Identification of drought-responsive hub genes and their related miRNAs in *Arabidopsis thaliana*

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Abstract

Drought is important environmental stress that reduces the yield and quality of crops all around the world. The drought-responsive regulatory network is very complex in plants. MicroRNAs are an important gene expression regulator under drought conditions. Identifying drought-responsive genes and their related miRNAs helps us to provide valuable information on plant stress regulation by miRNAs in stress conditions. In this study, a microarray dataset of drought stress and well-water samples of Arabidopsis were analyzed to identify the common root and shoot genes in drought. GEO2R tool was used for identifying differentially expressed genes. Gene ontology enrichment, protein-protein interaction, pathway analysis, and potential miRNAs for hub genes were performed using bioinformatics tools. There were 486 and 288 DEGs of root and shoot tissue respectively. Venn diagram analysis demonstrated that 59 DEGs were common in both root and shoot tissues, including 51 upregulated genes and 8 downregulated genes enriched in response to water deprivation, response to osmotic stress, response to abscisic acid, response to oxidative stress, response to salt stress and other related pathways. Gene ontology and protein network analysis showed that *TSPO*, *LTI78*, *AFP1*, *RAB18*, *LTI65*, *HAI1*, *HAI2*, *LEA4-5*, *LEA7*, and *F16B3.11* genes are hub genes in drought conditions. All hub genes except *LEA7* and *F16B3.11* were target genes of miRNAs. It seems that these genes and their related miRNAs can play a crucial role in drought response adaptation and they can be considered in breeding programs and genetic engineering for the production of drought-tolerant plants.

Introduction

Drought stress has a devastating effect on plant growth and development which reduces the yield and quality of crops all around the world. Drought induces morphophysiological, biochemical, and molecular responses in plants. Leaf area, shoot length, Photosynthetic rate, growth, and yield are reduced under drought conditions. Also, Reactive Oxygen Species (ROS) accumulation and stress metabolites are increased (Kumar et al. 2018).

ROS is a signal transduction molecule in plant cells under normal conditions (Mittler 2017). Whereas under stress conditions, ROS and scavenging are primary plant defense strategies to fight against biotic or abiotic stress (Nath et al. 2016). Drought conditions lead to the production of various ROS. ROS causes cellular damage, toxicity, and inhibition of photosynthesis (Rao and Chaitanya 2016).

The regulatory network involved in plants' response to drought conditions is complex and incorporates a wide range of transcription factors, molecular networks, comprising stress sensing, signaling, and regulation of gene expression. Many drought-responsive genes are down and up-regulated under drought conditions (Kim et al. 2020).

After signal transduction and sensing the drought stress by cell receptors, plants initiate drought recovery mechanisms (Mahmood et al. 2020). Abscisic acid (ABA) accumulation under drought stress conditions, is vital and causes adaptation to drought stress in plants. This leads to induce expression of drought-

responsive genes such as *RD29A/B*, *rd22*, and *RAB18* genes (Kumar et al. 2018). ABA binds to different ABA receptors in intracellular and extracellular sites like PYR/PYL/RCAR (Cutler et al. 2010). The ABA-induced genes are involved in drought stress tolerance, including dehydrins, enzymatic and nonenzymatic antioxidants, and regulatory factors. The accumulation of osmolyte solutes, dehydrins, and Late Embryogenesis Abundant proteins (LEAs) are crucial in protecting proteins and membranes under drought (Cutler et al. 2010; Rao and Chaitanya 2016).

Transcription factors such as *bZIP*, *AP2/ERF*, *MYB*, and *NAC* family TFs are important in gene expression regulation (Janiak et al. 2016; Mahmood et al. 2020). Drought induces signaling pathways like Mitogen-Activated Protein Kinase (MAPK), Ca^{2+}, and ABA-mediated signaling pathways (Mahmood et al. 2020).

Micro RNAs (miRNAs) are small and non-coding RNAs having a great impact on stress response in the plant (Guo et al. 2020). Several studies on Arabidopsis (Baek et al. 2016), Canola (Jian et al. 2016), Indian mustard (Bhardwaj et al. 2014), Wheat (Kantar et al. 2011), Rice (Balyan et al. 2017), Switchgrass (Hivrale et al. 2016), maize (Wei et al. 2009), tomato (Liu et al. 2018) and other plants showed that miRNAs are involved in plant response and adaptation to drought stress. Therefore, the Identification of drought-responsive miRNAs helps us to provide valuable information on plant stress physiology (Vakilian 2020).

nowadays, reanalyzing extensive transcriptome datasets is publicly available, and economical, and a valuable way to investigate important genes in drought. It helps identification of the involved genes network in drought stress. The purpose of our study was the identification of drought-responsive genes and associated pathways in both shoot and root tissues of Arabidopsis under drought stress conditions and identification of related miRNAs by in silico analysis.

Material And Methods

Data analysis and identification of DEGs

Microarray expression profiles of the GSE76827 datasets were obtained from the GEO database (http://www.ncbi.nlm.nih.gov/geo/). Drought treatment (5 days after a drought) and unstressed samples of the shoot and root tissues of *Arabidopsis thaliana* data files were downloaded. GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) was used to detect the differentially expressed genes (DEGs) between treatment and unstressed samples of shoot and root separately. GEO2R performs comparisons on two or more groups of samples in a GEO series dataset. Genes with $\log_2$ fold-change (FC)>2, p-value<0.05 and Adj p-value < 0.05 in expression were considered to be DEGs. FC> 2 and < -2 were identified as upregulated and downregulated genes respectively.

Venn diagram analysis

Venn diagram analysis was used for the determination of common DEGs in root and shoot tissues. Common genes help us to identify important and effective genes in drought stress conditions.

Identification of GO

Gene Ontology (GO) was conducted by DAVID (https://david.ncifcrf.gov). This online bioinformatics resource is a free database that investigated genes based on three terms including Molecular Function (MF), Biological Process (BP), and Cellular Component (CC). Therefore, It provides a comprehensive set of functional annotation tools for the understanding of GO and KEGG pathways for genes (Jiao et al. 2012).

Determination of drought-responsive hub genes

Network analyzer and CytoHubba plugins of Cytoscape software version 3.8.1 (https://cytoscape.org/) were used for the determination of drought-responsive hub genes with a high degree of connectivity in common DEGs in root and shoot by Maximal Clique Centrality (MCC) topological algorithms (Pezeshkian et al. 2020).

PPI network analysis

STRING database and STRING app of Cytoscape software was used for protein-protein interaction (PPI) network analysis (https://string-db.org). The scores of > 0.4 and the maximum number of interactors = 0 were selected to construct the PPI network.

Identification of potential miRNA for hub genes

Potential miRNA for hub genes identified by psRNATarget server (http://plantgrn.noble.org/psRNATarget/) (Dai et al. 2018). This web-based target prediction tool was developed to identify plant small RNA and their targets. This server is clear, compelling, free, and user-friendly. mRNA sequences of hub genes were used as candidate targets of 427 published miRNAs in *Arabidopsis thaliana* (miRBase Release 21, June 2014) with expectation ≤4 and penalty for other mismatches=1. Interactions between identified miRNAs and hub genes rechecked in RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/) and IntaRNA (http://rna.informatik.uni-freiburg.de/IntaRNA/Input.jsp). RNAhybrid is a fast and effective tool for finding the minimum free energy hybridization of mRNA and miRNA (Rehmsmeier et al. 2004). IntaRNA also is a good program for the fast and accurate prediction of RNA-RNA interactions (Mann et al. 2017).

Result

GEO2R analysis of 5 days after the onset of drought treatment and unstressed condition samples from the GSE76827 datasets showed that 252 genes were significantly up-regulated in root while 234 genes were significantly down-regulated. The number of up-regulated genes in the shoot was 224 and the number of down-regulated genes was 64. The statistical significance log2 fold change and relationship of genes indicated by volcano plot and Uniform Manifold Approximation and Projection (UMAP) (Fig. 1). Volcano plot of genes is useful for visualizing differentially expressed genes and UMAP displays sample relationships.

Among the 486 DEGs of root and 288 DEGs of the shoot, Venn diagram analysis demonstrated that 59 DEGs were common in both shoot and root tissues (Fig. 2 and Table 1). The protein-protein interaction network of common DEGs is shown in Fig. 3. It suggested that the gene expression regulation network is done in a coordinated manner between shoot and root. Therefore, common genes can be very important and effective in the drought stress regulation network.

Table1. Common DEGs in root and shoot of *Arabidopsis thaliana* under drought stress

Up-regulated DEGs in both root and shoot	*LTP3*, *AT5G01300*, *LTI78*, *SAG29*, *SBT3.14*, *AT2G37870*, *LTI65*, *PYD4*, *AT3G27250*, *VSP2*, *JAL19*, *K18L3.100*, *AT3G02480*, *AT4G33550*, *AT5G66400*, *LEA7*, *K19A23.1*, *SBT3.15*, *COR15A*, *LEA*, *NAC019*, *T17B22.14*, *AT3G62990*, *T14P8.17*, *LTP4*, *YUP8H12.4*, *TSPO*, *BFT*, *HB-7*, *AT4G01985*, *AT5G05220*, *GolS2*, *AT5G50360*, *LEA4-5*, *KIN1*, *AT2G21820*, *AT1G16850*, *F5A18.10*, *HAI2*, *SBT3.13*, *LEA18*, *HIS1-3*, *HAI1*, *AT3G56275*, *MNC17.24*, *SUS3*, *AFP1*, *SIS*, *AT5G63350*, *T6D22.8* LTP3,
Down-regulated DEGs in both root and shoot	F9D1 *F9D 12.7*, *DIN11*, *RL3*, *PRXCA*, *AT4G08780*, *MEE3*, *UPI*, *Prx37*

GO enrichment analysis of common DEGs in root and shoot tissues showed that the most of DEGs in root and shoot are related to the biological process of response to water deprivation and abscisic acid (Table 2). Some genes also were involved in other stress responses like a response to osmotic stress, oxidative stress, salt stress, cold response, and cold acclimation, etc, indicating that drought-responsive pathways are closely related to other stress responses mechanisms. GO analysis of drought DEGs in root and shoot of sorghum revealed that the GO categories were related to response to stimulus especially response to water deprivation and abscisic acid (Zhang et al. 2019). Drought responsive genes also play a similar role in other stresses. Gene Ontology of salt responsive genes in different Rice genotypes illustrated that the biological process of salinity stress similar to drought was a response to water deprivation, cold, salt, and abscisic acid (Kong et al. 2019).

Table 2
GO enrichment analysis of common DEGs in root and shoot tissues by DAVID

Category	Term	Description	Count	*p-Value*	Genes
BP	GO:0009414	response to water deprivation	17	8.38E-19	*LEA18*, *LTI65*, *LTI78*, *AT3G02480*, *AT5G66400*, *SUS3*, *KIN1*, *GolS2*, *LTP3*, *HIS1-3*, *LTP4*, *LEA4-5*, *NAC019*, *HB-7*
BP	GO:0009737	response to abscisic acid	12	1.11E-09	*LTP3*, *LTP4*, *LTI65*, *LTI78*, *AT5G66400*, *KIN1*, *TSPO*, *HB-7*, *GolS2*, *HAI1*, *COR15A*, *LEA*
BP	GO:0006970	response to osmotic stress	6	9.05E-06	*LEA18*, *LEA4-5*, *LTI78*, *KIN1*, *TSPO*, *COR15A*
BP	GO:0006979	response to oxidative stress	7	5.38E-05	*K18L3.100*, *PRXCA*, *VSP2*, *K19A23.1*, *PER38*, *GolS2*, *Prx37*
BP	GO:0010150	leaf senescence	5	6.09E-05	*LTI65*, *LTI78*, *SAG29*, *HAI1*, *COR15A*
BP	GO:0009651	response to salt stress	8	1.15E-04	*LTP4*, *LTI65*, *SIS*, *LTI78*, *TSPO*, *GolS2*, *COR15A*, *AT1G16850*
BP	GO:0009631	cold acclimation	4	2.25E-04	*AT5G66400*, *KIN1*, *COR15A*, *LEA*
BP	GO:0006869	lipid transport	5	4.01E-04	*LTP3*, *LTP4*, *MNC17.24*, *AT2G37870*, *AT4G33550*
BP	GO:0009409	response to cold	6	6.21E-04	LEA4-5, LTI65, LTI78, KIN1, GolS2, COR15A
BP	GO:0009790	embryo development	2	0.006957529	*LEA18*, *LEA4-5*
BP	GO:0042744	hydrogen peroxide catabolic process	3	0.018187202	*PRXCA*, *PER38*, *Prx37*

Category	Term	Description	Count	*p-Value*	Genes
BP	GO:0010555	response to mannitol	2	0.020731032	*LTI78*, *SUS3*
BP	GO:0009793	embryo development ending in seed dormancy	5	0.021538098	*MEE3*, *LEA18*, *LEA4-5*, *LEA7*, *SAG29*
BP	GO:0009415	response to water	2	0.027547633	*AT5G66400*, *LEA*
BP	GO:0006508	proteolysis	5	0.028362298	*UPI*, *SBT3.14*, *SBT3.13*, *SBT3.15*, *AT2G37870*
BP	GO:0002213	defense response to insect	2	0.038805777	*UPI*, *VSP2*
CC	GO:0005576	extracellular region	15	0.001041281	*UPI*, *PRXCA*, *SBT3.14*, *SBT3.13*, SBT3.15, *PER38*, *AT1G16850*, *AT2G37870*, *Prx37*, *T14P8.17*, *LTP3*, *LTP4*, *JAL19*, *MNC17.24*
MF	GO:0008289	lipid binding	6	2.15E-05	*LTP3*, *LTP4*, *MNC17.24*, *COR15A*, *AT2G37870*, *AT4G33550*
MF	GO:0008429	phosphatidylethanolamine binding	2	0.014004533	*T10O8.10*, *BFT*
MF	GO:0004601	peroxidase activity	3	0.017976021	*PRXCA*, *PER38*, *Prx37*
MF	GO:0004252	serine-type endopeptidase activity	3	0.01944604	*SBT3.14*, *SBT3.13*, *SBT3.15*
MF	GO:0020037	heme binding	4	0.029928688	*PRXCA*, *TSPO*, *PER38*, *Prx37*

Network analysis of common 59 DEGs in root and shoot in Cytoscape showed that AT2G47770 (TSPO), AT5G52310 (LTI78), AT1G69260 (AFP1), AT5G66400 (RAB18), AT5G52300 (LTI65), AT5G59220 (HAI1), AT1G07430 (HAI2), AT5G06760 (LEA4-5), AT1G52690 (LEA7) and AT3G02480 (*F16B3.11*) have the highest degree of connectivity in the protein-protein interaction network. PPI network of hub genes is shown in Fig. 4. DAVID gene ontology showed that these genes are related to drought response (Table 2).

Therefore, they were selected as hub genes in the gene network. Also in silico analysis of hub genes sequences illustrate that all hub genes except *LEA7* and *F16B3.11* are target genes of miRNAs (Fig. 5). we determined 10 types of related miRNA of hub genes. Identified miRNAs could be important in regulating the mechanisms of drought-responsive in plants. The finding of miRNAs of hub genes might help to identification of new drought-responsive miRNAs.

Data analysis illustrated that all of the hub genes were upregulated in drought stress. Most hub genes were related to water deprivation response and the ABA signaling pathway. The phytohormone ABA regulates plant responses to dehydration and regulation of water status. Dehydration signals in drought stimulate the accumulation of ABA in plant tissues. ABA plays an important role during stress by mediating signal cross-talk with other pathways (Gupta et al. 2020).

Data analysis demonstrated that the *TSPO* gene upregulated in both roots and shoot tissues in drought stress. A plant *TSPO* roles in various pathways of energy homeostasis in seeds. Overexpressing *TSPO* reduced the levels of lipid droplets in Arabidopsis (Jurkiewicz et al. 2018). *TSPO* plays an important role in other pathways. It can be induced by abiotic stress or ABA treatment and it is localized to the secretory pathway as a scavenger of toxic accumulates in plants (Guillaumot et al. 2009; Jurkiewicz et al. 2018). Therefore, upregulation of its could be important in plant response to drought. In silico investigation in this study showed that ath-miR415 and ath-miR5021 cleavage the *TSPO* gene. *TSPO* expression is upregulated in drought and the regulating factors for the expression level of *TSPO* could be ath-miR415 and ath-miR5021.

Also, *LTI65* and *LTI78* are upregulated in both roots and shoot tissues in drought stress. In silico study showed that both of them are cleaved by ath-miR5632-3p and ath-miR414. *LTI65* also is cleaved by ath-miR2934-5p.

LTI 65 and *LTI78* genes are important in drought, low temperature, and ABA treatment (Megha et al. 2018). The promoter of these genes contains the ABA-responsive element (ABRE), dehydration-responsive element (DRE), and C-repeat (CRT) element. DRE induces gene expression in response to low temperature and dehydration (Mishra et al. 2016).

The result of data analysis illustrated that the expression of ABI five binding protein 1 (*AFP1*) is upregulated in both roots and shoot in drought stress. It is a target of ath-miR414 and ath-miR5658. *AFP1* is one of the key regulators of ABA signaling in Arabidopsis in drought conditions (Garcia et al. 2008). It was increased by 24-hour drought and then decreased (Park et al. 2017). Mutations in *AFP1* lead to increased sensitivity to ABA and salt (Garcia et al. 2008). The target distribution of miRNAs showed that ath-miR5021 regulates the most number of mRNA targets.

Phosphatases 2C (*PP2Cs*) include *ABI1/2*, *HAB1/2*, *AHG1/3*, and *HAI1/2/3* (Xiang et al. 2017). *HAI-1* and *HAI-2* are upregulated in both root and shoot tissues and they are target genes of ath-miR5021 and ath-miR3440b-5p respectively. *HAI-1* and *HAI-2* genes are involved in ABA signaling and plant drought

response. These genes are negative regulators of ABA signaling. *HAI-1* gene is induced by environmental stresses such as ABA treatment and wound in Arabidopsis (Zhang et al. 2013).

LEA proteins are glycine-rich proteins that are important in drought stress tolerance in plants (Magwanga et al. 2018). LEA protein hub genes are *RAB18*, *LEA4-5*, *LEA7*, and *F16B3.11*. Results showed that all of these *LEA* genes upregulated in both root and shoot tissues in drought conditions. *RAB18* is cleaved by ath-miR826a, ath-miR393a-5p and ath-miR393b-5p. One of the most important LEA proteins is dehydrins. The expression of dehydrins can be induced by stress conditions and phytohormones. Some dehydrins can be induced by ABA, they are known as Response to ABA (RAB) proteins (Yu et al. 2018). *RAB18* is one of the dehydrins and it increases freezing tolerance (Hernández-Sánchez et al. 2019).

LEA4-5 is a kind of *LEA* gene that has the highest expression among ABA-inducible genes in plants (Dalal et al. 2009; Hoth et al. 2002). The result showed that *LEA4-5* is one of the hub genes in drought stress and it is the target gene of ath-miR5021 and ath-miR415. Regulation of target gene by ath-miR5021 and ath-miR415 are in the form of cleavage and Translation. Mature plant miRNAs regulate specific mRNAs to gene silencing via mRNA cleavage or translation (Wang et al. 2020).

In silico investigation showed that *LEA7* and *F16B3.11* are hub genes in drought conditions but they do not target genes of any 427 published miRNAs in Arabidopsis until now.

The miRNAs regulate responsive mechanisms to drought stress by the expression of their target genes (Megha et al. 2018). Therefore, investigation of miRNAs and their target genes are important to the understanding of the responsive mechanism in drought stress condition. Our result showed that *TSPO* and *LEA4-5* were target genes of ath-miR415.

TSPO, *HAI1*, and *LEA4-5* were cleaved by ath-miR5021. ath-miR5021 was identified as a miRNA with stress-related response function based on targets prediction in Arabidopsis (Xie et al. 2015). It plays an important role in other stress response like salinity and viral infection responses (Brosseau and Moffett 2015; Eren et al. 2015). Also, salinity stress increases the expression of *TSPO*, *LEA18*, *HAI1*, and *LEA4-5* genes in *Arabidopsis thaliana* (Seok et al. 2020).

Analysis of 198 AtMYB genes illustrated that one of the target genes of ath-miR5021 is the MYB gene. MYB transcription factors are primarily responsible for regulating drought stress response by ABA signals (Singh et al., 2017).

ath-miR5021 is the target of the gene of ARGONAUTE (AGO) proteins. These genes have an important role in virus defense response (Brosseau and Moffett 2015). Expression of ath-miR5021 of *Marsupenaeus japonicus* changes after virus infection (He and Zhang 2012). MicroRNA-microarray assay in Wheat (*Triticum aestivum*) showed that ath-miR5021 had differentially expressed upon salt-stress treatment (Eren et al. 2015). It suggested that ath-miR5021 could be stress-responsive miRNA and it is important in drought stress.

Our result showed that target genes of ath-miR5632-3p are *LTI78* and *LTI65*. Also, ath-miR414 cleaves *LTI78*, *LTI65*, and *AFP1*. The target gene of ath-miR5632 was drought-responsive gene *MAPK1* in *Zanthoxylum bungeanum* under drought stress (Fei et al. 2020). Nitrate stress-responsive genes were the targets of ath-miR5632 (Vidal et al. 2013). A study found that ath-miR414 was downregulated under individual and combined stress of drought and *Pseudomonas syringae* infection (Gupta et al. 2020). It suggested that miR5632 and ath-miR414 could be stress-responsive miRNAs and it may regulate important target genes under drought conditions.

ath-miR2934-5p and ath-miR5658 cleave *LTI65* and *AFP1* respectively. Targets of ath-miR5658 are important genes in the drought such as NAC family and bHLH (Sharma and Das 2019).

Our result demonstrated that *LTI65* is the target gene of ath-miR2934-5p. The identified target genes of ath-miR2934 are stress-responsive genes like histone methyltransferase *SUVH6*. Under stress conditions, *SUVH6* gene expression was reduced and its regulation is important (Grant-Downton et al. 2009). Therefore, ath-miR2934 could be drought-responsive miRNAs.

LTI78, *LTI65*, and *AFP1* are target genes of ath-miR414. ath-miR414 had an inverse relationship with the expression of their respective target genes in drought stress conditions (Gupta et al. 2020).

There is little information about the role of miR415 in different plants. miR415 cleaves proteasome subunit beta type-1 in Rice. It targeted the embryonic-related genes in Arabidopsis and Bambo (Jin et al. 2016). The proteasome system (UPS) is a regulatory mechanism for protein degradation and plays a crucial function in growth, development, and stress tolerance. The effect of miR415 on proteasome may be related to drought.

ath-miR3440b and ath-miR5021 were differentially expressed in salt treatment roots of Wheat (Eren et al. 2015). Our result showed that ath-miR3440b-5p cleaves the *HAI2* gene. miR3440 was nutrients stress-responsive miRNAs (Shahzad et al. 2018). Also, miR3440 was differentially expressed under boron treatment in Barley (Ozhuner et al. 2013).

RAB18 is target gene of ath-miR826a, ath-miR393a-5p and ath-miR393b-5p. In *Arabidopsis thaliana* miR826 was induced by N starvation. Overexpressing miR826 in transgenic plants showed enhanced tolerance to N deficiency (Chien et al. 2017; He et al. 2014). In Arabidopsis, the miR393 family is encoded by MIR393a/b. Exogenous indole-3-acetic acids (IAA) induced miR393 accumulation in *Arabidopsis thaliana*. Also, It has been shown that pathogen responsive genes are regulated by miR393 in response to biotic stress, and overexpression of miR393 caused enhanced antibacterial resistance (Chen et al. 2011). ath-miR393b is an important miRNA in plant defense against fungal pathogens (Cao et al. 2020). A study demonstrated that exogenous IAA treatment caused miR393 to accumulate in Arabidopsis. miR393 targets are related to auxin receptors genes and F-box proteins. Therefore, they could be important in stress conditions such as drought (Chen et al. 2011). ath-miR393a is one of TATA box-null stress-related miRNA genes (Xie et al. 2015). ath-miR393b plays important role in other conditions. It reported that ath-

miR393a was up-regulated and down-regulated at 5% at 20% glyphosate herbicide treatments respectively (Unver et al. 2010). Therefore, ath-miR393b could be regulating factor in different conditions.

Conclusion

Hub genes of common DEGs in root and shoot tissues could be critical genes in drought stress conditions. Also, microRNA-mediated stress regulatory networks are important in plant drought stress response (Balyan et al. 2017). Identification of hub genes and their related miRNAs gives us a more accurate and deeper understanding of the regulation pathway of drought-responsive mechanisms. Some of the identified miRNAs in this study are important in other stress conditions such as salt and cold stress. This indicates that similar pathways occur in stress conditions. Therefore, it is possible that there are similar miRNAs in stress conditions and they are drought-responsive miRNAs.

Declarations

Ethical Statement The authors declare that they have no conflict of interest and informed consent to publish this study.

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Figures

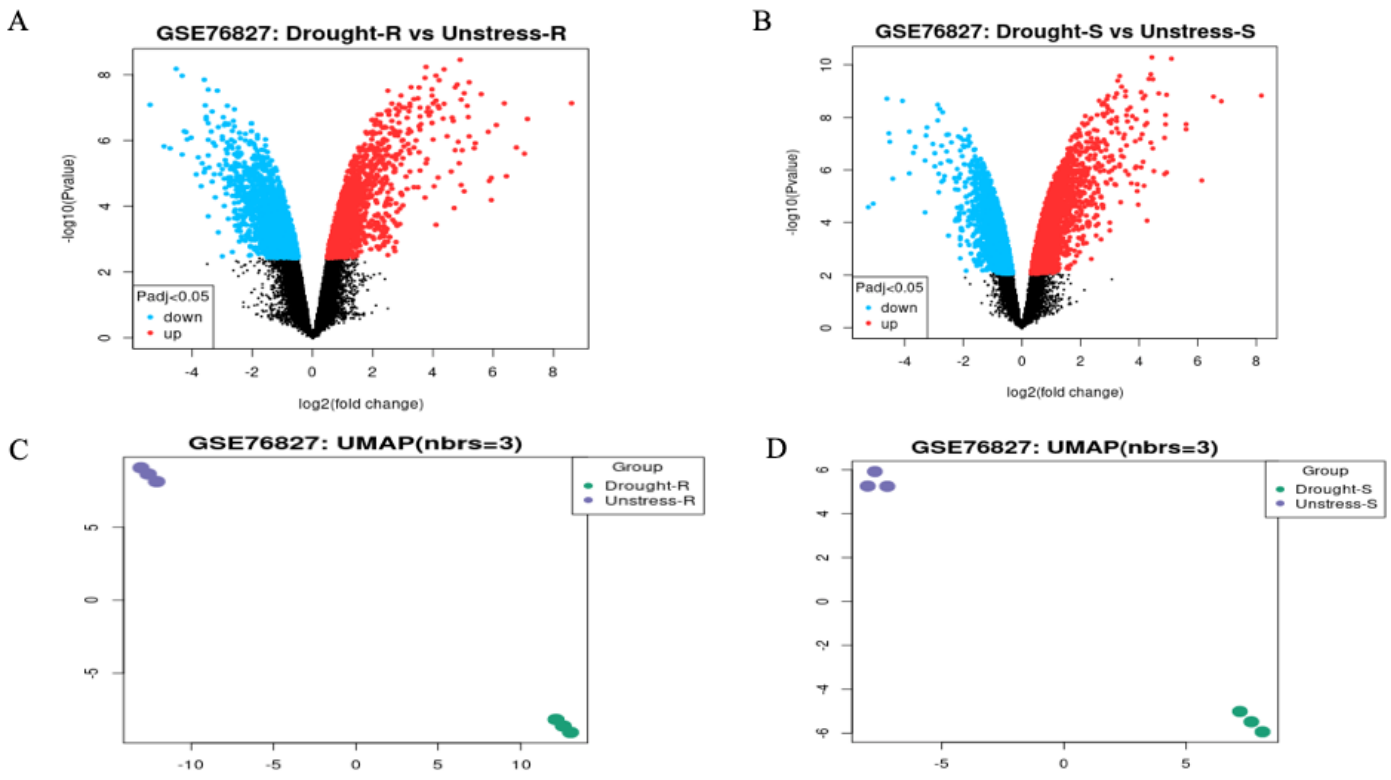

Figure 1

Volcano plot of DEGs in the root (A) and shoot (B), UMAP of DEGs in the root (C), and shoot (D)

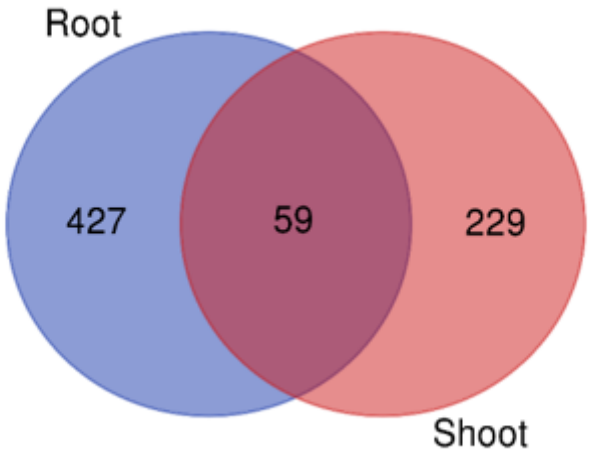

Figure 2

A Venn diagram for common DEGs in shoot compared with the root of Arabidopsis thaliana under drought stress

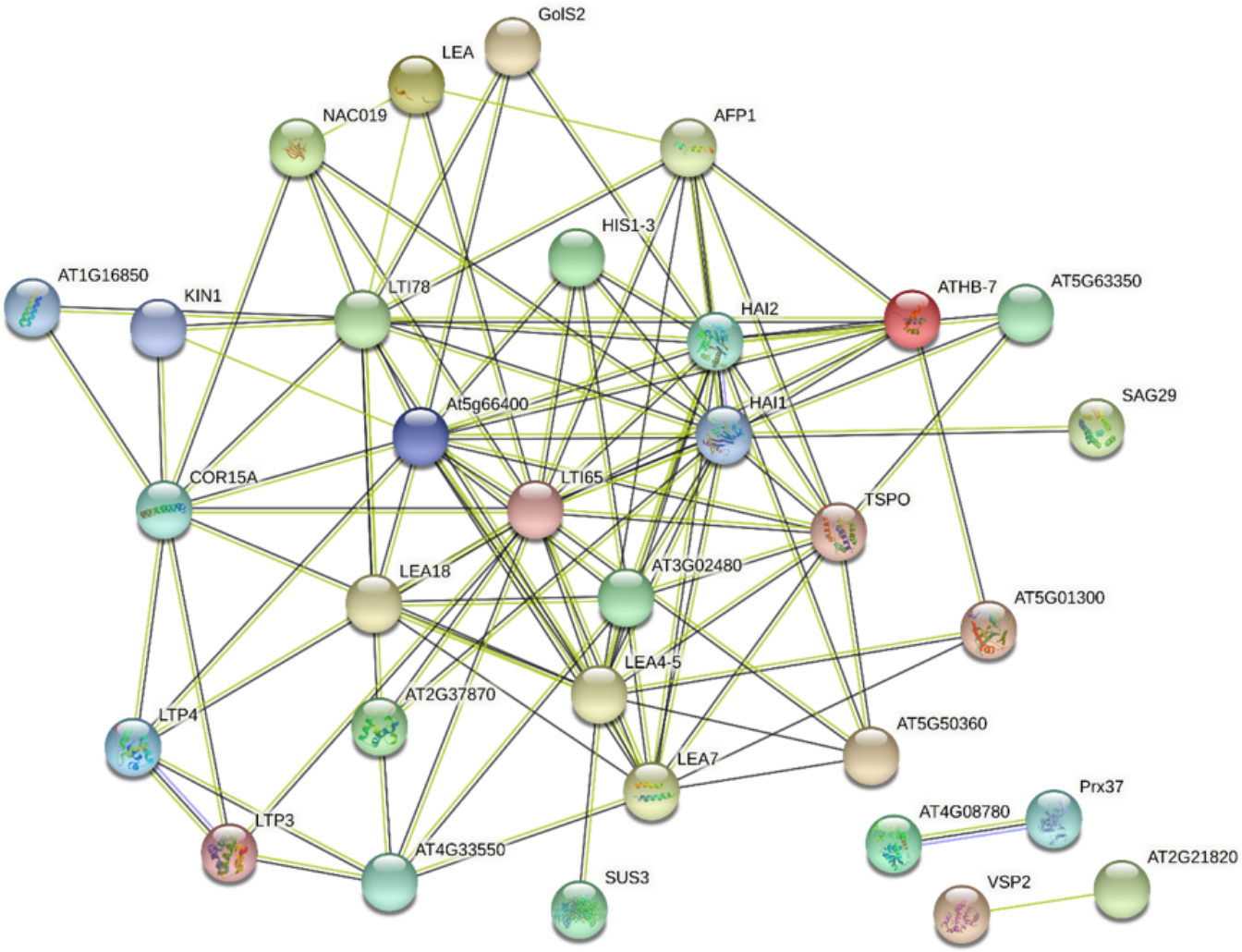

Figure 3

PPI network of common DEGs in root and shoot of Arabidopsis thaliana in STRING

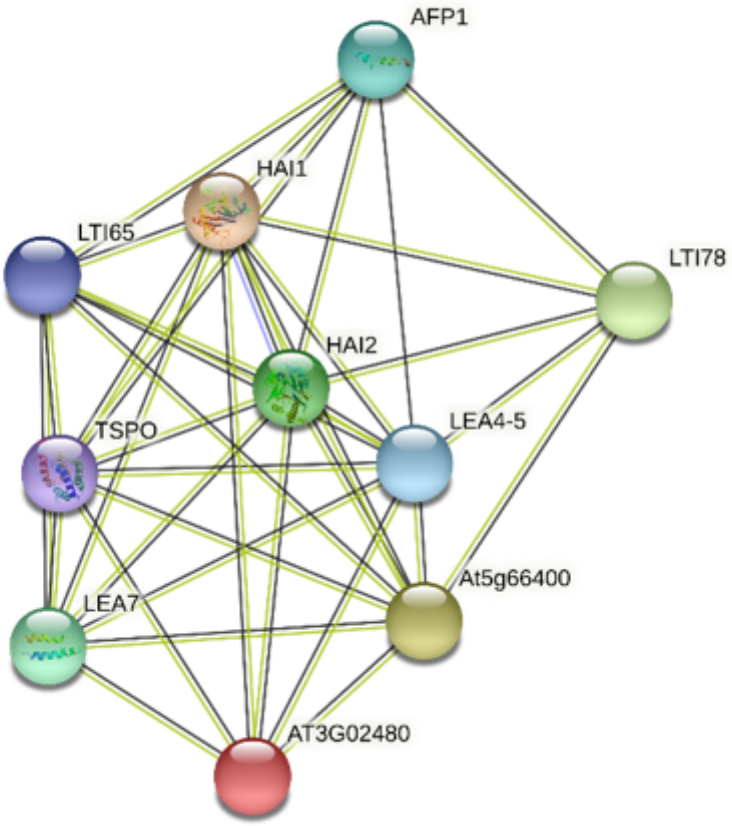

Figure 4

PPI network of hub genes in common DEGs in root and shoot of Arabidopsis thaliana in STRING

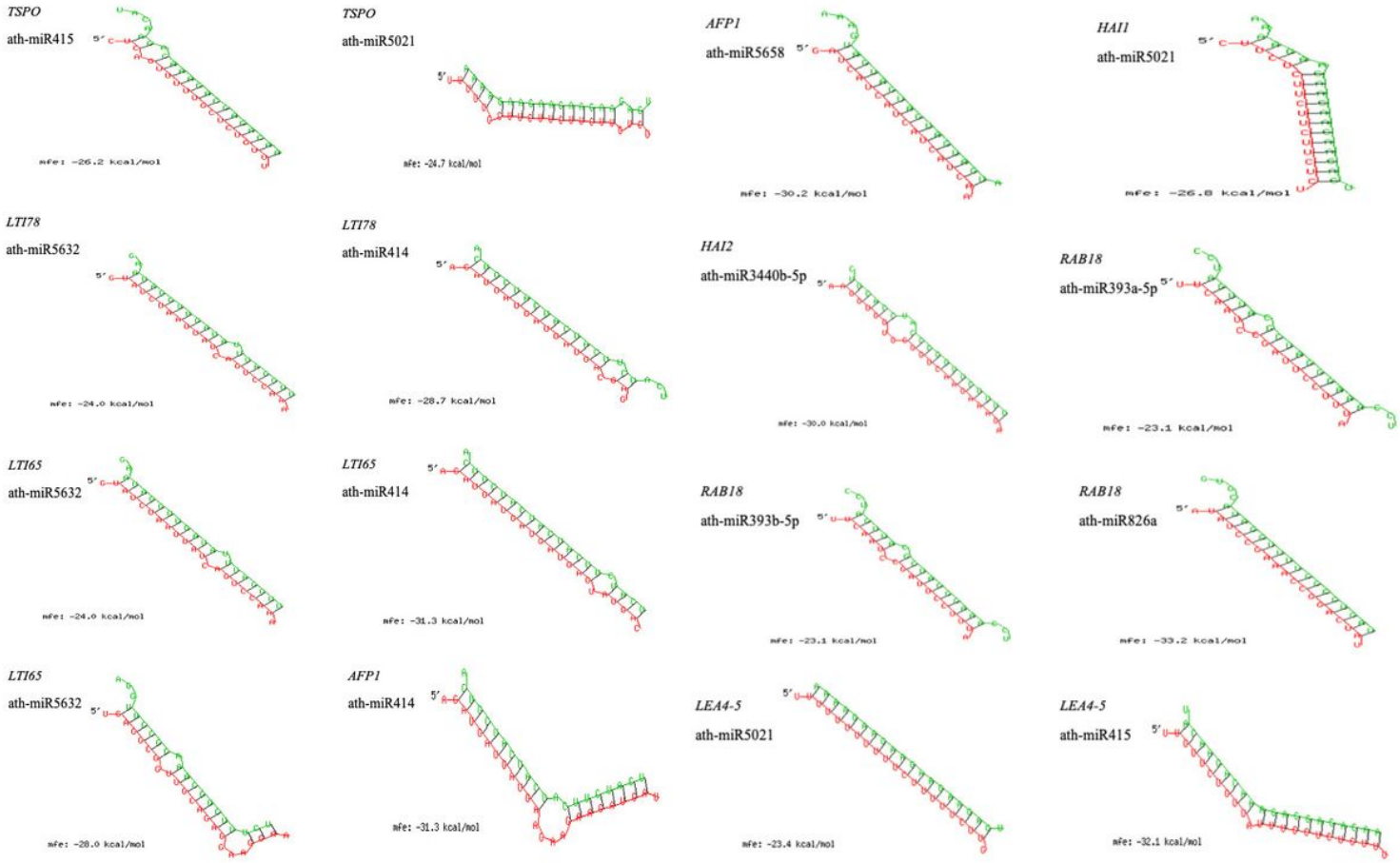

Figure 5

complementary of hub genes with miRNAs based on minimum free energy hybridization in RNAhybrid