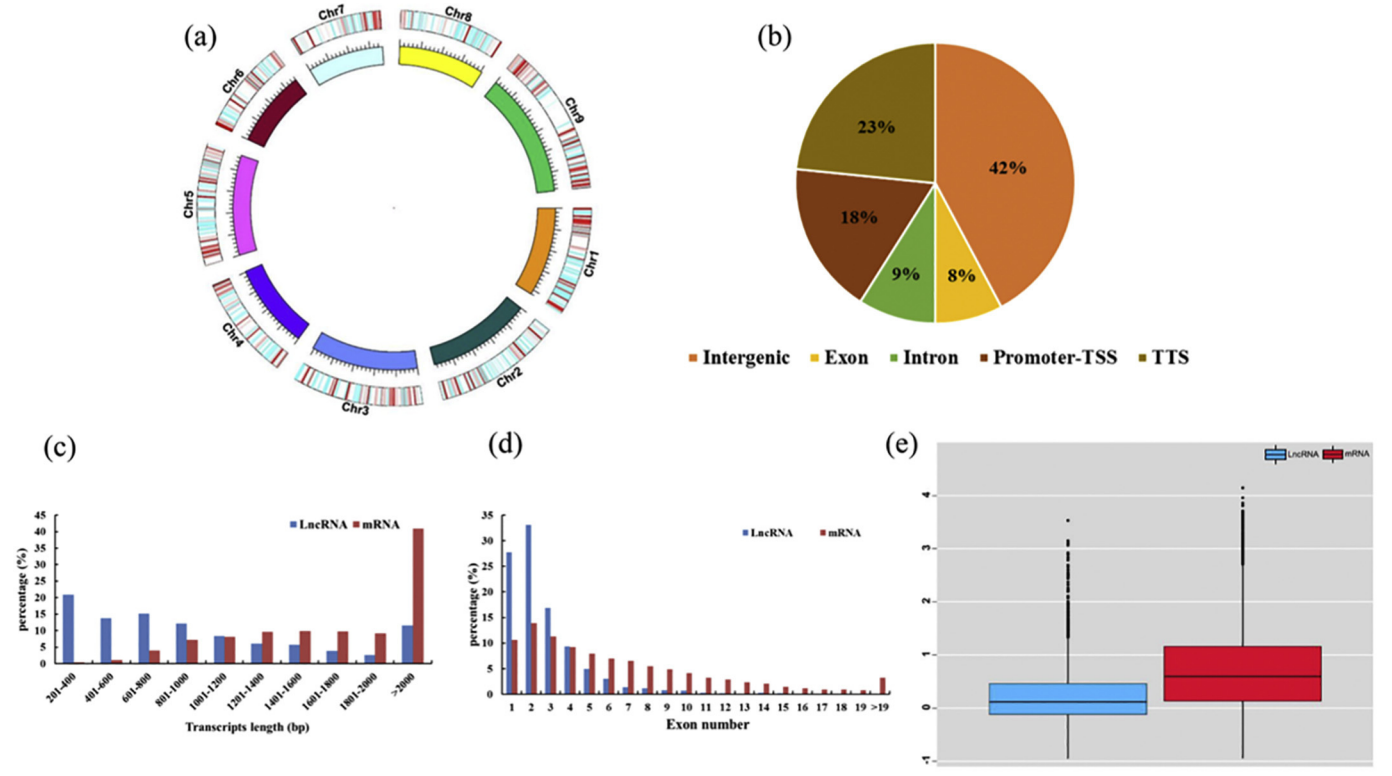

Fig. 2. Distribution and expression of lncRNA in foxtail millet. (A) Distribution of lncRNAs on each chromosome. (B) Genomic positions of lncRNAs. (C) Comparison of the transcript length between lncRNAs and mRNA. (D) Comparison of the exon number between lncRNAs and mRNA. (E) Comparison of the expression level between lncRNAs and mRNA.

2.5. LncRNA target gene prediction and functional enrichment analysis

To find the cis targets, the coding genes sitting at 10 kb/100 kb upstream and downstream of each identified lncRNAs were Blast searched. We identified trans target genes of lncRNAs through the expression level. The expressed correlation between lncRNAs and coding genes was calculated with custom scripts and the mRNAs with Pearson's correlation coefficients > 0.90 or < -0.90 were used for functional

Table 1
Numbers of differentially expressed lncRNAs (DEL).

Comparison[a]	Numbers of DELs	Number of up-regulated DELs	Number of down-regulated DELs
RT vs R0	41	28	13
ST vs S0	296	155	141
RT vs ST	161	88	73
R0 vs S0	98	58	40

[a] RT: herbicide treated 48 h of herbicide-resistant cultivar.
R0: untreated control for herbicide-resistant cultivar.
ST: herbicide treated 48 h of herbicide-sensitive cultivar.
S0: untreated control for herbicide-sensitive cultivar.

enrichment analysis [40].

GO (GO, http://www.geneontology.org/) and functional enrichment analysis were performed on all target genes of lncRNAs using TermFinder software (http://www.yeastgenome.org/help/analyze/go-term-finder). These target genes were also mapped to a pathway in the KEGG database (http://www.genome.jp/kegg/pathway.html) using Blast_v2.2.26 software. A hyper geometric distribution test was carried out to identify GO (p-value < 0.01), comparing to total expressed genes.

2.6. Co-expression network construction

To identify key lncRNAs and mRNAs related to herbicides tolerance, a co-expression correlation coefficient (PCC) of expression patterns were calculated using the expression values [47]. Then, the co-expression network was visualized by Cytoscape software [48].

2.7. Quantitative real-time PCR (qRT-PCR) analysis

qRT-PCR was employed to validate the expression of differentially expressed lncRNAs. Briefly, total RNAs were isolated from foxtail millet leaves using TRIzol reagent (Tiangen, Beijing, China). First-strand cDNA synthesis was performed using a FastKing RT kit (Tiangen). qRT-PCR was performed on an ABI 7500 (Applied Biosystems, Foster City, CA, USA) using SYBR Green PCR Master Mix Reagent (SuperReal PreMix Plus, Tiangen, China). Relative abundance of mRNAs was determined by qRT-PCR according to the $2^{-\Delta\Delta Ct}$ method [49]. The foxtail millet *Actin 7* (AF288226.1) gene was used as a reference gene. Three biological replicates were performed for each gene. The primers for PCR used in gene expression analysis were listed in the supplementary materials (Table S4).

3. Results

3.1. Identification of lncRNAs in foxtail millet

In order to identify the lncRNAs involved in herbicide resistance in foxtail millet, a strand-specific deep sequencing technology was employed to sequence all potential lncRNAs in two foxtail millet cultivars (one is herbicide-sensitive and another one is herbicide-resistant) by using the llumina Hiseq 4000 platform. Strand-specific deep sequencing has advantages over the non-stand specific sequencing, which can be used to distinguish the non-sense strand from a sense strand that gives

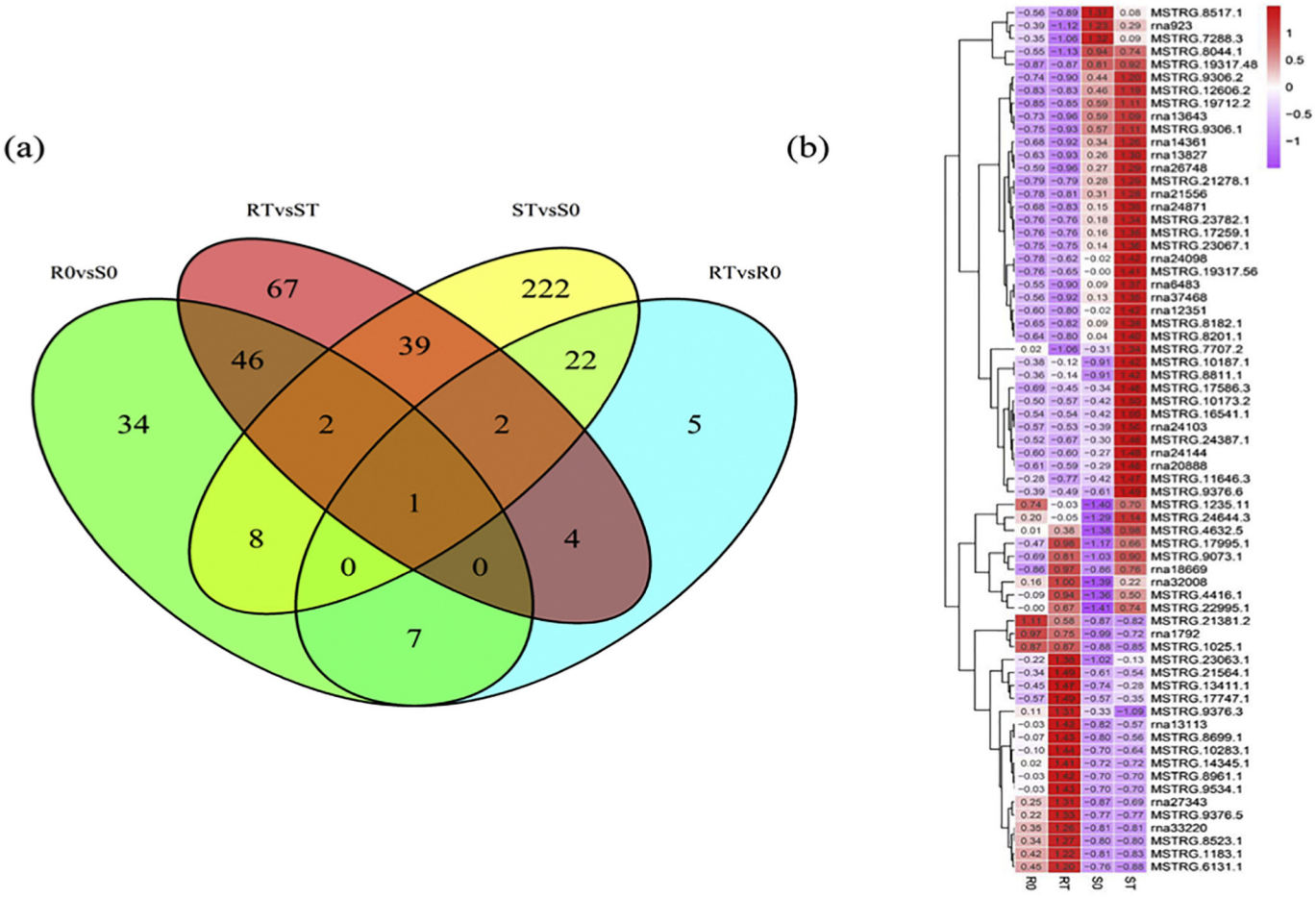

Fig. 3. Differently expressed lncRNAs in foxtail millet. (A) Venn diagram showing differently expressed lncRNAs. (B) Heatmap showing the expression levels of unique differently expressed lncRNAs between herbicide-resistant and sensitive cultivars after herbicide treatment.

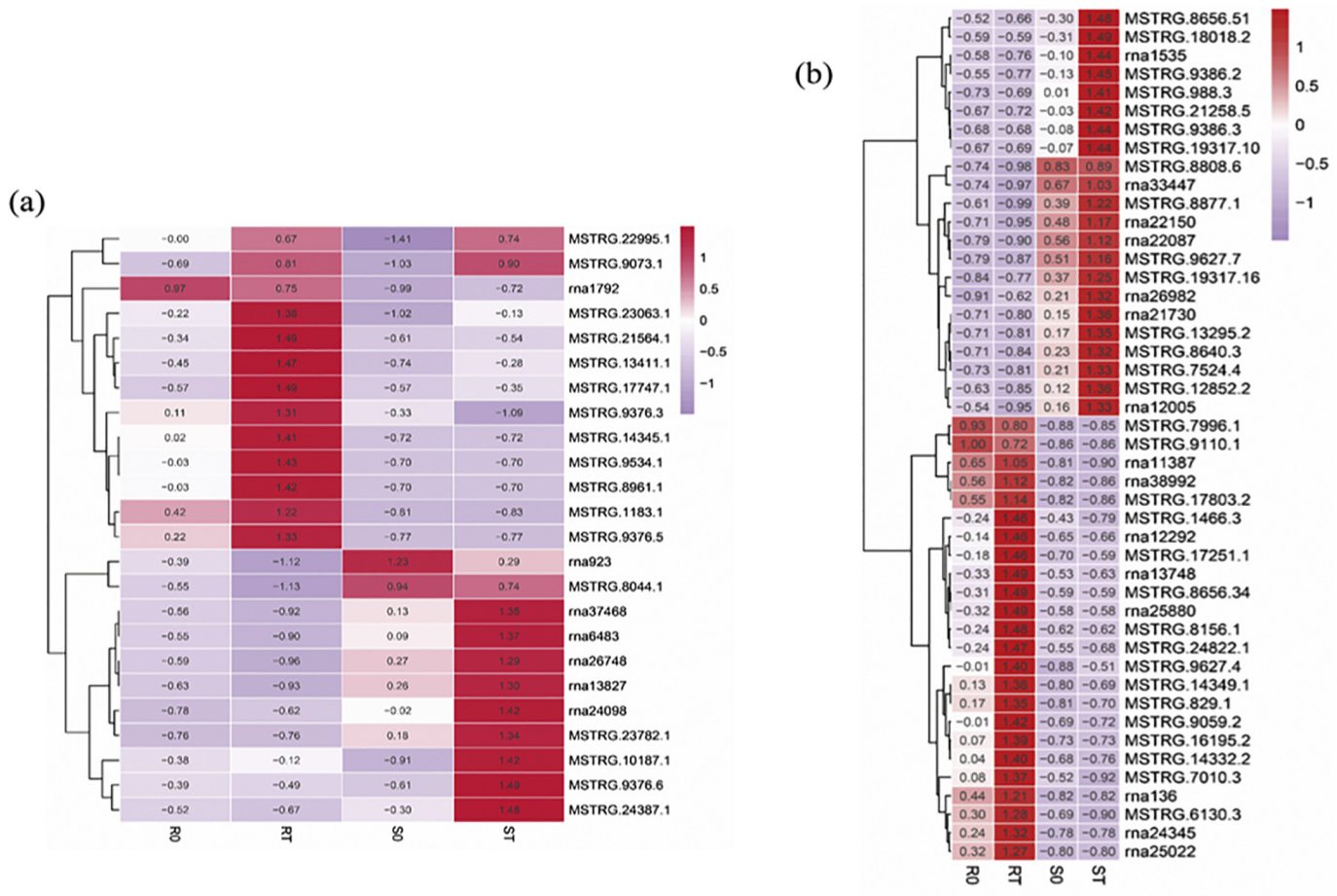

Fig. 4. Expression levels of lncRNA-mRNA pairs. (A) Heatmap showing the expression levels of 24 DELs. (B) Heatmap showing the expression levels of 46 DEGs.

more confidence on identifying lncRNAs. A total of 394,756,182 clean reads were obtained with an average of 32,896,348 clean read for each sample. Of these clean reads, 88.99%–93.53% were mapped to the foxtail millet genome (Supplementary Table S1). A strict pipeline was used to identify the lncRNAs as described in material and method (Fig. 1a). After removing housekeeping RNAs (tRNA, rRNA and snRNAs) and potential coding sequence and considering the criteria of lncRNAs, a total of 2547 lncRNAs were identified in foxtail millet. Among these identified lncRNAs, 787 were known lncRNAs and 1760 were novel lncRNAs. A novel lncRNA referred that an identified lncRNA has not been reported previously.

3.2. LncRNA characterization and expression analysis

The 2547 identified lncRNAs were distributed across all nine chromosomes. The majority of identified lncRNAs were located on the arm regions of chromosomes, and they are preferred to sit at the far end of the chromosomes with high densities (Fig. 2a).

LncRNAs were found to locate anywhere in the chromosome (Fig. 2b). Among these identified ncRNAs, 42% was located in the intergenic region, 18% was located in the promoter-TSS region (within 2000 bp upstream of the transcription start site), and 23% was located in TTS (within 2000 bp downstream of the transcription termination site). The remaining lncRNA was either located in the intron region (9%) or overlaps with the protein coding sequence (8%) (Fig. 2b). The distribution ratio was consistent with previous studies, and most lncRNA were located in the intergenic region.

Compared with mRNAs, the length distribution had different pattern (Fig. 2c). There are more than 20% of lncRNAs only had 200–400 nt in length. There are also about 15% of lncRNAs with 401–600, 601–800, 801–1000 nt in length, respectively. After that, the number of lncRNAs was significantly reduced. There was only 11.62% of identified lncRNAs with length more than 2000 nt (Fig. 2c). However, the length of mRNAs showed the reverses pattern with the majority of mRNAs were more than 2000 nt in length (Fig. 2c).

Both lncRNAs and mRNAs conatined introns and exons in foxtail millet (Fig. 2d). The exon numbers of lncRNAs and mRNAs were different and lncRNAs had less exons than that mRNAs had. A large proportion (60.86%) of lncRNAs had no more than two exons, while only 24.56% mRNAs contained only 1 to 2 exons, furthermore, 3.21% mRNAs had more than 19 exons (Fig. 2d).

3.3. Identification of differentially expressed lncRNAs

All 2547 identified lncRNAs were expressed in foxtail millet and the expression patterns were similar with each other in the two cultivars before and after herbicide treatment (Fig. 1b). Not all of the 2547 identified lncRNAs were always expressed in all samples. Compared with the two cultivars, there were more lncRNAs expressed in herbicide-resistant cultivars than that in the herbicide-sensitive cultivar; herbicide treatment induced more lncRNAs expressed (Fig. 1c). At the herbicide-sensitive without herbicide treatment, there were only 1944 lncRNAs expressed while there were 2117 lncRNAs expressed in the herbicide-resistant cultivar with herbicide treatment (Fig. 1c).

Compared the expression of protein-coding genes (mRNAs), the expression levels of lncRNAs were generally low (Fig. 1b and 2e). In the herbicide-resistant cultivar, without herbicide treatment, there were 500 lncRNAs no expressed, 830 lncRNAs were expressed with less than 1 RPKM, Transcripts per million reads), 525 lncRNAs were expressed with 1–2 RPKM, 220 with 2–3 RPKM, 103 with 3–4 RPKM; there were

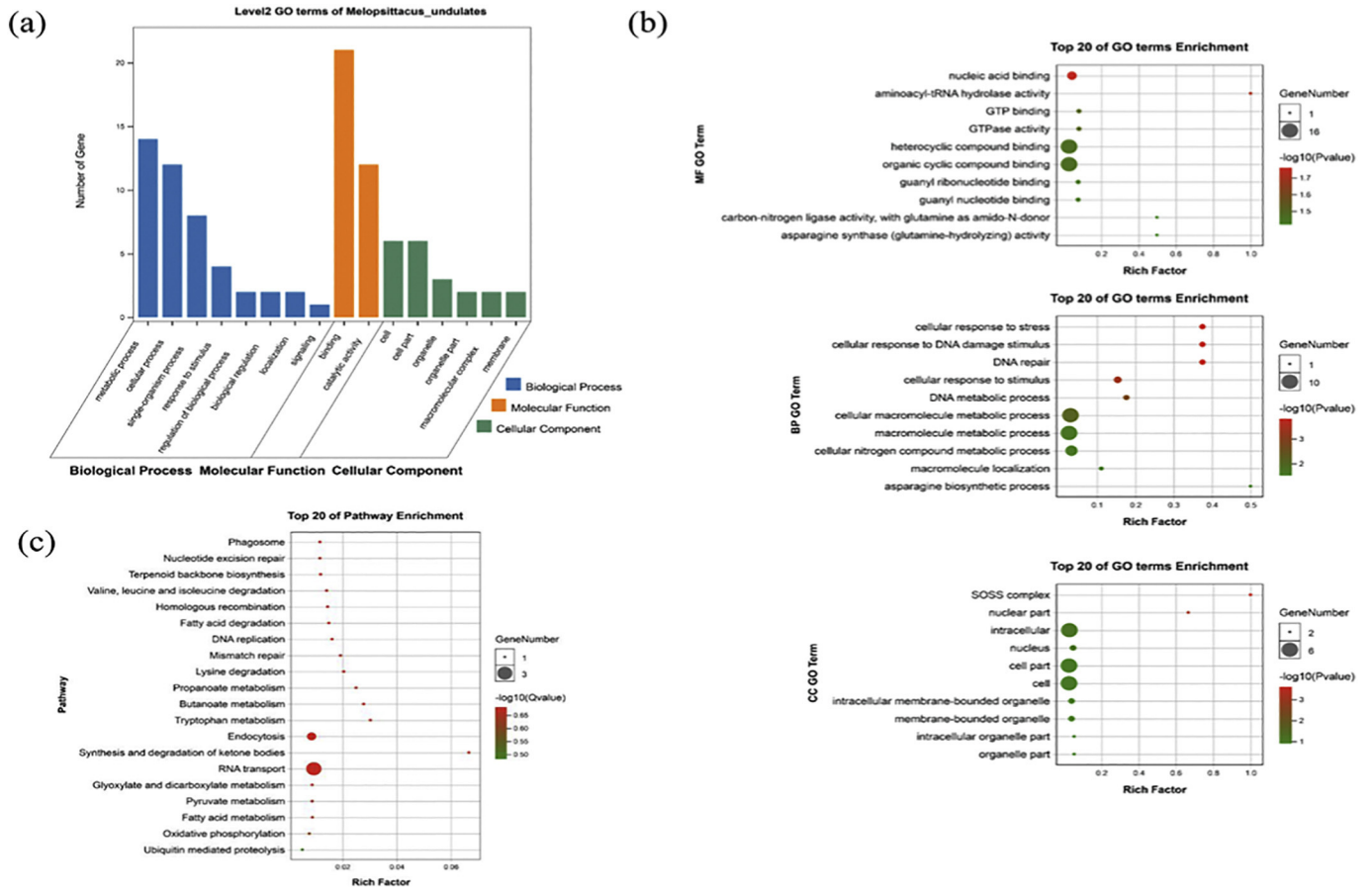

Fig. 5. Function analysis of unique DEL Targets. (A) GO analysis of target genes. (B) Gene functions significantly enriched in MF, BP and CC terms. (C) Gene functions significantly enriched KEGG pathways.

Table 2
lncRNAs and genes targeted by miRNAs.

miRNA	miRNA-sequence	Target	Expectation	UPE$	Inhibition Target_Desc
sit-miR62	UCGAAGUUCAUGGACCUGGAU	(LncRNA) MSTRG.10187.1	3.0	13.92	Cleavage
sit-miR399	UCUGUGCCAAAGGAGAGCUGC	(Gene) MSTRG.7524.4 (AT1G64140.1)	2.5	13.501	Cleavage

also 307 lncRNA with expression or more than 5 RPKM. Among them, there are 5 lncRNAs with expression of more than 1000 FPKM; these 5 lncRNAs were gene 32,039, MSTRG.26555.2, rna34170, gene32028 and MSTRG.39.1. In herbicide-sensitive cultivar and also in both treated and untreated conditions, the expression of lncRNAs show the similar pattern (Fig. 1b).

Imazapic herbicide treatment altered the expression of lncRNAs in both herbicide-resistant and herbicide-sensitive cultivars (Table 1). In herbicide-resistant cv 5058, imazapic herbicide treatment significantly altered the expression of 41 lncRNAs, among them, 28 were up-regulated and 13 were down-regulated by herbicide. Compared with the herbicide-resistant cultivar 5058, herbicide-sensitive cultivar Yu 18 was more sensitive to herbicide treatment, there were a total of 296 lncRNAs with differential expression after herbicide treatment in Yu 18; among them, 155 lncRNAs were up-regulated and 141 were down-regulated.

There was also difference between herbicide-resistant and herbicide-sensitive cultivars no matter under herbicide treatment or not (Table 1). Without herbicide treatment, the expression of 98 lncRNAs were different between herbicide-resistant and herbicide-sensitive cultivars; among them, 58 lncRNAs were expressed with higher levels in herbicide-resistant cultivars than that in herbicide-sensitive cultivar. In contrast, there were also 40 lncRNAs whose expressions were lower in herbicide-resistant cultivars than that in herbicide-sensitive cultivar. Herbicide treatment enhanced this difference. Under herbicide treatment, 161 lncRNAs were differentially expressed between herbicide-resistant and herbicide-sensitive cultivars; among those, 88 lncRNAs were expressed with higher levels in herbicide-resistant cultivars than that in herbicide-sensitive cultivar; at the same time, 73 lncRNAs were expressed with lower levels in herbicide-resistant cultivars than that in herbicide-sensitive cultivar. Among all treatment and gentypes, herbicide treatment induced more lncRNAs with differential expression in herbicide-sensitive cultivar than all others.

Venn diagram was performed to identify the common and unique DELs between different cultivars, treatment and between different comparisons of groups (Fig. 3a). For example, there are a total of 296 differentially expressed lncRNAs (DEL) identified in herbicide-sensitive cultivars with herbicide treatment and the controls. Among these 296 DELs, 222 was only differentially expressed in herbicide-sensitive cultivar between herbicide treatment and the controls. There were also 22 DEL lncRNAs with differential expression in both group of herbicide-resistant and -sensitive cultivars with/without herbicide treatments, respectively. Between the treatment group of herbicide-resistant and herbicide-sensitive cultivars, there were 67 unique DEL, of which 32

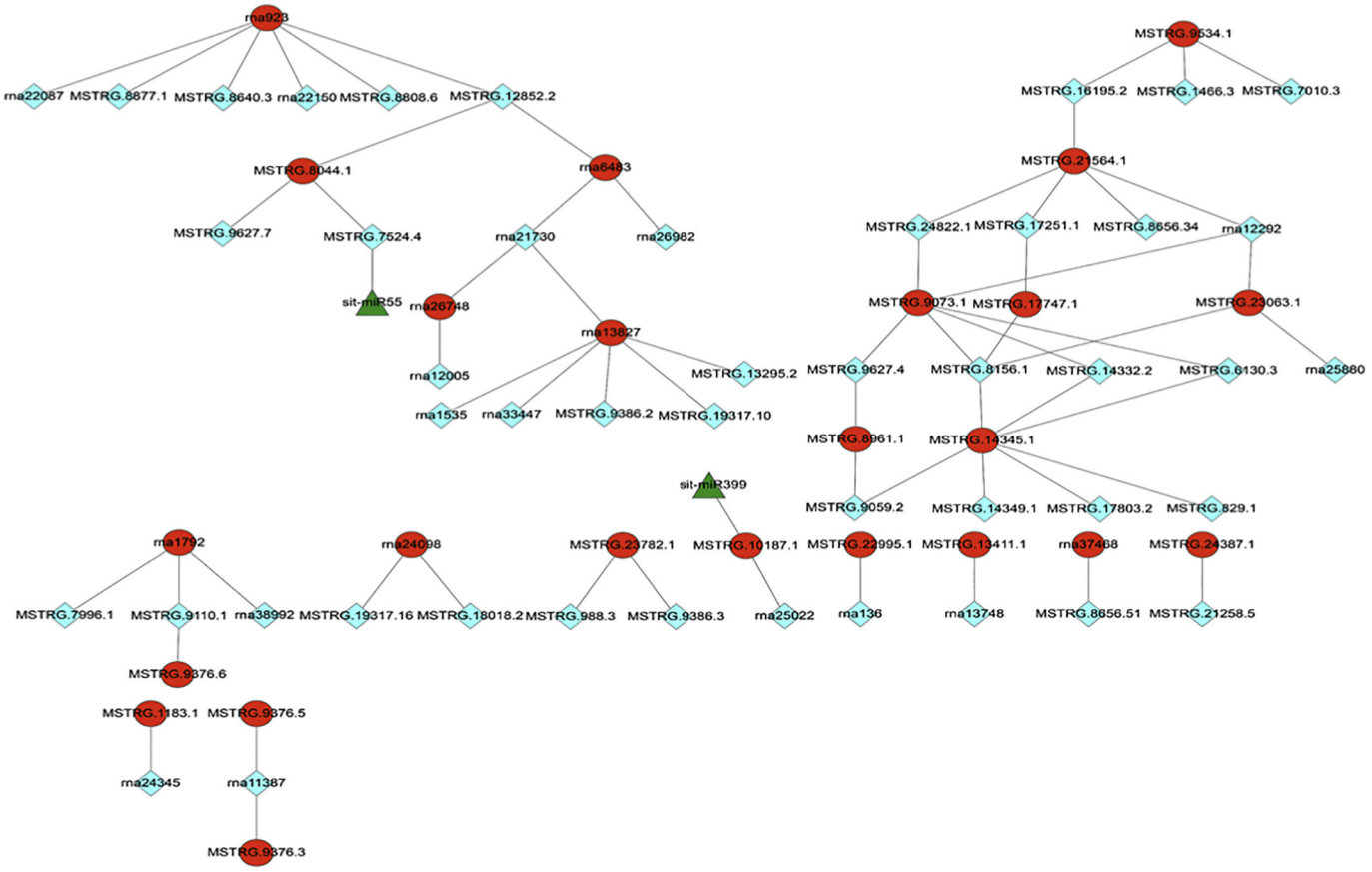

Fig. 6. A network of lncRNAs, miRNAs, and the genes between 24 DELs and 46 DEGs. Genes, lncRNAs, and miRNAs were represented by blue rhombus, red cycles, and green triangles, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

DEL were up-regulated and 35 DEL were down-regulated in the resistant cultivar compared to the sensitive cultivar (Table S2). The expression levels of these 67 DEL had different patterns in these two herbicide-responsible cultivars under the treatment and controls (Fig. 3b).

3.4. Identification and functional analysis of unique DEL Targets

In order to analyze potential functions of the DELs, we first searched the cis-regulatory targets of all identified DELs through examining DEGs (differentially expressed genes) 10 and 100 kb upstream and downstream of the lncRNAs, respectively. We also identified the trans-acting lncRNA targets in line accordance with expressed correlation coefficient (Pearson correlation ≥ 0.90 or ≤ -0.90; p-value $<$ 1e-5). A total of 187 cis-regulatory and 4165 target DEGs were found for 265 and 179 lncRNAs, respectively. GO enrichment analysis showed that many lncRNAs were annotated to many GO term, the major ones included antioxidant activities, response to stimulus, metabolic process and biological regulation, which may be related to plant response to herbicide treatment (Figs. S1). Many KEGG pathways were associated differentially expressed lncRNAs during the herbicide treatment, the major KEGG pathways included plant-environment interaction, signaling pathway, RNA transport and component metabolism (Fig. S2).

Between the herbicide-resistant and sensitive cultivars, there were a total of 64 lncRNA-mRNA pairs identified in trans between 24 DELs and 46 DEGs (Table S3). The expression levels of DELs and trans-acting lncRNA targets in two cultivars under normal and herbicide treatment were normalized read counts through heatmap (Fig. 4). Among the 64 lncRNA-mRNA pairs, the majority of lncRNAs were significantly up-regulated after herbicide treatment in both cultivars. The trans-acting 46 targets expression patterns were positively correlated with their lncRNAs (Fig. 4).

GO analysis of these target genes reveals that these genes are involved in the biologic process (BP), cellular components (CC) and molecular functions (MF) (Fig. 5a). For the GO MF terms, the targets were significantly enriched in the nucleic acid binding, catalytic activity, and aminoacyl-tRNA hydrolase activity. For the GO BP terms, the targets were significantly enriched in metabolic process, the cellular response to stress and cellular response to DNA damage stimulus. In addition, the targets were significantly enriched in the SOSS complex in the GO CC terms (Fig. 5b). Many these functions are related to foxtail millet plant response to herbicide treatment and metabolize herbicide molecule.

KEGG pathway analysis was also performed to investigate the function of DELs from both cultivars, the significantly enriched pathways were phagosome, nucleotide excision repair, terpenoid backbone biosynthesis, valine, leucine and isoleucine degradation, homologous recombination, fatty acid degradation, DNA replication and mismatch repair (Fig. 5c.

Based on these analyses, we found that some genes were both induced in both cultivars, such as disease resistance protein (*RPP13*) (rna24345 and rna33447), glycine-rich RNA-binding protein 1-like (MSTRG.9627.4 and MSTRG.988.3), elongation factor 1-alpha (MSTRG.8656.34 and MSTRG.8656.51), SOSS complex subunit C (MSTRG.14349.1 and rna22150). It is noteworthy that some genes related to plant resistance and growth were highly expressed in herbicide-resistant cultivar after herbicide treatment, such as polycomb group protein (*FIE2*), heat stress transcription factor (*HSF*), cellulose synthase-like protein G2 (*CSLG2*), nuclear ribonuclease Z (Rnase Z), non-specific lipid-transfer protein 1-like(*LTP1*), RNA-binding protein CP29B, (*CP29B*), probable glutamate carboxypeptidase (*LAMP1*), vacuolar protein sorting-associated protein 25 (*VPS25*), protein CHLOROPLAST

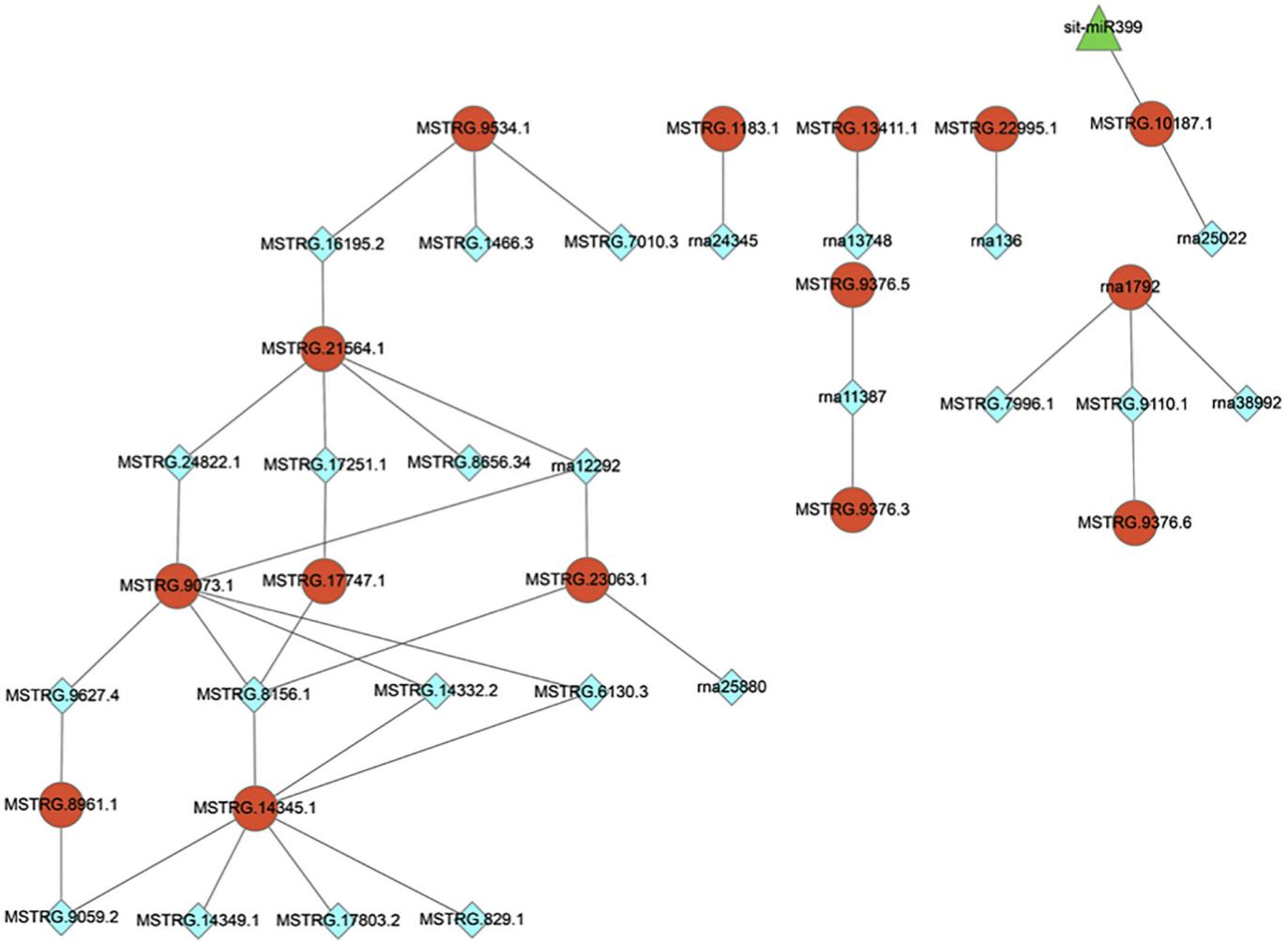

Fig. 7. A network of lncRNAs, miRNAs, and the genes between unique induced DELs and DEGs in resistant varieties. Genes, lncRNAs, and miRNAs were represented by blue rhombus, red cycles, and green triangles, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

IMPORT APPARATUS 2 (*CIA2*), and wall-associated receptor kinase (*WAKL*) (Table S3).

3.5. Certain lncRNAs may be regulated by miRNAs

LncRNAs may function through interacting with miRNAs. LncRNAs could act as precursor of miRNAs or be targeted by miRNAs [50]. In this study, we did not find any lncRNAs was the precursor of miRNAs. However, one lncRNA (MSTRG.10187.1) and one gene (MSTRG.7524.4, homologues of *Arabidopsis* AT1G64140.1) may be the targets of two different miRNAs, sit-miR62 and sit-miR399 (Table 2). This suggests that these two genes may be regulated by miRNAs. miR399 is a nutrient-related miRNA which regulate plant response to nutrient deficiency and uptake. AT1G64140.1 is one of WRKY transcription factor and play important role in plant response to stress.

3.6. LncRNA-miRNA-mRNA co-expression networks involved in imazapic herbicide response of foxtail millet

In order to understand the regulation of ncRNAs under imazapic herbicide stress in foxtail millet, a co-expression network was constructed, including 46 DEGs, 2 miRNAs and 24 lncRNAs. The results showed that 5 lncRNAs (MSTRG.9073.1, MSTRG.14345.1, MSTRG.21564.1, rna923 and rna13827) could interact with at least five genes, which were identified as hub lncRNAs (Fig. 6).

In order to explore the imazapic herbicide resistance difference of the two cultivars, we focused on the unique induced lncRNA in resistant varieties. Results showed that 3 lncRNAs (MSTRG.9073.1, MSTRG.14345.1 and MSTRG.21564.1) could interact with at least five genes. There were 13 genes targeted by only 1 lncRNA, and 11 genes were targeted by more than 2 lncRNAs. In addition, 1 lncRNA (MSTRG.10187.1) could positively regulate the expression of *WAKL3*, and also be targeted of sit-miR62 (Fig. 7).

3.7. Verification of RNA-seq lncRNAs expression by qRT-PCR

qRT-PCR was performed to verify the reliability of the RNA-seq data. Four DELs were randomly selected for investigation, including rna27343, rna14361, MSTRG.8201.1 and rna32008. As shown in Fig. 8, the expression of these DELs were affected after herbicide treatment, consistent with the results of RNA-seq data, confirming the reproducibility of the RNA-Seq data.

4. Discussion

Weeds are one major environmental issue in crop field, which seriously affect plant growth and development, and cause a serious threat to the yield and quality. Currently, herbicide treatment is the most effective approach for controlling weeds in the fields [8]. Studies in many plant species have demonstrated the important roles of lncRNA in

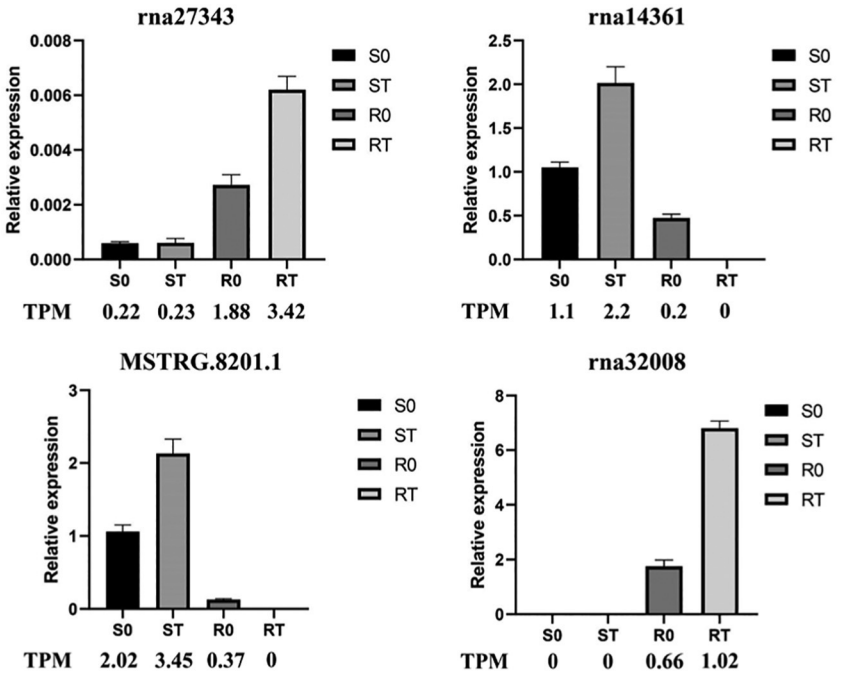

Fig. 8. Quantitative real-time PCR (qRT-PCR) validation and RNA-seq data of four selected DELs. Data shown were the mean of three independent repeated experiments ± standard deviation. Error bars represent standard deviations from three independent biological replicates.

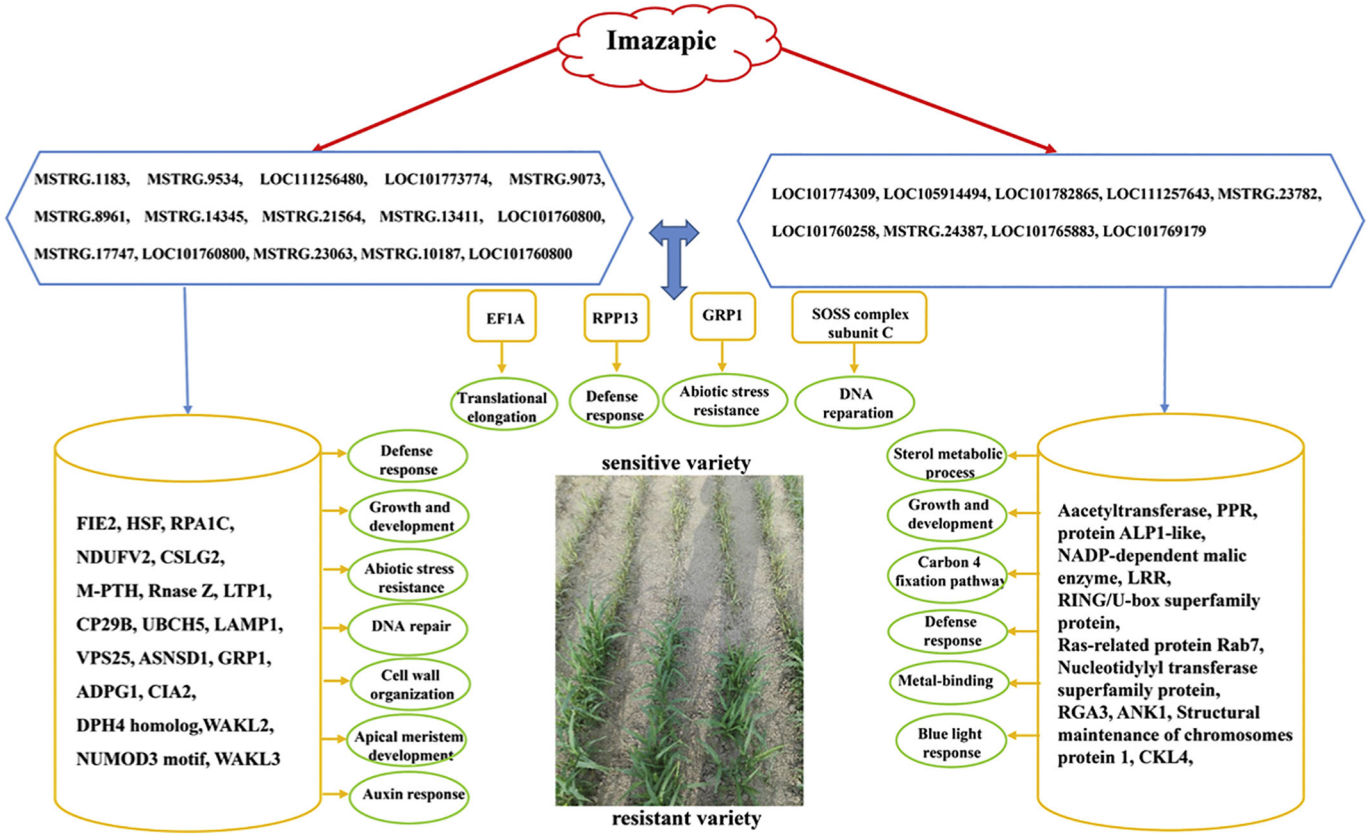

Fig. 9. A proposed mechanism for foxtail millet plant sensitive or resistance to herbicide.

various biological processes [12,14,15,41], however, our knowledge still remains limited regarding the imidazolinone herbicide response associated lncRNAs and the co-expression network of lncRNA-miRNA-mRNA in the regulation during imidazolinone herbicide treatment in plants, including foxtail millet. Understanding the regulation mechanism of lncRNAs will provide a molecular basis for better adaptation to herbicide treatment in sensitive cultivars and provide a novel approach for breeding new cultivars for high tolerance to herbicides.

In present study, strand-specific RNA-seq was performed to detect the expression changes of lncRNAs in both resistant and sensitive

cultivars of foxtail millet, that were treated with imazapic herbicide. In total, 2547 high-confidence lncRNAs, including 787 known and 1760 novel lncRNAs were identified based on strict criteria. LncRNAs in foxtail millet were unevenly distributed across all 9 chromosomes. The major of lncRNAs were located in the intergenic region and contained 1–2 exons, and lncRNAs also have shorter length and lower expression levels compared to protein-coding genes, these results were consistent with the previous studies [14,25,41].

More and more evidence shows that lncRNAs regulate the expression of target genes either in cis or in trans acting [14,41]. In our study, no cis-regulatory targets of 67 DELs through examining DEGs 10 and 100 kb upstream and downstream of the lncRNAs. Furthermore, we identified 64 lncRNA-mRNA pairs identified in trans between 24 DELs and 46 DEGs. This indicated that the target genes in trans-regulatory relationships may be more related to herbicide stress response. However, we found that the all trans lncRNA-mRNA pairs have positive expressional correlations in our study. In order to get a better understanding the function of these lncRNAs, GO term and KEGG pathway annotations were applied to their trans target genes. GO enrichment analysis show that these lncRNAs and targets were significantly enriched in the cellular response to stress and cellular response to DNA damage stimulus and so on. KEGG pathway analysis showed the significantly enriched pathways were phagosome, nucleotide excision repair, homologous [51]. Previous studies have revealed that lncRNAs can work as miRNA precursors or be targeted by miRNAs [14,41]. In our study, one lncRNAs (MSTRG.10187.1) may be targets of sit-miR399. miR399 is one important miRNA, which regulates plant response to lots of environmental abiotic stress, including the nutrient deficiency [52][53]. Because lncRNA MSTRG.10187.1 were differentially expressed during the herbicide treatment, it suggests that miR399 and its corresponding lncRNA MSTRG.10187.1 may play a role during foxtail millet response to herbicide treatment.

In our study, the expression of some deferentially expressed lncRNAs and their targets were altered in both herbicide-resistant and -sensitive cultivars of foxtail millet (Table S3). These genes include *RPP13* and *GRFs*. *RPP13* belongs to the nucleotide-binding site and leucine-rich repeat (NBS-LRR) family that contains the largest number of plant disease resistance genes in plants [54]. Glycine-rich proteins (*GRPs*) in plants play a wide role in protecting the organelles under stress conditions. Heterologous expression of rice RNA-binding glycine-rich gene *OsRBGD3* in transgenic *Arabidopsis thaliana* confers cold stress tolerance [51]. Additionally, some DELs and targets were only induced in resistant varieties after herbicide treatment, including *FIE2*, *HSF*, *CSLG2*, *Rnase Z*, *LTP1*, *CP29B*, *LAMP1*, *VPS25*, *CIA2* and *WAKL*. These genes have been reported to play important roles in plant growth and development. For example, *FIE2* belongs to polycomb group protein, *FIE2* in rice regulates various developmental processes during the entire growth period, including leaf, root and seed development [55,56]. Many studies have shown that most class-A HSF play a positive role under different abiotic stresses [57][58][59]. Wheat receptor-kinase-like protein (*WAKL*) controls gene-for-gene resistance to fungal pathogen *Zymoseptoria tritici* [62]. Plant LTPs also play a key role in plant growth and stress responses [63]. Although the functions of some lncRNAs or their targeted genes were not well characterized, such as rna21730 and MSTRG.13295.2, they were strongly induced after herbicide treatment, suggesting that they might play important roles. Thus, the specific induction of these genes in resistant cultivars may be related to their resistance to herbicides.

In summary, this study identified 2547 high-confidence lncRNAs, including 787 known and 1760 novel lncRNAs. These lncRNAs targets many protein-coding genes that regulate plant growth and development as well as response to different environmental stresses, including herbicide exposure; these lncRNA targets also regulate different metabolic and biological pathways and DNA repair mechanisms in plant cells for response to different stresses, which finally results in foxtail millet plant sensitive or resistance to herbicide (Fig. 9).

Author contributions

R.P., J.L., BZ., H.S. and T.W. designed the experiments. T.W., H.S., and B.Z. analyzed the data. T.W. and H.S. drafted the manuscript. Y.W., N.H. and P.L. examined the data. B.Z. reviewed and edited the manuscript. All authors agreed with the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygeno.2020.07.045.

References

[1] C. Lata, S. Gupta, M. Prasad, Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses, Crit. Rev. Biotechnol. 33 (2013) 328–343.
[2] A.N. Doust, E.A. Kellogg, K.M. Devos, J.L. Bennetzen, Foxtail millet: a sequence-driven grass model system, Plant Physiol. 149 (2009) 137–141.
[3] J.L. Bennetzen, J. Schmutz, H. Wang, R. Percifield, J. Hawkins, A.C. Pontaroli, M. Estep, L. Feng, J.N. Vaughn, J. Grimwood, J. Jenkins, K. Barry, E. Lindquist, U. Hellsten, S. Deshpande, X. Wang, X. Wu, T. Mitros, J. Triplett, X. Yang, C.Y. Ye, M. Mauro-Herrera, L. Wang, P. Li, M. Sharma, R. Sharma, P.C. Ronald, O. Panaud, E.A. Kellogg, T.P. Brutnell, A.N. Doust, G.A. Tuskan, D. Rokhsar, K.M. Devos, Reference genome sequence of the model plant Setaria, Nat. Biotechnol. 30 (2012) 551–561.
[4] G. Jia, X. Huang, H. Zhi, Y. Zhao, Q. Zhao, W. Li, Y. Chai, L. Yang, K. Liu, H. Lu, C. Zhu, Y. Lu, C. Zhou, D. Fan, Q. Weng, Y. Guo, T. Huang, L. Zhang, T. Lu, Q. Feng, H. Hao, H. Liu, P. Lu, N. Zhang, Y. Li, E. Guo, S. Wang, J. Liu, W. Zhang, G. Chen, B. Zhang, Y. Wang, H. Li, B. Zhao, J. Li, X. Diao, B. Han, A haplotype map of genomic variations and genome-wide association studies of agronomic traits in foxtail millet (*Setaria italica*), Nat. Genet. 45 (2013) 957–961.
[5] G. Zhang, X. Liu, Z. Quan, S. Cheng, X. Xu, S. Pan, M. Xie, P. Zeng, Z. Yue, W. Wang, Y. Tao, C. Bian, C. Han, Q. Xia, X. Peng, R. Cao, X. Yang, D. Zhan, J. Hu, Y. Zhang, H. Li, N. Li, J. Wang, C. Wang, R. Wang, T. Guo, Y. Cai, C. Liu, H. Xiang, Q. Shi, P. Huang, Q. Chen, Y. Li, Z. Zhao, Genome sequence of foxtail millet (*Setaria italica*) provides insights into grass evolution and biofuel potential, Nat. Biotechnol. 30 (2012) 549–554.
[6] R.L. Amor, T.M. Francisco, Survey of weeds in field peas, chickpeas and rapeseed in the Victorian Wimmera, Plant Protect. Q. 2 (1987) 124–127.
[7] E.C. Oerke, Crop losses to pests, J. Agr. Sci. Cambridge 144 (2005) 31–43.
[8] H. Wang, W. Liu, K. Zhao, H. Yu, J. Zhang, J. Wang, Evaluation of weed control efficacy and crop safety of the new HPPD-inhibiting herbicide-QYR301, Sci. Rep. 8 (2018) 7910.
[9] B. Bukun, S. Nissen, D. Shaner, J.D. Vassios, Imazamox absorption, translocation, and metabolism in red lentil and dry bean, Weed Sci. 60 (2012) 350–354.
[10] S. Tan, R.R. Evans, M.L. Dahmer, B.K. Singh, D.L. Shaner, Imidazolinone-tolerant crops: history, current status and future, Pest Manag. Sci. 61 (2005) 246–257.
[11] M. Harir, A. Gaspar, M. Frommberger, M. Lucio, M. El Azzouzi, D. Martens, A. Kettrup, P. Schmitt-Kopplin, Photolysis pathway of imazapic in aqueous solution: ultrahigh resolution mass spectrometry analysis of intermediates, J. Agr. Food Chem. 55 (2007) 9936–9943.
[12] P. Kapranov, J. Cheng, S. Dike, D.A. Nix, R. Duttagupta, A.T. Willingham, P.F. Stadler, J. Hertel, J. Hackermuller, I.L. Hofacker, I. Bell, E. Cheung, J. Drenkow, E. Dumais, S. Patel, G. Helt, M. Ganesh, S. Ghosh, A. Piccolboni, V. Sementchenko, H. Tammana, T.R. Gingeras, RNA maps reveal new RNA classes and a possible function for pervasive transcription, Science 316 (2007) 1484–1488.
[13] G. Karakülah, K. Yücebilgili Kurtoğlu, T. Unver, PeTMbase: a database of plant endogenous target mimics (eTMs), PLoS One 11 (2016) e0167698.
[14] M. Wang, W. Zhao, L. Gao, L. Zhao, Genome-wide profiling of long non-coding RNAs from tomato and a comparison with mRNAs associated with the regulation of

fruit ripening, BMC Plant Biol. 18 (2018) 75.
[15] X. Wang, G. Ai, C.L. Zhang, J.F. Wang, H.X. Li, J.H. Zhang, Z.B. Ye, Expression and diversification analysis reveals transposable elements play important roles in the origin of Lycopersicon-specific lncRNAs in tomato, New Phytol. 209 (2016) 1442–1455.
[16] Y. Wang, X. Fan, F. Lin, G. He, W. Terzaghi, D. Zhu, D. XW, Arabidopsis noncoding RNA mediates control of photomorphogenesis by red light, PNAS 111 (2014) 10359–10364.
[17] S.Y. Ng, L. Lin, B.S. Soh, L.W. Stanton, Long noncoding RNAs in development and disease of the central nervous system, Trends Genet. 29 (2013) 461–468.
[18] J.M. Couzigou, J.P. Combier, Plant microRNAs: key regulators of root architecture and biotic interactions, New Phytol. 212 (2016) 22–35.
[19] C. Li, B. Zhang, MicroRNAs in control of plant development, J. Cell. Physiol. 231 (2016) 303–313.
[20] X. Song, Y. Li, X. Cao, Y. Qi, MicroRNAs and their regulatory roles in plant-environment interactions, Annu. Rev. Plant Biol. 70 (2019) 489–525.
[21] F. Kopp, J.T. Mendell, Functional classification and experimental dissection of long noncoding RNAs, Cell 172 (2018) 393–407.
[22] L. Ma, V.B. Bajic, Z. Zhang, On the classification of long non-coding RNAs, RNA Biol. 10 (2013) 925–933.
[23] V. Pachnis, C.I. Brannan, S.M. Tilghman, The structure and expression of a novel gene activated in early mouse embryogenesis, EMBO J. 7 (1988) 673–681.
[24] S. Boerner, K.M. McGinnis, Computational identification and functional predictions of long noncoding RNA in Zea mays, PLoS One 7 (2012) e43047.
[25] J. Pang, X. Zhang, X. Ma, J. Zhao, Spatio-temporal transcriptional dynamics of maize long non-coding RNAs responsive to drought stress, Genes (Basel) 10 (2019) 138.
[26] L. Chen, S. Shi, N. Jiang, H. Khanzada, G.M. Wassan, C. Zhu, X. Peng, J. Xu, Y. Chen, Q. Yu, X. He, J. Fu, X. Chen, L. Hu, L. Ouyang, X. Sun, H. He, J. Bian, Genome-wide analysis of long non-coding RNAs affecting roots development at an early stage in the rice response to cadmium stress, BMC Genomics 19 (2018) 460.
[27] Y.C. Zhang, J.Y. Liao, Z.Y. Li, Y. Yu, J.P. Zhang, Q.F. Li, L.H. Qu, W.S. Shu, Y.Q. Chen, Genome-wide screening and functional analysis identify a large number of long noncoding RNAs involved in the sexual reproduction of rice, Genome Biol. 15 (2014) 512.
[28] C. Di, J. Yuan, Y. Wu, J. Li, H. Lin, L. Hu, T. Zhang, Y. Qi, M.B. Gerstein, Y. Guo, Z.J. Lu, Characterization of stress-responsive lncRNAs in Arabidopsis thaliana by integrating expression, epigenetic and structural features, Plant J. 80 (2014) 848–861.
[29] H. Budak, S.B. Kaya, H.B. Cagirici, Long non-coding RNA in plants in the era of reference sequences, Front. Plant Sci. 11 (2020) 276.
[30] H.B. Cagirici, B. Alptekin, H. Budak, RNA sequencing and co-expressed long non-coding RNA in modern and wild wheats, Sci. Rep. 7 (2017) 10670.
[31] T. Unver, H. Tombuloglu, Barley long non-coding RNAs (lncRNA) responsive to excess boron, Genomics 112 (2020) 1947–1955.
[32] L. Wang, S. Zhao, C. Gu, Y. Zhou, H. Zhou, J. Ma, J. Cheng, Y. Han, Deep RNA-Seq uncovers the peach transcriptome landscape, Plant Mol. Biol. 83 (2013) 365–377.
[33] J. Chen, M. Quan, D. Zhang, Genome-wide identification of novel long non-coding RNAs in Populus tomentosa tension wood, opposite wood and normal wood xylem by RNA-seq, Planta. 241 (2015) 125–143.
[34] T. Zhao, X. Tao, S. Feng, L. Wang, H. Hong, W. Ma, G. Shang, S. Guo, Y. He, B. Zhou, X. Guan, LncRNAs in polyploid cotton interspecific hybrids are derived from transposon neofunctionalization, Genome Biol. 19 (2018) 195.
[35] A.A. Golicz, M.B. Singh, P.L. Bhalla, The long intergenic noncoding RNA (lincRNA) landscape of the soybean genome, Plant Physiol. 176 (2018) 2133–2147.
[36] F. Bardou, F. Ariel, C.G. Simpson, N. Romero-Barrios, P. Laporte, S. Balzergue, J.W. Brown, M. Crespi, Long noncoding RNA modulates alternative splicing regulators in Arabidopsis, Dev. Cell 30 (2014) 166–176.
[37] P. Shuai, D. Liang, S. Tang, Z.J. Zhang, C.Y. Ye, Y.Y. Su, X.L. Xia, W.L. Yin, Genome-wide identification and functional prediction of novel and drought responsive lincRNAs in *Populus trichocarpa*, J. Exp. Bot. 65 (2014) 4975–4983.
[38] A.T. Wierzbicki, The role of long non-coding RNA in transcriptional gene silencing, Curr. Opin. Plant Biol. 15 (2012) 517–522.
[39] J.P. Yuan, Y. Zhang, J.S. Dong, Y.Z. Sun, B.L. Lim, D. Liu, Z.J. Lu, Systematic characterization of novel lncRNAs responding to phosphate starvation in Arabidopsis thaliana, BMC Genomics 17 (2016) 655.
[40] H. Liu, Y. Lu, J. Wang, J. Hu, T. Wuyun, Genome-wide screening of long non-coding RNAs involved in rubber biosynthesis in Eucommia ulmoides, J. Integr. Plant Biol. 60 (2018) 1070–1082.
[41] C. Zhang, G. Tang, X. Peng, F. Sun, S. Liu, Y. Xi, Long non-coding RNAs of switchgrass (*Panicum virgatum* L.) in multiple dehydration stresses, BMC Plant Biol. 18 (2018) 79.
[42] M. Martin, Cutadapt removes adapter sequences from high-throughput sequencing reads, EMBnet J. 17 (2011) 10–12.
[43] D. Kim, B. Langmead, S.L. Salzberg, HISAT: a fast spliced aligner with low memory requirements, Nat. Methods 12 (2015) 357–360.
[44] M. Pertea, G.M. Pertea, C.M. Antonescu, T.C. Chang, J.T. Mendell, S.L. Salzberg, StringTie enables improved reconstruction of a transcriptome from RNA-seq reads, Nat. Biotechnol. 33 (2015) 290–295.
[45] B. Li, C.N. Dewey, RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome, BMC Bioinformatics. 12 (2011) 323.
[46] C. Trapnell, A. Roberts, L. Goff, G. Pertea, D. Kim, D.R. Kelley, H. Pimentel, S.L. Salzberg, J.L. Rinn, L. Pachter, Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks, Nat. Protoc. 7 (2012) 562–578.
[47] J. Hu, T. Zeng, Q. Xia, Q. Qian, C. Yang, Y. Ding, L. Chen, W. Wang, Unravelling miRNA regulation in yield of rice (Oryza sativa) based on differential network model, Sci. Rep. 8 (2018) 8498.
[48] M.E. Smoot, K. Ono, J. Ruscheinski, P.L. Wang, K. Ideker, Cytoscape 2.8: new features for data integration and network visualization, Bioinformatics. 27 (2011) 431–432.
[49] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the the $2^{-\Delta\Delta Ct}$ method, Methods 25 (2001) 402–408.
[50] J.-H. Yoon, K. Abdelmohsen, M. Gorospe, Functional interactions among microRNAs and long noncoding RNAs Semin, Semin. Cell Dev. Biol. 34 (2014) 9–14.
[51] S.K. Lenka, A.K. Singh, S.K. Muthusamy, S. Smita, V. Chinnusamy, K.C. Bansal, Heterologous expression of rice RNA-binding glycine-rich (RBG) gene OsRBGD3 in transgenic Arabidopsis thaliana confers cold stress tolerance, Funct. Plant Biol. 46 (2019) 482–491.
[52] J. Park, D. Baek, M.C. Kim, H.J. Chun, S. Kang, H.C. Park, G. Shin, M. Shen, H. Hong, W.Y. Kim, D.H. Kim, S.Y. Lee, R.A. Bressan, H.J. Bohnert, D.J. Yun, Regulation of miR399f transcription by AtMYB2 affects phosphate starvation responses in Arabidopsis, Biochip J. Plant Physiol. 161 (2018) 362–373.
[53] B.H. Zhang, MicroRNA: a new target for improving plant tolerance to abiotic stress, J. Exp. Bot. 66 (2015) 1749–1761.
[54] J. Cheng, H. Fan, L. Li, B.Y. Hu, H.Y. Liu, Z. Liu, Genome-wide identification and expression analyses of RPP13-like genes in barley, Biochip J. 12 (2018) 102–113.
[55] S. Li, B. Zhou, X. Peng, Q. Kuang, X. Huang, J. Yao, B. Du, M.X. Sun, OsFIE2 plays an essential role in the regulation of rice vegetative and reproductive development, New Phytol. 201 (2014) 66–79.
[56] B.R. Nallamilli, J. Zhang, H. Mujahid, B.M. Malone, S.M. Bridges, Z. Peng, Polycomb group gene OsFIE2 regulates rice (Oryza sativa) seed development and grain filling via a mechanism distinct from Arabidopsis, PLoS Genet. 9 (2013) e1003322.
[57] V. Banti, F. Mafessoni, E. Loreti, A. Alpi, P. Perata, The heat-inducible transcription factor HsfA2 enhances anoxia tolerance in Arabidopsis, Plant Physiol. 152 (2010) 1471–1483.
[58] Y.C. Huang, C.Y. Niu, C.R. Yang, T.L. Jinn, The heat stress factor HSFA6b connects ABA signaling and ABA-mediated heat responses, Plant Physiol. 172 (2016) 1182–1199.
[59] D. Ogawa, T. Yamaguchi, T. Nishiuchi, High-level overexpression of the Arabidopsis HsfA2 gene confers not only increased themotolerance but also salt/osmotic stress tolerance and enhanced callus growth, J. Exp. Bot. 58 (2007) 3373–3383.
[62] C. Saintenac, W.S. Lee, F. Cambon, J.J. Rudd, R.C. King, W. Marande, S.J. Powers, H. Berges, A.L. Phillips, C. Uauy, K.E. Hammond-Kosack, T. Langin, K. Kanyuka, Wheat receptor-kinase-like protein Stb6 controls gene-for-gene resistance to fungal pathogen Zymoseptoria tritici, Nat. Genet. 50 (2018) 368–374.
[63] T.H. Kim, M.C. Kim, J.H. Park, S.S. Han, B.R. Kim, B.Y. Moon, M.C. Suh, S.H. Cho, Differential expression of rice lipid transfer protein gene (LTP) classes in response to abscisic acid, salt, salicylic acid, and the fungal pathogen Magnaporthe grisea, J. Plant Biol. 49 (2006) 371–375.