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Identification and functional prediction of lncRNAs in response to PEG and ABA treatment in cassava

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ABSTRACT

LncRNAs play crucial roles in plant drought stress, in which abscisic acid (ABA) was recognized as an essential signal molecule in stress regulatory networks. However, the mechanisms of lncRNAs participated in drought stress with or without the involvement of ABA signaling remain elusive in cassava, an important tropical and sub-tropical crop. In this study, a total of 1379 reliable lncRNAs were identified in cassava leaves using strand-specific RNA-seq method, of which 194 were differentially expressed between ABA or polyethylene glycol (PEG) treatment and the control condition. Trans-regulatory co-expression network revealed that ABA-uniquely-responsive DE lncRNAs were primarily participated in receptor kinases signaling, hormone metabolism, and cell wall modification; PEG-uniquely-responsive DE lncRNAs were mainly involved in jasmonate metabolism, biotic and abiotic stress, calcium signaling, and transport; and ABA/PEG-both-responsive DE lncRNAs were mainly referred to light reaction of photosynthesis, nitrogen metabolism, tetrapyrrole synthesis, secondary metabolism, RNA regulation of transcription, and calcium signaling. In addition, 41 lncRNA-mRNA pairs referred to cis-acting regulation were identified, and these lncRNAs regulated the expression of their neighboring genes through ABA signaling regulation, RNA regulation of transcriptions, and biotic and abiotic stress. Besides, 34 lncRNAs were identified as putative targets of cassava known miRNAs, and especially, TCONS_00129136, TCONS_00122745, TCONS_00088201, and TCONS_00067612 were targeted by functionally well-known miRNAs (such as miR156 and miR159) that involved in ABA- and drought-response, suggesting their roles in cassava drought response via ABA-dependent pathways with the participation of miRNA regulation. These findings provide a comprehensive view of cassava lncRNAs in response to PEG and ABA treatment, which will enable in-depth functional analysis in the near future.

1. Introduction

Long non-coding RNAs (lncRNAs) are widespread in plants and are usually defined as transcripts of non-protein-coding potential and longer than 200 nucleotides (Henz et al., 2007; Kung et al., 2013). LncRNAs are primarily transcribed from RNA polymerase II transcripts, and modified with 5′-cap and/or 3′-polyA-tail, and largely localized in the nucleus (Guttman et al., 2009; Kim and Sung, 2012; Zhang and Chen, 2013). According to their generated locations from genome, lncRNAs can be generally divided into main categories of long intergenic non-coding RNAs (lincRNAs), intronic non-coding RNAs (incRNAs), and long non-coding natural antisense transcripts (lncNATs) (Ariel et al., 2015; Zhao et al., 2018).

LncRNAs execute biological functions via various ways, including

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organization of nuclear domains, transcriptional regulation, and post-transcriptional regulation (Li et al., 2017; Kopp and Mendell, 2018). LncRNAs can regulate gene expression by cis-acting and trans-acting through a series of complex mechanisms, including activating or transporting proteins and epigenetic silencing and repression, modifying promoter activities by nucleosome repositioning, epigenetic modification by regulating the level of DNA methylation and histone modifications (Ponting et al., 2009; Liu et al., 2015; Deng et al., 2018). In cis-acting, one lncRNA, named *MAS*, positively regulated the expression of *MAF4* to regulate flowering time in *Arabidopsis*, through recruiting and interacting with *WDR5a* that was a core component of the COMPASS-like complexes (Zhao et al., 2018). Similarly, lncRNA *LDMAR* regulated *pms3* to effect pollen development to realize photoperiod-sensitive male sterility (PSMS) in hybrid rice (Ding et al., 2012). LncRNA33732 activated by WRKY1 induced *RBOH* expression to increase H_2O_2 accumulation in early defense reaction of tomato (Cui et al., 2018). In trans-acting, a long intronic noncoding RNA was required for the cold epigenetic repression of *FLC* through recruiting a component PRC2 to FLC in *Arabidopsis* (Csorba et al., 2014). PRP8 influenced sense *FLC* expression through effects on *COOLAIR* (a long noncoding RNA) splicing to regulate flowering time in *Arabidopsis* (Marquardt et al., 2014). In addition, lncRNA can also function as microRNA (miRNA) targets. For example, lncRNA *IPS1* bound to miR399 to destroy the miR399-mediated target *PHO2* mRNA to control of Pi homeostasis in *Arabidopsis* (Franco-Zorrilla et al., 2007). To date, many lncRNAs have been identified in response to drought stress in *Arabidopsis* (Zhao et al., 2018), maize (Zhang et al., 2014), cotton (Lu et al., 2016), *Populus trichocarpa* (Shuai et al., 2014), and *Pyrus betulifolia* (Wang et al., 2018). These results suggest that lncRNAs play significant roles in drought stress in plants.

As the most important hormone, ABA plays crucial roles in plant development and abiotic stress such as drought. Under drought condition, the contents of ABA that was a significant signal molecule and H_2O_2 that was a harmful active oxygen species were quickly increased, while the activities of several anti-oxidative enzymes, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were enhanced (Li et al., 2010; Zeng and Luo, 2012; Daszkowska-Golec and Szarejko, 2013). At the same time, the expression levels of many genes involved in ABA biosynthesis and signal transduction were induced (Daszkowska-Golec and Szarejko, 2013). Previous studies have demonstrated that lncRNAs participate in drought stress regulation through ABA signaling pathway. A lncRNA, DROUGHT INDUCED lncRNA (*DRIR*), positively regulated ABA-mediated responses to drought and salt stress in *Arabidopsis thaliana* (Qin et al., 2017). In addition to ABA-dependent pathway, accumulated evidences also demonstrate that ABA-independent pathway plays important roles in expression regulation of drought stress response (Yamaguchi-Shinozaki and Shinozaki, 2005). OsZFP245, as a C2H2-type zinc finger protein, was strongly induced under cold and drought stress through an ABA-independent pathway because of cis-element CRT/DRE being in OsZFP245 promoter region (Huang et al., 2005). Over-expression of *SiNAC110*, as a NAC-like transcription factor, in *Arabidopsis* conferred tolerance to drought stress through an ABA-independent response pathway (Xie et al., 2016).

Cassava is an important tropical and sub-tropical crop and provides food for over 600 million people worldwide because of its high starch production (Oliveira et al., 2014). Moreover, cassava has strong tolerance to drought and low-fertility soil environment due to its effective use of light, heat, and water resources (Maran et al., 2014). However, the mechanism underlying cassava drought response currently remains elusive, and less is known about the lncRNAs in cassava drought stress with the involvement of ABA-dependent or ABA-independent signaling pathways. In this study, in order to characterize the cassava lncRNAs in drought response with or without the participation of ABA signaling, cassava seedlings were treated by ABA and PEG treatment, respectively, and then the leaf samples were collected and subjected to strand-specific RNA-seq (ssRNA-seq) sequencing. Afterward, the lncRNAs responsive to ABA and/or PEG treatment were identified, their expression patterns were investigated, and their potential functions were also predicted and analyzed. These findings will increase our understanding of lncRNAs participating in drought stress of cassava, and also provide a reference for functional characterization of lncRNAs in abiotic stress in other species.

2. Materials and methods

2.1. Plant materials and treatments

Cassava (*Manihot esculenta*) genotype Argentina 7 (Arg7) was obtained from Dr. Wenquan Wang's lab and used in this study. Twelve months old cassava stems were cut into 10~15 cm segments containing two to three buds and cultured into pots filled with soil and vermiculite (1:1) in the green house of Chinese Academy of Tropical Agricultural Sciences (Haikou, China). The growth conditions were 16 h/35 °C in the day and 8 h/20 °C in the night with a light intensity of 200 μmol m^{-2} s^{-1} and a relative humidity of 70%. Three months later, the cassava seedlings with uniform growth status were chosen and watered with 100 μM abscisic acid (ABA) for ABA treatment and 20% polyethylene glycol (PEG) 6000 solution for drought treatment, respectively. The first expanded leaves of three independent cassava seedlings were collected at different duration (0, 0.5, 1, 3, 5, 7, 9, and 11 d after treatment) to measure several physiological indicators including superoxide dismutase (SOD) activity, catalase (CAT) activity, peroxidase (POD) activity, hydrogen peroxide (H_2O_2), and ABA content with three replicates. According to the physiological measurement, the samples at 0, 3, 5, and 7 d after treatment were selected for high-throughput strand-specific RNA-seq sequencing (ssRNA-seq). To reveal the difference of physiological parameters across different time-points, Duncan's multiple range tests were applied, and data were shown as mean ± standard deviation with different letters indicating a significant difference at the $P < 0.05$ level.

2.2. Determination of physiological indicators

The ABA content was measured according to the method described in previous study (Ding et al., 2011). 0.5 g leaves were ground in liquid nitrogen and homogenized in 5 mL extracting solution (90% v/v methanol, 200 mg/L sodium diethyldithiocarbamate trihydrate). Following the pre-processing steps described previously (Ding et al., 2011; Liu et al., 2014), a plant enzyme-linked immunosorbent assay (ELISA) kit (Jianglai Biotechnology, Shanghai, China) was used for the determination of ABA content according to the manufacturer's instructions.

H_2O_2 content and the enzyme activities of SOD, CAT and POD were determined as previously described (Huang et al., 2015; Hu et al., 2016). 0.5 g leaves were homogenized with 4.5 ml phosphate buffer (0.1 mol/L, pH7.8) at 4 °C. Then the crude extract was centrifuged at 10 000 *g* for 10 min at 4 °C and the supernatant was collected to measure H_2O_2 content, SOD, CAT and POD enzyme activities by corresponding detection kits (A064, A001, A007and A084, Nanjing Jiancheng, Nanjing city, China), respectively.

2.3. Library preparation and high-throughput sequencing

Total RNA extraction, transcriptome library construction, and ssRNA-seq sequencing were accomplished by Majorbio BioTech Co., Ltd. (Shanghai, China). Total RNA was isolated from leaves using Plant RNA Purification Reagent kit (Invitrogen, Waltham, Massachusetts, USA) and then Ribo-Zero Magnetic kit (Illumina, San Diego, California, USA) was used for rRNA removal. Illumina Truseq™ RNA sample prep kit (Illumina, San Diego, California, USA) was used to construct ssRNA-Seq libraries according to the Illumina instructions. HiSeq 4000

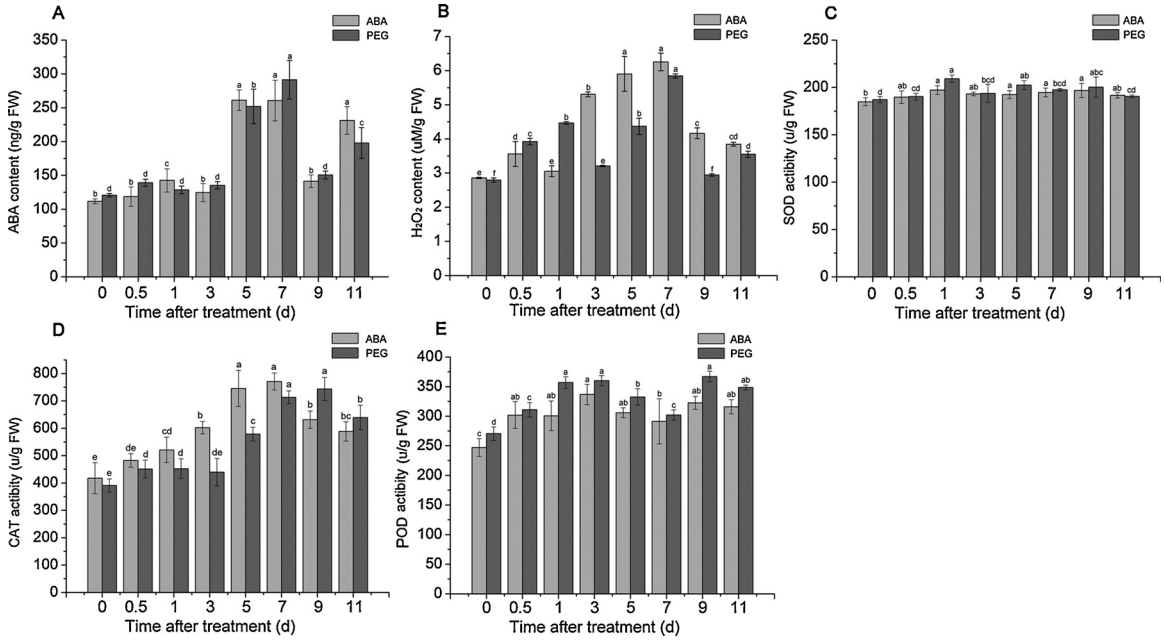

Fig. 1. Physiological changes of cassava leaves under ABA and PEG treatment. ABA content (A), H_2O_2 content (B), SOD activity (C), CAT activity (D) and POD activity (E) were investigated under ABA or PEG treatment across eight time points (0, 0.5, 1, 3, 5, 7, 9 and 11 d). Data are means ± SD of n = 3 biological replicates. Different lower-case letters indicate that means are significant at $P < 0.05$ level among different time-points of the same treatment.

instrument (Illumina, San Diego, California, USA) was used for strand-specific sequencing. The generated sequence data were deposited in NCBI's SRA database under the accession of SRP182603.

2.4. Bioinformatics identification of cassava lncRNAs

Adapter sequences and low-quality reads were removed from raw reads to obtain clean reads. Tophat 2.0 soft was applied to map clean reads to cassava genome with '-library-type fr-firststrand' parameter (Trapnell et al., 2009). Cufflinks soft was used to assemble reads into transcripts (Trapnell et al., 2012), and the transcripts detected in at least two samples were retained. The expression levels were estimated based on fragments per kilobase per million mapped reads (FPKM).

To identify lncRNAs, the following three rules were abided: 1) the transcripts with nucleotide length < 200 bp, minimum read coverage < 3, ORF length > 300, and overlapping with known protein-coding genes on the same nucleotide chain were discarded; 2) CPC (Kong et al., 2007), CPAT (Wang et al., 2013), and CNCI (Sun et al., 2013) were applied to exclude the transcripts with coding potential; 3) Pfam (Finn et al., 2016) and HMM (Eddy, 2011) were used to remove the transcripts with known protein domain. Finally, the remaining transcripts were recognized as the reliable lncRNAs. Differentially expressed (DE) lncRNAs were identified setting $|\log2FC| > 1$ and FDR < 0.05.

2.5. Prediction of DE lncRNA targets in cis-regulation

To predict the potential targets in cis-regulation, protein-coding genes located on 10k/100k up-stream and down-stream of DE lncRNAs were selected to perform co-expression analysis (Liao et al., 2011). The lncRNA-mRNA pairs co-expressed and adjacent located were in cis-regulatory relationships.

2.6. Prediction of DE lncRNA targets in trans-regulation

To identify the lncRNA targets in trans-regulatory relationship, the standard procedure of WGCNA (Langfelder and Horvath, 2008) was performed for co-expression analysis. The lncRNAs and genes with similar expression patterns were co-expressed and classified into the same group. To further understand the function of lncRNA in trans-regulation, MapMan (Thimm et al., 2004) soft was used to annotate the cassava genes, subsequently over-represented functional categories were identified based on Fisher's exact test as previously described (Ding et al., 2016).

2.7. Prediction of lncRNAs as miRNA targets

All discovered DE lncRNAs were used to predict as miRNA targets by psRNATarget (Dai and Zhao, 2011). The mature cassava miRNAs were obtained from miRBase database (Kozomara and Griffiths-Jones, 2013). The lncRNA-miRNA pairing regions were allowed with less than three mismatches according to Wu et al. (2013). The Cytoscape software (Shannon et al., 2003) was used to visualize the interaction network of microRNAs and target lncRNAs.

2.8. Quantitative real-time PCR analyses

To verify the accuracy of ssRNA-seq results, four lncRNAs exhibited different expression patterns in response to PEG and ABA treatments, and three lncRNAs and three genes involved in cis-regulatory lncRNA-mRNA pairs, were selected to perform qRT-PCR analysis. The 20 μL of qRT-PCR reaction solution contained 1 μL cDNA, 0.5 μL of each specific primer (300 nmol·μL^{-1}), 10 μL 2 × ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China) and 8 μL RNase-free water. The qRT-PCR thermal cycling was as follows: 95 °C for 5 min; followed by 40 cycles of 95 °C for 10 s, 56 °C for 30 s and 72 °C for 30 s in the Mx3005 P Real-Time PCR System (Stratagene, California, USA). The β-tubulin gene (TUB) was selected as an internal control and these specific primers used for qRT-PCR were listed in Table S1. All qRT-PCR experiments were measured in triplicate, and $2^{-\Delta\Delta Ct}$ method was used to analyze the relative expression level.

3. Results

3.1. Physiological indicators of cassava leaves

To study dynamic changes of cassava respond to ABA and PEG treatment, five physiological traits including ABA content, H_2O_2 content, SOD, CAT and POD enzyme activities were measured after ABA and PEG treatment at eight time-points (0, 0.5, 1, 3, 5, 7, 9, and 11d) in leaves, respectively (Fig. 1).

Overall, ABA content exhibited a very similar trend under ABA and PEG treatment: the values were slightly changed at the first 3 days, then sharply increased at 5 and 7 d, and then declined until the end of treatment. Similar changes were observed between the two treatments for H_2O_2 content, CAT activity and POD activity, although obvious differences were found at a few time-points, e.g., 3 d and 5 d for H_2O_2 and CAT activity. POD activity was rapidly induced from 0 d to 1 d and then maintained at a constant level until the end of treatment, while H_2O_2 content and CAT activity were significantly induced and reached its highest level at 7 d and then declined until 11 d expected for 9d time-point of POD activity under PEG treatment. However, unlike the former traits, SOD activity was only slightly fluctuated through the entire treatment, indicating its minor contribution to PEG and ABA treatment.

Together, these results suggested significantly physiological changes of cassava during the period of ABA or PEG treatment. Subsequently, leaf samples were collected at 0, 3, 5, and 7 d, respectively, after ABA or PEG treatment and subjected to high-throughput strand-specific RNA sequencing to further investigate the expression response of lncRNAs.

3.2. Identification and characterization of lncRNAs in cassava

In total, ~1064 million clean reads with 2 × 150 bp in length were obtained from 14 RNA-seq libraries. Then, they were aligned to the cassava reference genome, generating 153 417 transcripts with 1893 bp on average (total assembled length = 211.3 Mb, N50 = 2527 bp). Of which, 96 612 (~62.9%) transcripts overlapped with 31532 protein-coding genes (representing ~95.5% genes of the cassava genome) based on the reference gene annotation were removed. Subsequently, the remaining transcripts were progressively filtered according to the parameters (e.g., transcript length, exon number, and coding potential et al.) described in the methods, and ultimately, a total of 1379 reliable lncRNAs containing 1063 intergenic lncRNAs and 316 anti-sense lncRNAs were identified according to the location of these lncRNAs in cassava genome.

To investigate the characterization of cassava lncRNAs, they were mapped to the 18 chromosomes of cassava genome. It was clearly observed that more anti-sense lncRNAs were located on chromosome 12 and 13 than other chromosomes, while intergenic lncRNAs preferred to locate on chromosome 5 and 6 (Fig. 2A). Subsequently, the nucleotide length and exon number of anti-sense and intergenic lncRNAs were analyzed. Overall, most intergenic (89%) and anti-sense (86%) lncRNAs were shorter than 3000 nt with the median length of 1116 and 1045 nt for intergenic and anti-sense lncRNAs, respectively (Fig. 2B). More than half of the intergenic and anti-sense lncRNAs contained two exons. In addition, the number of lncRNAs was greatly decreased as the number of exons increased (Fig. 2C). Taken together, these results reveal a general characterization of cassava lncRNAs under abiotic stress.

3.3. Validation of lncRNA expression using qRT-PCR

To confirm ssRNA-seq results, four lncRNAs with different responses to ABA and PEG treatments, e.g., TCONS_00129088 was up-regulated and TCONS_00114791 was down-regulated by both ABA and PEG treatments, TCONS_00135265 was induced by PEG treatment but decreased by ABA treatment, and TCONS_00062095 was suppressed by PEG treatment but induced by ABA treatment, were analyzed by qRT-PCR (Fig. 3). In addition, three lncRNAs and three genes involved in cis-regulation (see below) were also analyzed. Of which, TCONS_00085215-Manes.10G067700 pair showed positive correlation under both ABA and PEG treatment, TCONS_00018832-Manes.02G066400 pair displayed positive correlation under ABA treatment but negative correlation under PEG treatment, while TCONS_00001528-Manes.01G052500 pair showed positive correlation under PEG treatment but negative correlation under ABA treatment (Fig. 4). Overall, high correlation coefficients (R = 0.81-0.99) were revealed between ssRNA-seq and qRT-PCR results (Table S2), suggesting the reliable of ssRNA-seq for lncRNA expression analysis. These results also implied that lncRNAs exhibited different responses to ABA and PEG treatments, in addition to the common responses under these two treatments.

3.4. Identify stress-responsive DE lncRNAs

To further understand the transcriptome changes of lncRNAs in response to abiotic stresses, DE lncRNAs were identified based on pair-wise comparison of lncRNA expression levels between PEG/ABA treatment and the control, respectively. In total, 194 DE lncRNAs were identified (Table S3). Of which, 75 (39%) DE lncRNAs were specially identified under ABA treatment, 74 (38%) DE lncRNAs were specially identified under PEG treatment, and 45 (23%) DE lncRNAs were identified in response to both ABA and PEG treatments (Fig. 2D).

3.5. Functional analysis of DE lncRNAs and their targets in cis-regulation

To investigate the potential functions of cassava DE lncRNAs, protein-coding genes, which located 10k/100k upstream and downstream of these lncRNAs, were selected to perform co-expression analysis. The lncRNA-mRNA pairs, which were closely located and co-expressed, were in cis-acting regulatory relationships and deserved to study in depth.

A total of 41 lncRNA-mRNA pairs involved in cis-regulatory relationships were identified (Table S4). Of which, TCONS_00114791 was placed 10 179 bp upstream of a SDR protein (Manes.13G126100) related to ABA synthesis and TCONS_00085215 was located 549 bp upstream of Manes.10G067700 coding 8-hydroxylase protein related to ABA metabolism (Fig. 4A), implying these two lncRNAs play major roles in ABA signaling regulation respond to abiotic stresses. TCONS_00015102, TCONS_00045569, and TCONS_00103761 were located 3 627 bp, 1 712 bp, and 9 399 bp downstream of Manes.02G145400, Manes.05G200500, and Manes.12G031000 coding bHLH transcription factors, respectively, and TCONS_00005423 was located 6 920 bp downstream of a NAC transcription factor (Manes.01G238300), and TCONS_00018832 was placed 1 625 bp upstream of a TCP transcription factor (Manes.02G066400) (Fig. 4B), suggesting these five DE lncRNAs might participate in RNA regulation of transcription under ABA and/or PEG treatment. Additionally, we also observed that TCONS_00001528 was spaced 257 bp upstream of Manes.01G052500 coding a COPINE protein involved in disease resistance (Fig. 4C), and TCONS_00084119 was located 121 bp downstream of a NBS-LRR protein (Manes.10G023100), indicating these two lncRNAs might be referred to biotic and abiotic stress response. Taken together, these results implied that the DE lncRNAs, which were involved PEG and/or ABA stresses in cis-regulatory relationships, regulated the expression of their neighboring gene through ABA signaling regulation, RNA regulation of transcriptions, and biotic and abiotic stress.

3.6. Expression analysis and functional characterization of DE lncRNAs in trans-regulatory relationships

To study the possible functions of DE lncRNAs responding to abiotic stresses, 194 DE lncRNAs, together with 1150 protein-coding genes

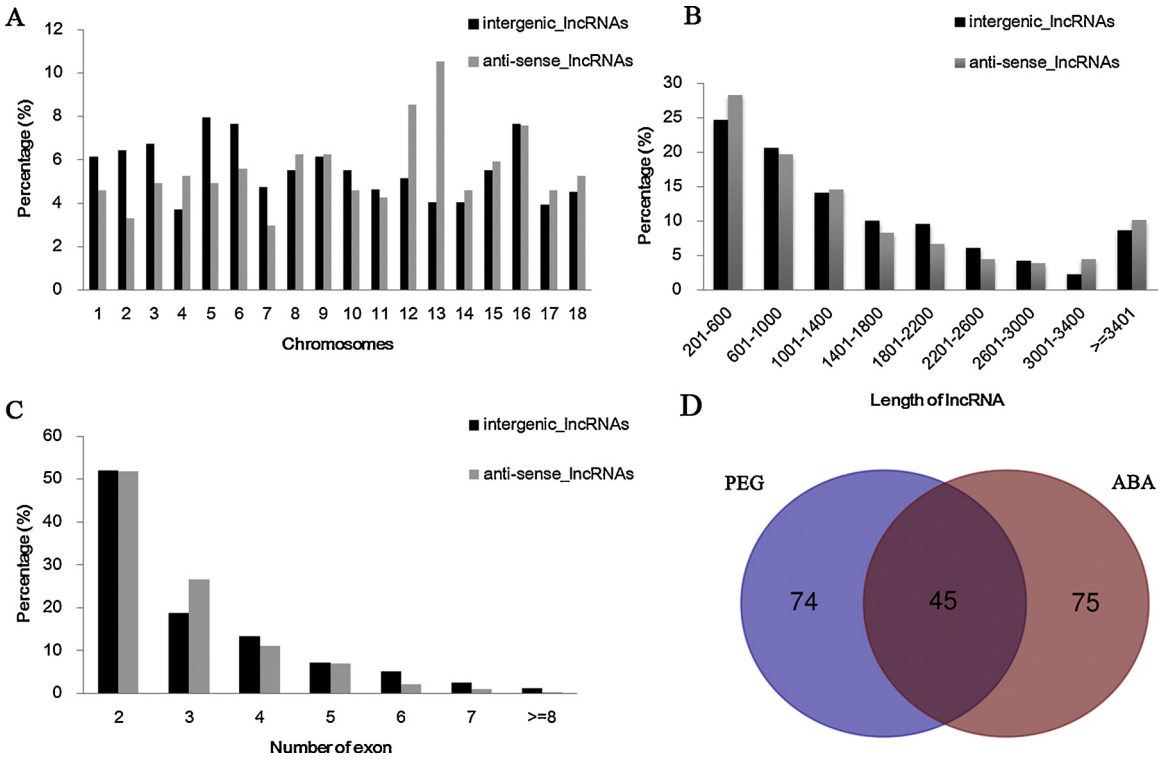

Fig. 2. Characteristics of cassava lncRNAs. (A) Distribution of intergenic lncRNAs and antisense lncRNAs on each chromosome. (B) Distribution of lncRNAs length. (C) Distribution of exon number of lncRNAs. (D) Venn diagram of DE lncRNAs in response to ABA and PEG treatment.

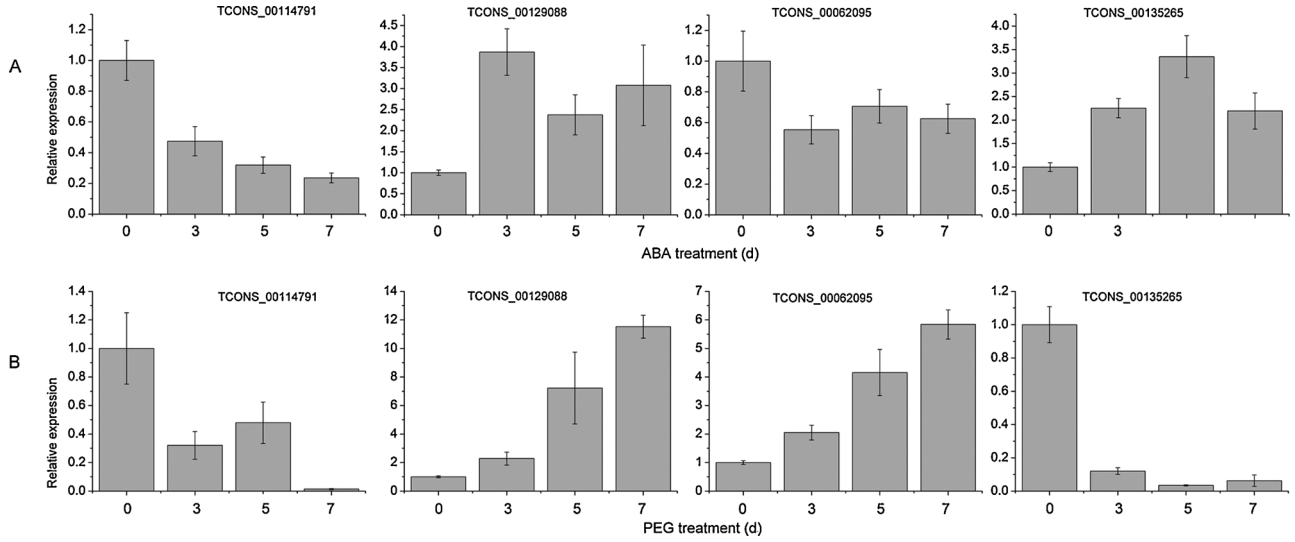

Fig. 3. Validation of the expression patterns of lncRNAs using qRT-PCR. (A) The expression pattern of lncRNAs under ABA treatment. (B) The expression pattern of lncRNAs under PEG treatment. Control (no treatment control) was normalized as "1". Data are means ± SD of n = 3 replicates.

differentially expressed under PEG or ABA treatment, were carried out by co-expression analysis, and then functional enrichment was performed for these DE genes within each group/module, respectively, based on MapMan annotation. The functions of DE lncRNAs can be predicted by the enriched functions of co-expressed DE genes. In total, six groups (G1-G6) were identified according to the expression patterns of lncRNAs and the co-expressed DE genes under ABA and PEG treatment (Fig. 5, S5).

There were four and nine DE lncRNAs in group G1 and G2, respectively. The expression levels of lncRNAs/genes in G2 were expressed highest in the control (CK), indicating that these lncRNAs/genes were significantly suppressed in both ABA and PEG treatments. The enriched categories of this group included tetrapyrrole synthesis, light reaction of photosynthesis, secondary metabolism, nitrogen metabolism, and lipid metabolism. However, no obvious expression trend was observed in G1, although the expression of lncRNAs/genes in this group was slightly down-regulated under PEG treatment.

There were 12 DE lncRNAs in group G3, in which the expression of these lncRNAs/genes were notably induced in response to both ABA and PEG stresses. The functional enrichment exhibited that these lncRNAs/genes were involved in cellulose synthesis of cell wall, ethylene metabolism, RNA regulation of transcription, and calcium signaling.

There were 29 DE lncRNAs in group G4. The expression levels of

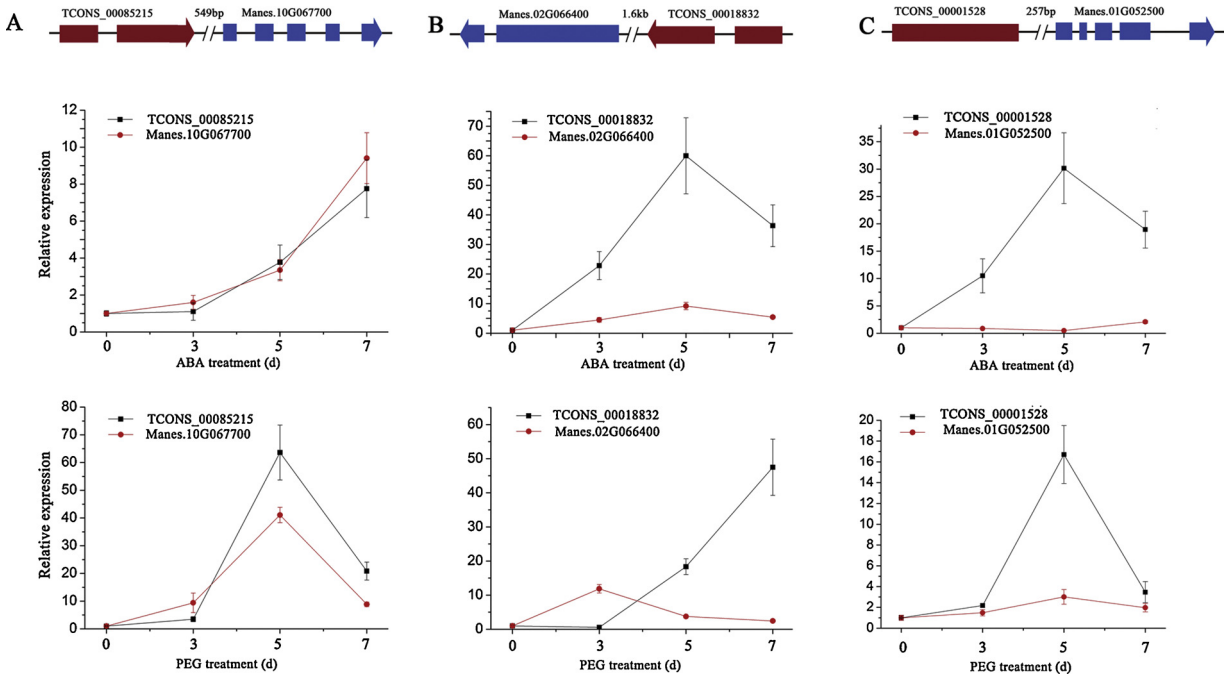

Fig. 4. Comparison of the expression patterns of lncRNAs and their neighboring protein-coding genes in cis-regulatory relationship. Structures and expression patterns of three lncRNAs-mRNA pairs, including TCONS_00085215-Manes.10G067700 (A), TCONS_00018832-Manes.02G066400 (B), TCONS_00001528-Manes.01G052500 (C). Control (no treatment control) was normalized as "1". Data are means ± SD of n = 3 replicates.

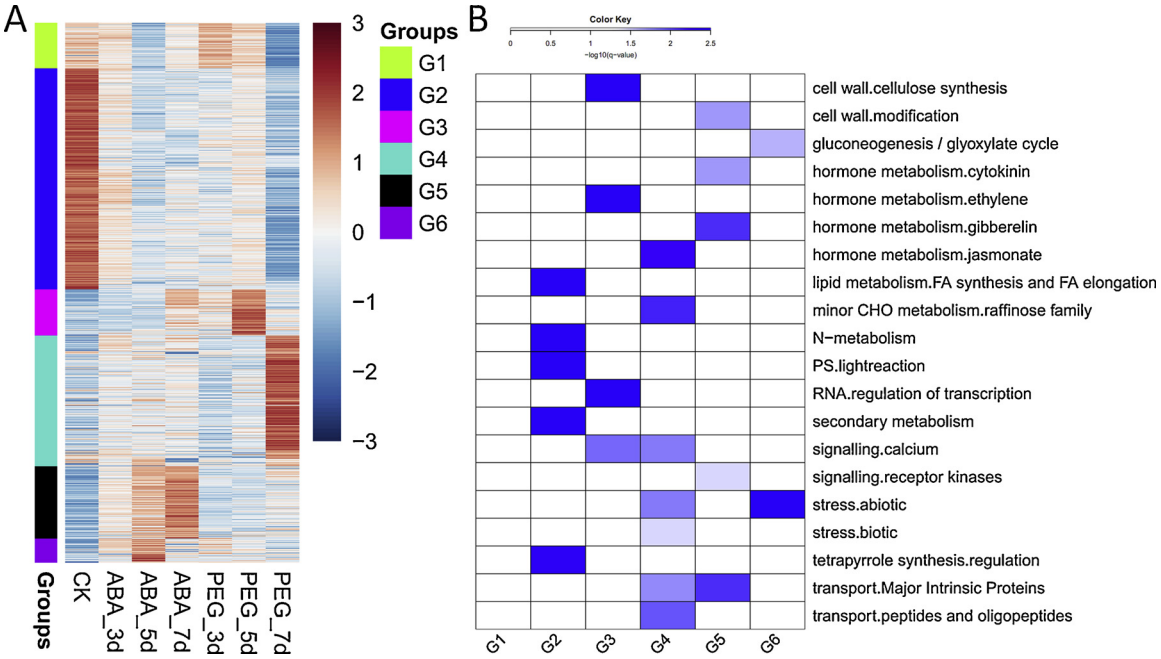

Fig. 5. Co-expression analysis of lncRNAs and mRNAs. (A) Heatmap of 103 DE lncRNAs and 866 DE mRNAs, which were clustered into six main groups. (B) Functional category enrichment of each group presented in (A).

these lncRNAs were significantly up-regulated at 7 d under PEG treatment but without significantly changes in response to ABA treatment. The enriched categories in this group included jasmonate metabolism, biotic and abiotic stress, minor CHO metabolism of raffinose family, calcium signaling, and transport.

There were 13 and 12 DE lncRNAs respectively in group G5 and G6, in which the expression of lncRNAs/genes was greatly increased under ABA treatment but without significantly changes in response to PEG treatment. The lncRNAs/genes in G5 were significantly enriched in cell wall modification, hormones such as cytokinin and gibberelin, receptor kinases signaling, and transport, while those in G6 were significantly enriched in gluconeogenesis/glyoxylate cycle and abiotic stress.

Taken together, these results suggested that ABA-uniquely-responsive DE lncRNAs were primarily participated in receptor kinases signaling, hormone metabolism, and cell wall modification; PEG-uniquely-responsive DE lncRNAs were mainly involved in jasmonate metabolism, biotic and abiotic stress, calcium signaling, and transport; and ABA/PEG-both-responsive DE lncRNAs were mainly referred to light reaction of photosynthesis, nitrogen metabolism, tetrapyrrole synthesis, secondary metabolism, RNA regulation of transcription, and

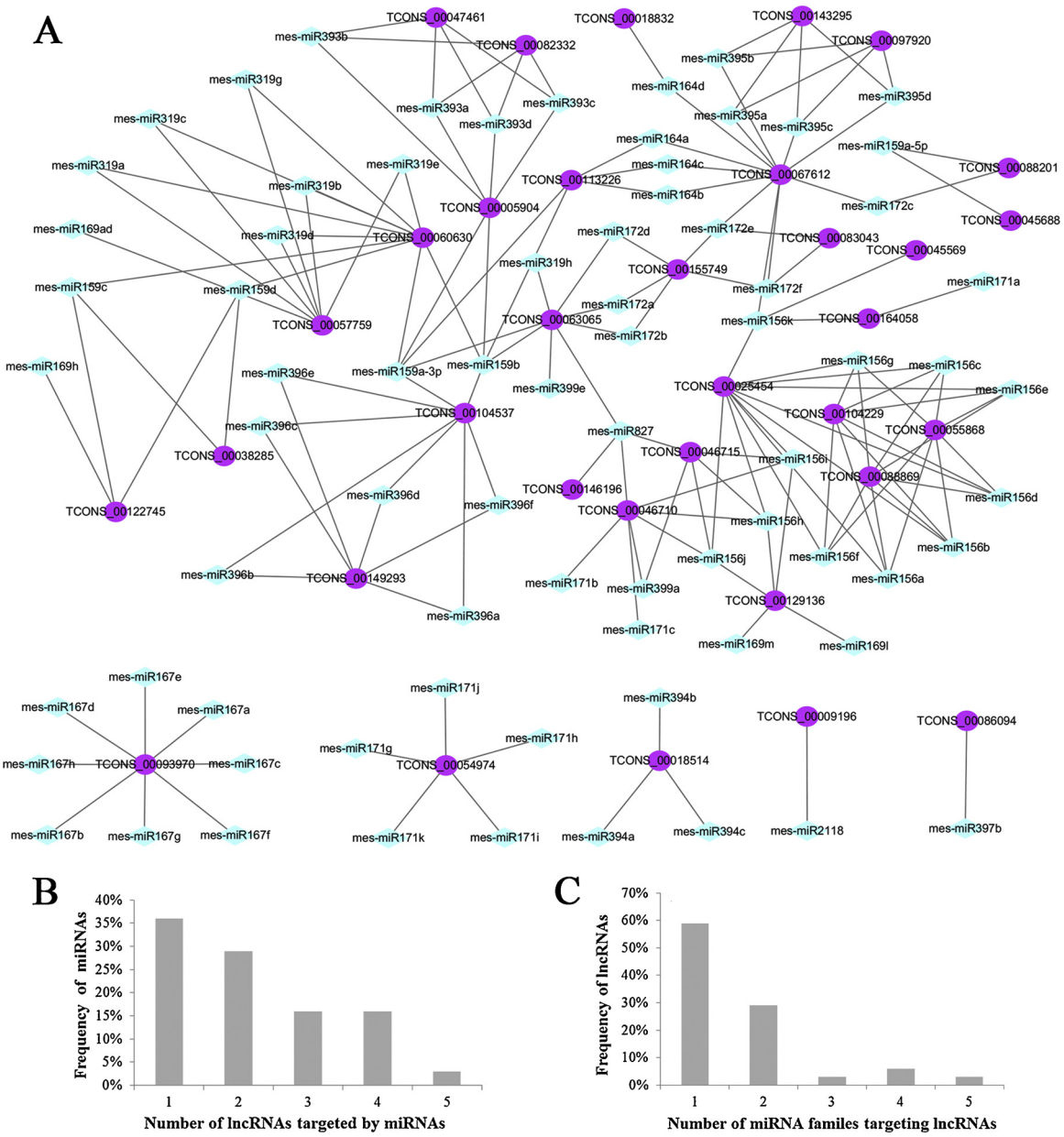

Fig. 6. MiRNAs target on lncRNAs. (A) Interaction network of miRNA and their targets of lncRNAs. The network was drawn by Cytoscape. (B) Frequency of miRNAs targeting on lncRNAs. (C) Frequency of lncRNAs targeted by miRNA families.

calcium signaling.

3.7. LncRNAs as putative targets of miRNAs

To understand the association between miRNAs and lncRNAs in response to drought in cassava, psRNATarget server was used to identify DE lncRNAs as the potential targets of miRNAs. In total, 34 DE lncRNAs, which were targeted by 75 miRNAs distributed from sixteen families, were identified, and the network of miRNAs and lncRNAs were shown in Fig.6A. The details of miRNAs and targeting lncRNAs were provided in the Table S6. The number of lncRNAs targeted by miRNAs ranged from one to five, and most (36%) miRNAs only targeted one lncRNA (Fig. 6B). Notably, miR159a, which was induced by ABA and drought treatment by directing the degradation of MYB mRNAs in *Arabidopsis* (Reyes and Chua, 2007), targeted five lncRNAs including TCONS_00005904, TCONS_00060630, TCONS_00063065, TCONS_00104537, and TCONS_00113226. In addition, miR827 and miR156, which were both involved in drought stress response (Ferdous et al., 2017; González-Villagra et al., 2017), targeted four lncRNAs, respectively. These results suggested that miR159, miR827, and miR156 might be key players in cassava drought stress via lncRNA-miRNA interaction.

We also investigated the number of miRNA families having the potential to target lncRNAs (Fig. 6C). Specifically, TCONS_00063065 was targeted by as many as five miRNAs, and TCONS_00046710 and TCONS_00067612 were targeted by four each miRNAs, respectively, strongly suggested the participation of these three lncRNAs in cassava drought stress with the involvement of miRNA regulation.

4. Discussion

Drought is one of the most widespread abiotic stresses affecting plant growth and crop yield in the world (Fu et al., 2016). Furthermore, ABA biosynthesis and signaling transduction are considered to be crucial for plants abiotic stress, including salt, cold and drought (Ben-Ari, 2012). Under drought stress or ABA treatment, the content of ABA, as

an essential signal molecule, was quickly induced (Daszkowska-Golec and Szarejko, 2013). In addition, the H_2O_2 acting as an important reactive oxygen species (ROS) was also rapidly accumulated to cause oxidative damage during the period of drought or ABA treatment. To deal with the generated oxidative stress, several anti-oxidative enzymes such as SOD, CAT and POD were rapidly activated (Li et al., 2010; Zeng and Luo, 2012). In this study, we observed that the content of ABA and H_2O_2 were significantly increased at 3, 5, and 7 d under ABA and PEG treatment, and accordingly, CAT and POD activities were greatly increased at these time-points compared with control (Fig. 1). These results were consistent with the descriptions in previous studies (Ding et al., 2011; Huang et al., 2015; Fu et al., 2016).

LncRNAs can function via cis-acting regulation (Deng et al., 2018; Kopp and Mendell, 2018). For example, lncRNA33732 located 1752 bp adjacent to RBOH and induced the expression of RBOH to defense *Phytophthora infestans* attack through accumulating H_2O_2 in tomato (Cui et al., 2018). In this work, we observed that many DE lncRNAs regulated their adjacent target genes involved in ABA biosynthesis/signaling and RNA transcriptional regulation in response to PEG or ABA treatment. For examples, TCONS_00085215 located adjacently and co-expressed with Manes.10G067700, which encodes a 8-hydroxylase protein that plays crucial roles in expression regulating of ABA metabolism genes (Zheng et al., 2015; Saika et al., 2007), Moreover, the ABA content was varied accordingly with the expression change of TCONS_00085215 and Manes.10G067700. We also found that TCONS_00114791 regulated a SDR protein (Manes.13G126100) that acts as an important ABA biosynthesis gene (Cheng et al., 2002), TCONS_00018832 regulated a TCP transcription factor (Fig. 4B) referred to ABA signaling and drought stress (Lei et al., 2017; Wu et al., 2018), TCONS_00005423 regulated its neighboring gene (Manes.01G238300) encoding a NAC transcription factor related to ABA biosynthesis and abiotic stress tolerance (Mao et al., 2017; Shen et al., 2017). Collectively, these results strongly suggested that these DE lncRNAs participated in cassava drought stress via ABA-dependent signaling pathway. In addition, a few lncRNAs (e.g., TCONS_00055300) were exclusively differentially expressed under PEG treatment and it seems that their neighboring targets were not relevant to ABA biosynthesis and signaling, indicating these lncRNAs might participate in cassava drought stress via ABA-independent pathways.

LncRNAs can function via trans-acting regulation (Deng et al., 2018; Kopp and Mendell, 2018). For examples, *Arabidopsis* lncRNA *HID1* acts in trans and as a component of RNA–protein complex associated with the chromatin of the transcription factor *PIF3* to repress its transcription (Wang et al., 2014); moreover, tomato lncRNA16397 acts as an antisense transcript of SlGRX22 to affect its expression during tomato *P. infestans* interactions (Cui et al., 2017). In this work, a total of 103 DE lncRNAs were identified to be involved in trans-acting regulation by gene co-expression analysis, and they were classified into six groups according to their expression patterns under ABA and PEG treatment (Fig. 5). The lncRNAs in group G2 were greatly suppressed while those in group G3 were dramatically induced by both ABA and PEG treatment, suggesting the involvement of these lncRNAs in drought stress via ABA signaling. Consistently, one SDR gene (Manes.09G050600) and two CYP707A genes (Manes.01G170900 and Manes.10G071600) related to ABA biosynthesis and signaling (Cheng et al., 2002; Zheng et al., 2015) were included in group G2. Similarly, another two CYP707A genes (Manes.07G081100 and Manes.10G067700) were also included in group G3. In contrast to G2 and G3, the lncRNAs in group G4 were greatly induced by PEG treatment but not significantly changed under ABA treatment, suggesting these lncRNAs might participate in drought response via ABA-independent signaling regulation. As expected, a few transcription factors including ERF/AP2 (Manes.13G033200 and Manes.16G030600) and HD-Zip (Manes.01G242300 and Manes.05G018700) and hormone signaling genes such as GID (Manes.05G070500) and JAZ (Manes.15G133000) involved in ABA-independent signaling pathways (Yamaguchi-Shinozaki and Shinozaki, 2005; Fu et al., 2016; Liu et al., 2018) were found in this group.

LncRNAs can also function as targets of miRNAs (Franco-Zorrilla et al., 2007). To date, many miRNAs have been functionally characterized in drought stress in plants, therefore, their functions can be directly used to predict the roles of lncRNAs. For examples, miR156 was up-regulated as a drought stress-responsive gene in many species and its expression was significantly induced by endogenous ABA treatment (González-Villagra et al., 2017); miR159 was rapidly induced by ABA and drought treatment accompanied by the degradation of MYB mRNA levels (Reyes and Chua, 2007). In addition, miR167, miR169, miR172, and miR394 were also involved in ABA response and drought stress (Ni et al., 2012; Ding et al., 2013; Li et al., 2016). In this study, a total of 34 lncRNAs acting as miRNA targets were identified, of which 24 were targeted by the above six miRNAs (Supplemental Tables S6). It's noteworthy that TCONS_00129136 was targeted by miR156i and miR169 l, TCONS_00122745 was targeted by miR159d and miR169 h, TCONS_00088201 was targeted by miR159a and miR172c, and TCONS_00067612 was targeted by miR156k and miR172c. Moreover, the expression levels of these four lncRNAs were significantly altered in response to both ABA and PEG treatment, strongly suggested that these four lncRNAs might participate in cassava drought response via ABA-dependent pathways with the involvement of miRNA regulation. However, currently it's very hard for us to distinguish whether the remaining lncRNAs were involved in ABA-dependent or ABA-independent signaling pathways in drought via miRNA-lncRNA interaction without adequate evidences.

5. Conclusions

In summary, this work provides a comprehensive identification of cassava lncRNAs under ABA and PEG treatment, and systematically analyzes the functions of lncRNAs through cis-regulation, trans-regulation, and miRNA targets. These results were finally summarized in Fig.7, and the important lncRNA candidates in each regulatory type were proposed. Especially, lots of lncRNAs were identified to participate in drought response via ABA-dependent or ABA-independent signaling pathways in cassava, which will enable in-depth functional analysis of these lncRNAs in the near future.

Author contributions

G.H., W.H., G.Y., M.C., conceived and designed the study. C.W., Z.D., Y.Y., W.T. and J.Z., performed the experiments. C.W. and Z.D. carried out the data. C.W., Z.D. and W.H. wrote the manuscript.

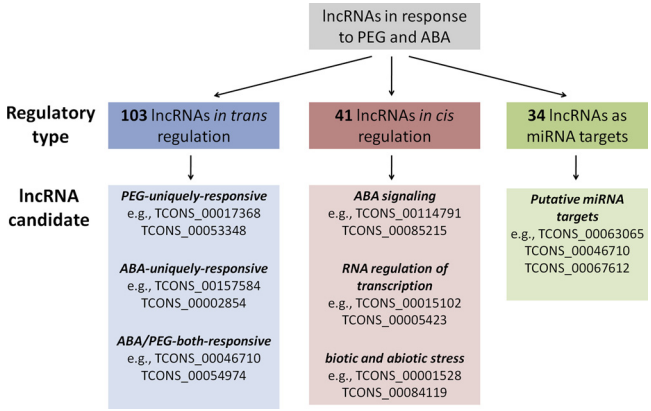

Fig. 7. Summarize of lncRNAs in response to PEG and ABA treatment in cassava. In total, 103 lncRNAs *in trans* regulation, 41 *in cis* regulation, and 34 as miRNA targets were identified, respectively.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2019.103809.

References

Ariel, F., Romero-Barrios, N., Jégu, T., Benhamed, M., Crespi, M., 2015. Battles and hijacks: noncoding transcription in plants. Trends Plant Sci. 20, 362–371. https://doi.org/10.1016/j.tplants.2015.03.003.

Ben-Ari, G., 2012. The ABA signal transduction mechanism in commercial crops: learning from *Arabidopsis*. Plant Cell Re. 31, 1357–1369. https://doi.org/10.1007/s00299-012-1292-2.

Cheng, W.H., Endo, A., Zhou, L., Penney, J., Chen, H.C., Arroyo, A., et al., 2002. A unique short-chain dehydrogenase/reductase in *Arabidopsis* glucose signaling and abscisic acid biosynthesis and functions. Plant Cell 14, 2723–2743. https://doi.org/10.1105/tpc.006494.

Csorba, T., Questa, J.I., Sun, Q., Dean, C., 2014. Antisense COOLAIR mediates the coordinated switching of chromatin states at FLC during vernalization. Proc. Natl. Acad. Sci. U. S. A. 111, 16160–16165. https://doi.org/10.1073/pnas.1419030111.

Cui, J., Luan, Y., Jiang, N., Bao, H., Meng, J., 2017. Comparative transcriptome analysis between resistant and susceptible tomato allows the identification of lncRNA16397 conferring resistance to phytophthora infestans by co-expressing glutaredoxin. Plant J. 89, 577. https://doi.org/10.1111/tpj.13408.

Cui, J., Jiang, N., Meng, J., Yang, G., Liu, W., Zhou, X., et al., 2018. LncRNA33732-respiratory burst oxidase module associated with WRKY1 in tomato- Phytophthora infestans interactions. Plant J. 89, 577–589. https://doi.org/10.1111/tpj.14173.

Dai, X., Zhao, P.X., 2011. PsRNATarget: a plant small RNA target analysis server. Nucleic Acids Res. 39, W155–9. https://doi.org/10.1093/nar/gkr319.

Daszkowska-Golec, A., Szarejko, I., 2013. The Molecular Basis of ABA-Mediated Plant Response to Drought. InTech Open Access Publisher, Rijeka, Croatia. https://doi.org/10.5772/53128.

Deng, F., Zhang, X., Wang, W., Yuan, R., Shen, F., 2018. Identification of *Gossypium hirsutum* long non-coding RNAs (lncRNAs) under salt stress. BMC Plant Biol. 18, 23. https://doi.org/10.1186/s12870-018-1238-0.

Ding, Y., Avramova, Z., Fromm, M., 2011. The Arabidopsis trithorax-like factor ATX1 functions in dehydration stress responses via ABA-dependent and ABA-independent pathways. Plant J. 66, 735–744. https://doi.org/10.1111/j.1365-313X.2011.04534.x.

Ding, J., Lu, Q., Ouyang, Y., Mao, H., Zhang, P., Yao, J., et al., 2012. A long noncoding RNA regulates photoperiod-sensitive male sterility, an essential component of hybrid rice. Proc. Natl. Acad. Sci. U. S. A. 109, 2654–2659. https://doi.org/10.1073/pnas.1121374109.

Ding, Y., Tao, Y., Zhu, C., 2013. Emerging roles of micrornas in the mediation of drought stress response in plants. J. Exp. Bot. 64, 3077–3086. https://doi.org/10.1093/jxb/ert164.

Ding, Z., Zhang, Y., Xiao, Y., Liu, F., Wang, M., Zhu, X., et al., 2016. Transcriptome response of cassava leaves under natural shade. Sci. Rep. 6, 31673. https://doi.org/10.1038/srep31673.

Eddy, S.R., 2011. Accelerated profile HMM searches. PLoS Comput. Biol. 7, e1002195. https://doi.org/10.1371/journal.pcbi.1002195.

Ferdous, J., Whitford, R., Nguyen, M., Brien, C., Langridge, P., Tricker, P.J., 2017. Drought-inducible expression of hv -miR827 enhances drought tolerance in transgenic barley. Funct. Integr. Genomics 17, 279–292. https://doi.org/10.1007/s10142-016-0526-8.

Finn, R.D., Coggill, P., Eberhardt, R.Y., Eddy, S.R., Mistry, J., Mitchell, A.L., et al., 2016. The Pfam protein families database: towards a more sustainable future. Nucleic Acids Res. 44, D279–D285. https://doi.org/10.1093/nar/gkv1344.

Franco-Zorrilla, J.M., Valli, A., Todesco, M., Mateos, I., Puga, M.I., Rubio-Somoza, I., Leyva, A., et al., 2007. Target mimicry provides a new mechanism for regulation of microRNA activity. Nat. Genet. 39, 1033–1037. https://doi.org/10.1038/ng2079.

Fu, L., Ding, Z., Han, B., Hu, W., Li, Y., Zhang, J., 2016. Physiological investigation and transcriptome analysis of polyethylene glycol (PEG)-induced dehydration stress in cassava. Int. J. Mol. Sci. 17, 283. https://doi.org/10.3390/ijms17030283.

González-Villagra, J., Kurepin, L.V., Reyes-Díaz, M.M., 2017. Evaluating the involvement and interaction of abscisic acid and miRNA156 in the induction of anthocyanin biosynthesis in drought-stressed plants. Planta 246, 299–312. https://doi.org/10.1007/s00425-017-2711-y.

Guttman, M., Amit, I., Garber, M., French, C., Lin, M.F., Feldser, D., et al., 2009. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 458, 223–227. https://doi.org/10.1038/nature07672.

Henz, S.R., Cumbie, J.S., Kasschau, K.D., Lohmann, J.U., Carrington, J.C., Weigel, D., et al., 2007. Distinct expression patterns of natural antisense transcripts in *Arabidopsis*. Plant Physiol. 144, 1247–1255. https://doi.org/10.1104/pp.107.100396.

Hu, W., Kong, H., Guo, Y., Zhang, Y., Ding, Z., Tie, W., et al., 2016. Comparative physiological and transcriptomic analyses reveal the actions of melatonin in the delay of postharvest physiological deterioration of cassava. Front. Plant Sci. 7, 736. https://doi.org/10.3389/fpls.2016.00736.

Huang, J., Wang, J.F., Wang, Q.H., Zhang, H.S., 2005. Identification of a rice zinc finger protein whose expression is transiently induced by drought, cold but not by salinity and abscisic acid. DNA Seq. 6, 130–136. https://doi.org/10.1080/10425170500061590.

Huang, Q., Wang, Y., Li, B., Chang, J., He, G., 2015. TaNAC29, a NAC transcription factor from wheat, enhances salt and drought tolerance in transgenic *Arabidopsis*. BMC Plant Biol. 15, 268. https://doi.org/10.1186/s12870-015-0644-9.

Kim, E.D., Sung, S., 2012. Long noncoding RNA: unveiling hidden layer of gene regulatory networks. Trends Plant Sci. 17, 0–21. https://doi.org/10.1016/j.tplants.2011.10.008.

Kong, L., Zhang, Y., Ye, Z.Q., Liu, X.Q., Zhao, S.Q., Wei, L., et al., 2007. CPC: assess the protein-coding potential of transcripts using sequence features and support vector machine. Nucleic Acids Res. 35, W345–W349. https://doi.org/10.1093/nar/gkm391.

Kopp, F., Mendell, J.T., 2018. Functional classification and experimental dissection of long noncoding RNAs. Cell 172, 393–407. https://doi.org/10.1016/j.cell.2018.01.011.

Kozomara, A., Griffiths-Jones, S., 2013. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res. 42, D68–73. https://doi.org/10.1093/nar/gkt1181.

Kung, J.T.Y., Colognori, D., Lee, J.T., 2013. Long noncoding RNAs: past, present, and future. Genetics 193, 651–669. https://doi.org/10.1534/genetics.112.146704.

Langfelder, P., Horvath, S., 2008. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9, 559. https://doi.org/10.1186/1471-2105-9-559.

Lei, N., Yu, X., Li, S., Zeng, C., Zou, L., Liao, W., et al., 2017. Phylogeny and expression pattern analysis of TCP transcription factors in cassava seedlings exposed to cold and/or drought stress. Sci. Rep. 7, 10016. https://doi.org/10.1038/s41598-017-09398-5.

Li, D., Li, C., Sun, H., Wang, W., Liu, L., Zhang, Y., 2010. Effects of drought on soluble protein content and protective enzyme system in cotton leaves. Front. Agric. China 4, 56–62. https://doi.org/10.1007/s11703-010-0102-2.

Li, W., Wang, T., Zhang, Y., Li, Y., 2016. Overexpression of soybean miR172c confers tolerance to water deficit and salt stress, but increases ABA sensitivity in transgenic Arabidopsis thaliana. J. Exp. Bot. 67, 175. https://doi.org/10.1093/jxb/erv450.

Li, S., Yu, X., Lei, N., Cheng, Z., Peng, M., 2017. Genome-wide identification and functional prediction of cold and/or drought-responsive lncRNAs in cassava. Sci. Rep. 7, 45981. https://doi.org/10.1038/srep45981.

Liao, Q., Liu, C., Yuan, X., Kang, S., Miao, R., Xiao, H., et al., 2011. Large-scale prediction of long non-coding RNA functions in a coding-non-coding gene co-expression network. Nucleic Acids Res. 39, 3864–3878. https://doi.org/10.1093/nar/gkq1348.

Liu, N., Ding, Y., Fromm, M., Avramova, Z., 2014. Endogenous ABA extraction and measurement from *Arabidopsis* leaves. Bioprotocol 4.

Liu, J., Wang, H., Chua, N.H., 2015. Long noncoding RNA transcriptome of plants. Plant Biotechnol. J. 13, 319–328. https://doi.org/10.1111/pbi.12336.

Liu, S.W., Lv, Z.Y., Liu, Y.H., Li, L., Zhang, L.D., 2018. Network analysis of ABA-dependent and ABA-independent drought responsive genes in *Arabidopsis thaliana*. Genet. Mol. Biol. 41, 624–637. https://doi.org/10.1590/1678-4685-GMB-2017-0229.

Lu, X., Chen, X., Mu, M., Wang, J., Wang, X., Wang, D., et al., 2016. Genome-wide analysis of long noncoding RNAs and their responses to drought stress in Cotton (*Gossypium hirsutum* L.). PLoS One 11, e0156723. https://doi.org/10.1371/journal.pone.0156723.

Mao, C., Lu, S., Lv, B., Zhang, B., Shen, J., He, J., et al., 2017. A Rice NAC transcription factor promotes leaf senescence via ABA biosynthesis. Plant Physiol. 174, 1747–1763. https://doi.org/10.1104/pp.17.00542.

Maran, J.P., Sivakumar, V., Thirugnanasambandham, K., Sridhar, R., 2014. Degradation behavior of biocomposites based on cassava starch buried under indoor soil conditions. Carbohydr. Polym. 30, 20–28. https://doi.org/10.1016/j.carbpol.2013.08.080.

Marquardt, S., Raitskin, O., Wu, Z., Liu, F., Sun, Q., Dean, C., 2014. Functional consequences of splicing of the antisense transcript *COOLAIR* on *FLC* transcription. Mol. Cell 54, 156–165. https://doi.org/10.1016/j.molcel.2014.03.026.

Ni, Z., Hu, Z., Jiang, Q., Zhang, H., 2012. Overexpression of gma-MIR394a confers tolerance to drought in transgenic *Arabidopsis thaliana*. Biochem. Biophys. Res. Commun. 427, 330–335. https://doi.org/10.1016/j.bbrc.2012.09.055.

Oliveira, E.J., Santana, F.A., Oliveira, L.A., Santos, V.S., 2014. Genetic parameters and prediction of genotypic values for root quality traits in cassava using REML/BLUP. Genet. Mol. Res. 13, 6683–6700. https://doi.org/10.4238/2014.

Ponting, C.P., Oliver, P.L., Reik, W., 2009. Evolution and functions of long noncoding RNAs. Cell 136, 629–641. https://doi.org/10.1016/j.cell.2009.02.006.

Qin, T., Zhao, H., Cui, P., Albesher, N., Xiong, L., 2017. A nucleus-localized long non-coding RNA enhances drought and salt stress tolerance. Plant Physiol. 175, 1321–1336. https://doi.org/10.1104/pp.17.00574.

Reyes, J.L., Chua, N.H., 2007. ABA induction of miR159 controls transcript levels of two

MYB factors during *Arabidopsis* seed germination. Plant J. 49, 592–606. https://doi.org/10.1111/j.1365-313X.2006.02980.x.

Saika, H., Okamoto, M., Miyoshi, K., Kushiro, T., Shinoda, S., Jikumaru, Y., et al., 2007. Ethylene promotes submergence-induced expression of OsABA8ox1, a gene that encodes ABA 8-hydroxylase in rice. Plant Cell Physiol. 48, 287. https://doi.org/10.1093/pcp/pcm003.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., et al., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504. https://doi.org/10.1101/gr.1239303.

Shen, J., Lv, B., Luo, L., He, J., Mao, C., Xi, D., et al., 2017. The NAC-type transcription factor OsNAC2 regulates ABA-dependent genes and abiotic stress tolerance in rice. Sci. Rep. 7, 40641. https://doi.org/10.1038/srep40641.

Shuai, P., Liang, D., Tang, S., Zhang, Z., Ye, C.Y., Su, Y., et al., 2014. Genome-wide identification and functional prediction of novel and drought-responsive lincRNAs in *Populus trichocarpa*. J. Exp. Bot. 65, 4975–4983. https://doi.org/10.1093/jxb/eru256.

Sun, L., Luo, H., Bu, D., Zhao, G., Yu, K., Zhang, C., et al., 2013. Utilizing sequence intrinsic composition to classify protein-coding and long non-coding transcripts. Nucleic Acids Res. 41, e166. https://doi.org/10.1093/nar/gkt646.

Thimm, O., Bläsing, O., Gibon, Y., Nagel, A., Meyer, S., Krüger, P., et al., 2004. MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J. 37, 914–939. https://doi.org/10.1111/j.1365-313X.2004.02016.x.

Trapnell, C., Pachter, L., Salzberg, S.L., 2009. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25, 1105–1111. https://doi.org/10.1093/bioinformatics/btp120.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., et al., 2012. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. 7, 562–578. https://doi.org/10.1038/nprot.2012.016.

Wang, L., Park, H.J., Dasari, S., Wang, S., Kocher, J.P., Li, W., 2013. CPAT: coding-potential assessment tool using an alignment-free logistic regression model. Nucleic Acids Res. 41, e74. https://doi.org/10.1093/nar/gkt006.

Wang, Y., Fan, X., Lin, F., He, G., Terzaghi, W., Zhu, D., et al., 2014. *Arabidopsis* noncoding RNA mediates control of photomorphogenesis by red light. Proc. Natl. Acad. Sci. 111, 10359–10364. https://doi.org/10.1073/pnas.1409457111.

Wang, J., Lin, J., Kan, J., Wang, H., Li, X., Yang, Q., et al., 2018. Genome-wide identification and functional prediction of novel drought-responsive lncRNAs in *Pyrus betulifolia*. Genes 9, 311. https://doi.org/10.3390/genes9060311.

Wu, H.J., Wang, Z.M., Wang, M., Wang, X.J., 2013. Widespread long noncoding RNAs as endogenous target mimics for microRNAs in plants. Plant Physiol. 161, 1875–1884. https://doi.org/10.1104/pp.113.215962.

Wu, J., Wu, W., Liang, J., Jin, Y., Gazzarrini, S., He, J., et al., 2018. GhTCP19 transcription factor regulates corm dormancy release by repressing GhNCED expression in *Gladiolus*. Plant Cell Physiol. pcy186. https://doi.org/10.1093/pcp/pcy186.

Xie, L.N., Chen, M., Min, D.H., Feng, L., Xu, Z.S., Zhou, Y.B., et al., 2016. The NAC-like transcription factor SiNAC110 in foxtail millet (Setaria italica L.) confers tolerance to drought and high salt stress through an ABA independent signaling pathway. J. Integr. Agric. 15, 60345–60347. https://doi.org/10.1016/S2095-3119(16)61429-6.

Yamaguchi-Shinozaki, K., Shinozaki, K., 2005. Organization of cisacting regulatory elements in osmotic- and cold-stress-responsive promoters. Trends Plant Sci. 10, 88–94. https://doi.org/10.1016/j.tplants.2004.12.012.

Zeng, D., Luo, X., 2012. Physiological effects of chitosan coating on wheat growth and activities of protective enzyme with drought tolerance. Open J. Soil Sci. 2, 282–288. https://doi.org/10.4236/ojss.2012.23034.

Zhang, Y.C., Chen, Y.Q., 2013. Long noncoding RNAs: new regulators in plant development. Biochem. Biophys. Res. Commun. 436, 111–114. https://doi.org/10.1016/j.bbrc.2013.05.086.

Zhang, W., Han, Z., Guo, Q., Liu, Y., Zheng, Y., Wu, F., et al., 2014. Identification of maize long non-coding RNAs responsive to drought stress. PLoS One 9, e98958. https://doi.org/10.1371/journal.pone.0098958.

Zhao, X., Li, J., Lian, B., Gu, H., Li, Y., Qi, Y., 2018. Global identification of *Arabidopsis* lncRNAs reveals the regulation of *MAF4* by a natural antisense RNA. Nat. Commun. 9, 5056. https://doi.org/10.1038/s41467-018-07500-7.

Zheng, C., Halaly, T., Acheampong, A.K., Takebayashi, Y., Jikumaru, Y., Kamiya, Y., et al., 2015. Abscisic acid (ABA) regulates grape bud dormancy, and dormancy release stimuli may act through modification of ABA metabolism. J. Exp. Bot. 66, 1527–1542. https://doi.org/10.1093/jxb/eru519.