Identification Of Arabidopsis genes associated with cold tolerance based on integrated bioinformatics analysis

Meili Guo, Xin Li, Yusu Jiang, Jinhuan Yu and Tao Meng

ABSTRACT
Cold stress is a major environmental factor that limits plant growth and productivity. Plants have evolved various strategies to adapt to these environmental conditions. To better explain the mechanisms used to survive environmental challenges, we retrieved the cold-responsive genes of Arabidopsis thaliana from the Gene Expression Omnibus (GEO) database. The GEO raw data were normalized by the quantile method, and then the differentially expressed genes (DEGs) under cold stress were screened using the robust rank aggregation (RRA) algorithm, including 261 upregulated and 177 downregulated genes out of more than 20,000 genes. Further, the integrated bioinformatics analyses of PUBMED, PANTHER, DAVID, and STRING indicated that the upregulated DEGs were involved in cellular response to red light, negative regulation of circadian rhythm, photoprotection, monosaccharide transport, cold acclimation, and phosphate ion homeostasis, while the downregulated DEGs were associated with the indole glucosinolate biosynthetic process, regulation of RNA splicing, water transport, cell wall modification, cell wall loosening, cellular water homeostasis, and cell wall homeostasis. Furthermore, the up-regulated DEGs had about four times protein-protein-interactions (PPIs) than the down-regulated DEGs, and the cold-responsive genes were identified using Cytoscape software. Furthermore, qRT-PCR of low-temperature-responsive protein 78 (LTI78), transducin family protein (SWA1), and arginine methyltransferase 11 (PRMT11) were performed to validate the outcome of integrated bioinformatics analysis. Our work will improve our knowledge of cold-responsive mechanisms and these DEGs might be targets for plant cold stress-resistance research.

Introduction
Extreme low temperatures negatively affect crop productivity and threaten food security. Plants can perceive extreme temperatures and adjust their growth, reproduction, and development to adapt them. To better understand plant cold-resistance mechanisms and increase crop yield, Arabidopsis thaliana has been used as an ideal model plant to explore the response of differentially expressed genes (DEGs) to cold stress (Gong et al. 2020).

In previous studies, several important Arabidopsis genes and pathways, including calcium signaling, mitogen-activated protein kinase (MAPK) signaling, jasmonic acid (JA) signaling, and C-repeat/DREB binding factor (CBF) signaling, have been reported to be involved in the cold response (Shi et al. 2018; Wu et al. 2019; Yuan et al. 2018). For example, glutamate receptor family proteins (ATGLR1.2 and ATGLR1.3) are glutamate-like receptors related to cold stress. Overexpression of ATGLR1.2 and ATGLR1.3 could enhance the gene expression of the CBF signaling pathway, which positively improves cold adaption in Arabidopsis (Zheng et al. 2018). The β-expansin gene (TaEXPB7-B) is another cold-responsive gene located in the Arabidopsis cell wall. Overexpression of TaEXPB7-B improved cellulose and lignin content, and increased antioxidant activity to survive at low-temperatures (Feng et al. 2019). The gene open stomata 1 (OST1) also plays an important role in enhancing low-temperature tolerance in Arabidopsis, and interacts with a plasma membrane-localized clade-E growth-regulating 2 (EGR2) phosphatase to facilitate better adaptation of plants under cold stress (Ding et al. 2019). Abscisic acid (ABA) is associated with plant adaptation to cold stress. Overexpression of rice pyrabactin resistance-like gene 3 (OsPYL3) in Arabidopsis could increase the sensitivity of the ABA signaling pathway. As a result, the plant cold stress was enhanced (Lenka et al. 2018). Arabidopsis thaliana DEAD-box RNA helicase 7 (AtRH7) is a DEAD-box RNA helicase that interacts with cold shock domain protein 3 (AtCSP3), which plays a considerable role in cold tolerance. Mutants of AtRH7 negatively affect pre-RNA processing and cause delay in first leaf emergence in Arabidopsis (Liu et al. 2016). Ubiquitin-conjugating enzyme 13 (UBC13) is an important cold-responsive gene that participates in programmed cell death pathways in Arabidopsis. The UBC13 mutant was involved in the lesion mimic phenotype and interacted with F-box and associated interaction domains-containing protein 1 (CPR1), which is an F-box protein that regulates TIR-NBS-LRR class disease resistance protein 1 (SNC1) degradation under cold stress (Wang et al. 2019). Arabidopsis cystatin A (AtCYSa) and cystatin B (AtCYSb) are cysteine...
proteinase inhibitors that can be induced by cold stress. Their promoter regions included a dehydrogenation-responsive element (DRE) and abscisic acid-responsive element (ABRE). Therefore, AtCYSa and AtCYSb are target genes of the dehydrogenation response element B1A (DREB1A) and AREG. Overexpression of AtCYSa and AtCYSb enhances plant tolerance to environmental stress (Zhang et al. 2008). By regulating the expression of cold-responsive salicylic acid and bZIP transcription factor family protein (TGA), the regulatory protein (NPR1) also plays a vital role in enhancing the cold acclimation of Arabidopsis. Interacting with HSF1 significantly promoted cold tolerance in plants, and NPR1 might be an essential gene during plant cold acclimation (Olate et al. 2018).

Although cold-responsive genes have been reported by different laboratories, more comprehensive DEGs will better disclose the mechanisms of cold acclimation in plants. Recently, 5742 genes were differentially expressed to reveal the gene regulators and pathways involved in cold tolerance in Brassica napus. These DEGs were related to the inhibition of photosynthesis and the primary biological processes (Ke et al. 2020). To identify early responsive events in Oryza sativa under cold stress, a transcriptome profile was performed to identify 516 DEGs that were involved in Ca^{2+} and ROS-mediated signaling, and the DREB/COB pathway (Dasgupta et al. 2020).

In the present study, to disclose the details of cold-responsive DEGs in Arabidopsis, the Gene Expression Omnibus database (GEO) was used to retrieve the DEGs of plant cold tolerance. Then, 261 upregulated and 177 downregulated genes from the GEO database were identified using a rank aggregation method. Integrated bioinformatics strategies have been applied to demonstrate the molecular functions, signaling pathways, and PPI interaction network of cold-responsive DEGs. These DEGs will greatly improve our knowledge of plant cold-response mechanisms and might become useful targets for crop plant growth, development, and crop output.

Materials and Methods

Retrieval of cold-responsive genes in Arabidopsis from GEO database

The eight responsive gene expression profiles of the microarray and RNA-seq data related to cold stress in Arabidopsis (Table 1) were downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/).

Microarray data normalization and robust rank aggregation (RRA) algorithm in Arabidopsis

Microarray data were downloaded from the GEO database in TXT format (https://www.ncbi.nlm.nih.gov/geo/). The R software package was used to process the matrix files and filter low-quality data. The resultant data were log2 transformed and processed with the limma package (http://www.bioconductor.org/) to retain the DEGs that had a p-value < 0.05, and a |log2 fold change (FC)| > 1. Furthermore, the DEGs identified above were integrated into the robust rank aggregation (RRA) software package (https://cran.r-project.org/web/packages/RobustRankAggreg/index.html). Using a null hypothesis of uncorrelated inputs, the RRA algorithm defines genes ranked consistently better than expected, and then assigns a significance score to each gene. The hypothesis of the RRA method is that each gene is in a random order in each experiment. If a gene highly ranked in all experiments, its p-value is smaller (Niu et al. 2019).

Cluster analysis of cold-responsive DEGs in Arabidopsis

The resultant cold-responsive DEGs were clustered by Gene Cluster 3.0, using the C Clustering Library version 1.24 created by Michael de Hoon, Seiya Imoto and Satoru Miyano, and the results were viewed using Java TreeView software created by Alok (Version:1.0.4; http://jtreeview.sourceforge.net).

Gene ontology classification and KEGG Pathway in Arabidopsis

The bioinformatics tools of the PANTHER (PROtein Analysis TROUGH Evolutionary Relationships) classification system (Version 15.0 released 2019_04) (http://pantherdb.org/) and DAVID (Database for annotation, visualization and integrated discovery, Version 6.8) (https://david.ncifcrf.gov/) were applied for gene ontology enrichment analysis of the DEGs. Each gene was classified into a single category.

Table 1. Detailed information about GEO data were showed in this study.

| Dataset       | Stress | Platform | Number of samples (Treatment/Control) | Experimental set-up | Organism          | Data         | Tissue           |
|---------------|--------|----------|--------------------------------------|---------------------|------------------|--------------|-----------------|
| GSE3326       | cold   | GPL198   | 4(2/2)                               | 0°C, 24h            | Arabidopsis thaliana | RNA-seq      | seedlings       |
| GSE31837      | cold   | GPL198   | 6(3/3)                               | 4°C, 3h             | Arabidopsis thaliana | Microarray   | T87 cells       |
| GSE39090      | cold   | GPL198   | 4(2/2)                               | 4°C, 24h            | Arabidopsis thaliana | Microarray   | seedlings       |
| GSE43818      | cold   | GPL198   | 6(3/3)                               | 4°C, 24h            | Arabidopsis thaliana | Microarray   | leaves          |
| GSE59907      | cold   | GPL198   | 4(2/2)                               | 4°C, 24h            | Arabidopsis thaliana | Microarray   | seedlings       |
| GSE106635     | cold   | GPL198   | 4(2/2)                               | 4°C, 8h             | Arabidopsis thaliana | Microarray   | seedlings       |
| GSE112389     | cold   | GPL198   | 4(2/2)                               | 4°C, 24h            | Arabidopsis thaliana | Microarray   | leaves          |
| GSE113547     | cold   | GPL198   | 6(3/3)                               | 4°C, 24h            | Arabidopsis thaliana | Microarray   | roots           |

Table 2. Primers Used in qRT-PCR.

| gene names | forward primers (5' to 3') | reverse primers (5' to 3') |
|------------|----------------------------|---------------------------|
| Llt78      | CACCCAGCGTTCCTACAGAATGAG   | AACGTGTCCTTCACAGAATGAG    |
| Swa1       | GCACTAGAGCTATGTTT          | CCAACATGGGCGCTTCCCTCT    |
| Prmt11     | CAGTACACCAAGACCAGATGAG     | GTAATCCCAGACTTGGTATC      |
| Act2       | ACCCTGGTGGACCTGACCTTACATG | GTGTCCTGCTGATCCTCCAGACGCTT |
Protein-protein-interaction (PPI) networks in Arabidopsis

PPI networks were analyzed using the STRING (search tool for recurring instances of neighbouring genes) database (Version 11.0, released January 19, 2019) (http://string-db.org/). Subsequently, the maximal clique centrality (MCC) app in Cytoscape software was used to screen modules within the PPI network with the default parameters.

Growth conditions and harvest of Arabidopsis

According to a previous publication (Guo et al. 2020b), ecotype Col-0 Arabidopsis seeds were germinated on a normal medium plate at 22/20°C day/night, an 8/16 h light/dark cycle, and 60 μmol·m⁻²·s⁻¹ of light intensity. After 7 days, the A. thaliana seedlings individually underwent cold stress by exposure to a temperature of 4°C for 12 h. Then, the Arabidopsis roots and leaves were harvested and stored at −80°C.

qRT-PCR analysis

According to the previous method of RNA isolation and qRT-PCR analysis (Guo et al. 2020b), the relative expression levels of Arabidopsis gene low-temperature-responsive protein 78 (LTI78), transducin family protein (SWA1), and arginine methyltransferase 11 (PRMT11) were determined and Arabidopsis gene actin 2 (AT3G18780) was used as an endogenous reference in Arabidopsis roots and leaves. Briefly, total RNA was extracted using the RNeasy Plus reagent (TaKaRa). RNA concentrations were determined by spectrophotometry (NanoDrop 2000/2000C, Thermo Scientific). Then, 2 μg of total RNA was reverse transcribed using ReverTra Ace (TOYOBO). Real-time RT–PCR analysis was performed in a Roter-Gene Q (QIAGEN) using the Platinum

Figure 1. Differential expression genes of Arabidopsis responding to cold stress. A. GSE3326 (control: GSM74894, GSM74895, cold: GSM74900, GSM74901); B. GSE31837 (control: GSM789668, GSM789675, GSM789682, cold: GSM789671, GSM789678, GSM789685); C. GSE39090 (control: GSM955985, GSM955986, cold: GSM955989, GSM955990); D. GSE43818 (control: GSM1071668, GSM1071669, GSM1071670, cold: GSM1071671, GSM1071672, GSM1071673); E. GSE5907 (control: GSM1348266, GSM1348268, GSM1348269); F. GSE106635 (control: GSM2844128, GSM2844129, cold: GSM2844132, GSM2844133); G. GSE112389 (control: GSM3069395, GSM3069396, cold: GSM3069397, GSM3069398); H. GSE113547 (control: GSM3108856, GSM3108857, GSM3108858, cold: GSM3108862, GSM3108863, GSM3108864). The red points represent up-regulated genes (fold change > 2.0, P-value < 0.05). The green points represent down-regulated genes (fold change < 0.5, P-value < 0.05). The black points represent unchanged genes difference.
SYBR Green qPCR SuperMix-UDG kit (Life Technologies Corporation). Cycling conditions were as follows: 50°C for 2 min, 95°C for 5 min, followed by 40 cycles of 95°C for 10 s and 60°C for 45 s. The 2-ΔΔCt calculation was used to determine the differences in the cold-responsive genes. All experiments were performed in triplicate (Table 2).

**Statistical analysis**

The data of qRT-PCR analysis were statistically analyzed by means of an unpaired t-test using GraphPad Prism 7 (GraphPad Prism, La Jolla, CA, USA). Statistical significance was set at p < 0.05.

**Results**

**Microarray data retrieving from GEO and raw data normalization in Arabidopsis**

In the present study, eight retrieval cold-responsive microarray data (GSE3326 (control: GSM74894, GSM74895, cold: GSM74900, GSM74901); B. GSE31837 (control: GSM789668, GSM789675, GSM789682, cold: GSM789671, GSM789678, GSM789685); C. GSE39090 (control: GSM955985, GSM955986, cold: GSM955989, GSM955990); D. GSE43818 (control: GSM1071668, GSM1071669, GSM1071670, cold: GSM1071671, GSM1071672, GSM1071673); E. GSE55907 (control: GSM1348266, GSM1348267, cold: GSM1348268, GSM1348269); F. GSE106635 (control: GSM2844128, GSM2844129, cold: GSM2844132, GSM2844133); G. GSE112389 (control: GSM3069395, GSM3069396, cold: GSM3069397, GSM3069398); H. GSE113547 (control: GSM3108856, GSM3108857, GSM3108858, cold: GSM3108862, GSM3108863, GSM3108864).

**Figure 2.** Hierarchial clustering of differential expression genes in Arabidopsis under cold stress. A. GSE3326 (control: GSM74894, GSM74895, cold: GSM74900, GSM74901); B. GSE31837 (control: GSM789668, GSM789675, GSM789682, cold: GSM789671, GSM789678, GSM789685); C. GSE39090 (control: GSM955985, GSM955986, cold: GSM955989, GSM955990); D. GSE43818 (control: GSM1071668, GSM1071669, GSM1071670, cold: GSM1071671, GSM1071672, GSM1071673); E. GSE55907 (control: GSM1348266, GSM1348267, cold: GSM1348268, GSM1348269); F. GSE106635 (control: GSM2844128, GSM2844129, cold: GSM2844132, GSM2844133); G. GSE112389 (control: GSM3069395, GSM3069396, cold: GSM3069397, GSM3069398); H. GSE113547 (control: GSM3108856, GSM3108857, GSM3108858, cold: GSM3108862, GSM3108863, GSM3108864).
GSM1348266, GSM1348267, cold: GSM1348268, GSM1348269); GSE106635 (control: GSM2844128, GSM2844129, cold: GSM2844132, GSM2844133); GSE112389 (control: GSM3069395, GSM3069396, cold: GSM3069397, GSM3069398); GSE113547 (control: GSM3108856, GSM3108857, GSM3108858, cold: GSM3108862, GSM3108863, GSM3108864)). These cold-responsive gene datasets were screened using the limma package in R with the following condition: p-value < 0.05 and |log2FC| > 1 (Figure 1). In GSE3326, 2193 downregulated and 2452 upregulated DEGs were screened (Figure 3).

Quantification of cold stress-responsive DEGs in Arabidopsis

These cold-responsive gene datasets were screened using the limma package in R with the following condition: p-value < 0.05 and |log2FC| > 1 (Figure 1). In GSE3326, 2193 downregulated and 2452 upregulated DEGs were screened in R. Then, the eight GEO datasets were standardized as shown in Supplementary Figure 1.
Arabidopsis. In GSE31837, 1872 downregulated and 1959 upregulated DEGs were screened in Arabidopsis. In GSE39090, 402 downregulated and 466 upregulated DEGs were screened in Arabidopsis. In GSE43818, 875 downregulated and 843 upregulated DEGs were identified in Arabidopsis. In GSE55907, 608 downregulated and 689 upregulated DEGs were screened in Arabidopsis. In GSE106635, 367 downregulated and 485 upregulated DEGs were identified in Arabidopsis. In GSE112389, 677 downregulated and 906 upregulated DEGs were identified in Arabidopsis. In GSE113547, 1003 downregulated and 962 upregulated DEGs were screened in Arabidopsis. These cold-responsive DEGs were further clustered using Cluster 3.0 software, and viewed using Java TreeView software (Figure 2).

**Cold-responsive DEGs screening by RRA algorithm in Arabidopsis**

After the RRA method was used to integrate the above DEGs, 261 upregulated and 177 downregulated genes were...
identified as the cold stress-responsive DEGs of Arabidopsis (Figure 3, Supplementary Table 1).

Bioinformatics enrichment of cold stress-responsive DEGs in Arabidopsis

Bioinformatics tools were further used to analyze 261 upregulated and 177 downregulated genes. For biological processes, the upregulated DEGs were mainly related to the regulation of transcription (20.3%), response to cold (14.2%), response to abscisic acid (11.5%), circadian rhythm (5.4%) and response to oxidative stress (4.2%) (Figure 4A), while the downregulated DEGs were involved in the oxidation-reduction process (12.4%) (Figure 4B). For molecular function, the upregulated DEGs were associated with transcription factor activity (17.6%), DNA binding (13.1%), and transferase activity (5.4%) (Figure 4A), and the downregulated DEGs were associated with transcription factor activity (11.9%), protein dimerization activity (4.5%) and oxidoreductase activity (4.5%) (Figure 4B). For the cellular component, the up-regulated DEGs were associated with transcription factor activity (17.6%), DNA binding (13.1%), and transferase activity (5.4%) (Figure 4A), and the downregulated DEGs were associated with transcription factor activity (11.9%), protein dimerization activity (4.5%) and oxidoreductase activity (4.5%) (Figure 4B).

KEGG pathway of cold-responsive DEGs in Arabidopsis

Based on the KEGG pathway, the upregulated DEGs were involved in circadian rhythm (p-value=2.18E-05), inositol phosphate metabolism (p-value=8.07E-04), phosphatidylinositol signaling system (p-value=8.07E-04), and galactose metabolism (p-value=0.0019), while the down-regulated DEGs were related to metabolic pathways (p-value=0.016) and tropane, piperidine and pyridine alkaloid biosynthesis (p-value=0.067) (Figure 5).

PPI network and genes of cold-responsive DEGs in Arabidopsis

Through the analysis of the online software STRING, 261 up-regulated cold stress-responsive DEGs were connected with 646 edges (PPI enrichment p-value=< 1.0e-16) (Figure 6A), and 177 downregulated cold stress-responsive DEGs were connected with 242 edges (PPI enrichment p-value=< 1.0e-16) (Figure 6B). Genes encoding PMRTI1, fibrillarin 2 (FIB2), transducin (YAO), protein kinase
superfamily protein (AT3G57640), P-loop containing nucleoside triphosphate hydrolases superfamily protein (AT4G34910), pumilio 24 (PUM24), ribosomal RNA processing Brix domain protein (AT3G15460), GTP-binding family protein (NSN1), LTI78, and SWA1 were upregulated. Meanwhile, NAD(P)-binding Rossmann-fold superfamily protein (AT2G29290), transmembrane protein (AT5G52780), hydroxypyruvate reductase (HPR), 2-phosphoglycolate phosphatase 1 (PGLP1), P-loop containing nucleoside triphosphate hydrolases superfamily protein (AT2G03750), strictosidine synthase-like 4 (SSL4), magnesium-protoporphyrin IX methyltransferase (CHLM), glucose-6-phosphate dehydrogenase 1 (G6PD1), photosystem I subunit D-2 (PSAD-2) and plastocyanin 1 (PETE1) were downregulated.

**qRT-PCR analysis of cold-responsive genes in Arabidopsis**

Our results showed that cold-responsive *Arabidopsis* genes LTI78, SWA1, and PRMT11 were upregulated under cold stress. Therefore, the relative mRNA expression of *Arabidopsis* genes LTI78, SWA1, and PRMT11 in both *Arabidopsis* roots and leaves were assayed under cold stress to validate the confidence of the bioinformatic outcome. The results indicated that the relative mRNA expression levels of *Arabidopsis* genes LTI78, SWA1, and PRMT11 in *Arabidopsis* roots and leaves were all increased under cold stress (Figure 7).

**Discussion**

Cold stress negatively affects plant growth and crop yield. *Arabidopsis* has been used as a model plant for research on cold-responsive mechanisms. Our previous work indicated that there are complex signaling pathways and PPI interaction networks under salt stress in *Arabidopsis* (Guo et al. 2014; Guo et al. 2019a; Guo et al. 2020a; Guo et al. 2020b). However, it is still not clear how plants respond to other stresses, such as low temperatures, and further research is still needed.

Here, we retrieved the cold-responsive DEGs from the GEO database. To integrate the DEGs from the eight datasets, an RRA method was executed according to a previous publication (Niu et al. 2019). As a result, 261 upregulated and 177 downregulated genes were screened using the RRA algorithm. The integrated bioinformatics methods indicated that the DEGs were involved in biological processes, KEGG pathways, and PPI interaction networks. The upregulated DEGs were involved in the negative regulation of circadian rhythm, photoprotection, monosaccharide transport, cold acclimation, and phosphate ion homeostasis, while the downregulated DEGs were mainly involved in regulation of RNA splicing, water transport, cell wall modification,

![Figure 6. PPI networks of DEGs under cold stress and the interaction network of top 10 genes in *Arabidopsis*. (A) up-regulated DEGs, (B) down-regulated DEGs, (C) top 10 genes of up-regulated DEGs, (D) top 10 genes of down-regulated DEGs. DEG, differentially expressed genes; PPI, protein-protein interaction.](image-url)
cell wall loosening, cellular water homeostasis, and cell wall homeostasis. Among the upregulated DEGs under cold stress, zinc finger protein ZAT12 (ZAT12) is a protein that is necessary for the expression of ascorbate peroxidases (APXs), maintaining the balance of ROS (Rizhsky et al. 2004). In the ABA signaling pathway, ABI3/VP1 1 (RAV1) is activated by serine/threonine-protein kinase (SRK2), which then downregulates the expression of ABI3, ABI4 and ABI5. In the present study, RAV1 was upregulated, which was consistent with previous findings (Feng et al. 2014) and might play an important role in response to cold stress.

The ICE-CBF-COR pathway is an important signaling pathway associated with low-temperature stress (Guo et al. 2019b; Jin et al. 2018). In the present study, the CBFs, inducer of CBF expression (ICEs), and cold-responsive (COR) genes were differentially expressed under cold stress. Jasmonate zim-domain protein 1 (JAZ1) also increased in response to low temperature. It can inhibit the JA signaling pathway and regulate ICE1 expression (Hu et al. 2013). Furthermore, JAZ1 interacted with ICE1 and regulated the expression of the CBF. CBF is regulated by the circadian clock late elongated hypocotyl (LYH) gene, related to plant circadian rhythm. LYH positively binds to the promoter of CBF. Furthermore, under cold stress, osmotically responsive gene 1 (HOS1) ubiquitinated ICE1, and SUMO E3 ligase (SIZ1) sumoylated ICE1. Then, the resultant ICE1 was degraded by the 26S proteasome pathway (Dong et al. 2006; Dong et al. 2011; Miura et al. 2007). Additionally, cold-regulated protein 28 (COR28) interacts with protein CCA1 (CCA1) and negatively regulates the expression of CBF. In the present study, JAZ1, ICE1, dehydration-responsive element-binding protein 1A/1B/1C (CBF1/2/3), LHY, CCA1, and COR27 were all overexpressed and interacted with each other, which played an important role in adapting to low temperature (Li et al. 2016). Furthermore, to compare with a previous publication, the DEGs involved in the CBF pathway under cold stress could also be found in rice through comparative transcriptome analysis (Dasgupta et al. 2020), which indicated the confidence and applicability of our results.

Conclusion
This work provides a new understanding of the details involved in tolerance of Arabidopsis under cold stress, which showed new signaling pathways, more cold-responsive DEGs, and more comprehensive interaction networks. This study has been helpful in demonstrating how plants survive under low temperature, and the mechanisms involved in cold tolerance might be potential targets for the research on cold-response in plants.

Figure 7. qRT-PCR analysis of Arabidopsis genes Lti78 (At5g52310), Swa1 (At2g47990), and Prmt11 (At4g29510) response to cold stress (Actin 2 (AT3G18780) was used as endogenous reference). (A) mRNA expression of Lti78 (At5g52310) in leaf, (B) mRNA expression of Swa1 (At2g47990) in leaf, (C) mRNA expression of Prmt11 (At4g29510) in leaf, (D) mRNA expression of Lti78 (At5g52310) in root, (E) mRNA expression of Swa1 (At2g47990) in root, (F) mRNA expression of Prmt11 (At4g29510) in root.
Acknowledgements

MG conceived the study, and designed the experiments. MG, XL, YJ, JY, and TM performed the experiments. MG, XL, YJ, JY and TM analyzed the raw data, and drafted the manuscript. All the authors participated in the revision of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This project was supported by grants from Shandong Provincial Natural Science Foundation, China (grant no. ZR2010CQ024), Shandong Provincial Universities Science and Technology Programme, China (grant no. J10LC72), and School based scientific research project of Yantai Vocational College (grant no. 2021XBYB010).

Notes on contributors

Melili Guo is an Associate Professor of Food and Biochemistry Engineering Department at Yantai Vocational College. Her research interest focuses on Arabidopsis growth and development, and the responses to cold stress responses.

Dr Xin Liu is an Associate Professor of Central Laboratory at the Affiliated Yantai Yuhuangding Hospital of Qingdao University. His research interest focuses on genomics, proteomics and bioinformatics.

Yusu Jiang is an Associate Professor of Food and Biochemistry Engineering Department at Yantai Vocational College. Her work focuses on investigations of plant growth and development, and stress physiology in plants.

Jinhuan Yu is an Associate Professor of Food and Biochemistry Engineering Department at Yantai Vocational College. Her research interest focuses on plant physiology.

Tao Meng is an assistant lecturer of Food and Biochemistry Engineering Department at Yantai Vocational College. His work focuses on plant physiology and plant cold stress responses.

References

Dasgupta P, Das A, Datta S, Banerjee I, Tripathy S, Chaudhuri S. 2020. Understanding the early cold response mechanism in IR64 indica rice variety through comparative transcriptome analysis. BMC Genomics. 21(1):425–442.

Ding Y, Li J, Shi Y, Gao J, Hua J, Song C, Gong Z, Yang S. 2019. EGR2 phosphatase regulates OST1 kinase activity and freezing tolerance in Arabidopsis. EMBO J. 38(1):e99819–99835.

Dong CH, Agarwal M, Zhang Y, Xie Q, Zhu JK. 2006. The negative regulation of Arabidopsis cold stress responses by the JAZ proteins. Arabidopsis Research. 3:674–688.

Feng CZ, Chen Y, Wang C, Kong YH, Wu WH, Chen YF. 2014. Arabidopsis RAV1 transcription factor, phosphorylated by SnRK2 kinases, regulates the expression of AB13, AB14, and AB5 during seed germination and early seedling development. Plant J. 80(4):654–668.

Feng X, Xu Y, Peng L, Yu X, Zhao Q, Feng S, Zhao Z, Li F, Hu B. 2019. TaEXPB7-B, a beta-expansin gene involved in low-temperature stress and abscisic acid responses, promotes growth and cold resistance in Arabidopsis thaliana. J Plant Physiol. 240:153004–153018.

Gong Z, Xiong L, Shi H, Yang S, Herrera-Estrella LR, Xu G, Chao DY, Li J, Wang PY, Qin F, et al. 2020. Plant cold stress response and nutrient use efficiency. Sci China Life Sci. 63(5):635–674.

Guo J, Ren Y, Tang Z, Shi W, Zhou M. 2019a. Characterization and expression profiling of the ICE-CBF-COR genes in wheat. PeerJ. 7:e8190–8208.

Guo ML, Li H, Li L, Cheng XM, Gao WX, Xu YL, Zhou CX, Liu FJ, Liu X. 2014. Comparative proteomic analysis of Arabidopsis thaliana between wild type and its salt-tolerant mutant. J Plant Interact. 9(1):330–337.

Guo ML, Liu X, Li L, Jiang YS, Xu YJ, Meng T, Zhou CX. 2020a. Integrated bioinformatics analysis reveals the response of Arabidopsis to salt stress through multiple gene expression omni-bus datasets. J Plant Interact. 15(1):313–321.

Guo ML, Liu X, Wang JH, Li L, Jiang YS, Xu YJ, Meng T. 2020b. In-depth investigation on abiotic stress-responsive differentially expressed genes in Arabidopsis s through GEO database. J Plant Interact. 15(1):294–302.

Guo ML, Liu X, Wang JH, Li L, Zhang WD, Gong BJ, Zhang CL, Zhou CX. 2019b. Investigation on salt-response mechanisms in Arabidopsis thaliana from UniProt protein knowledgebase. J Plant Interact. 14(1):21–29.

Hu Y, Jiang L, Wang F, Yu D. 2013. Jasmonate regulates the inducer of cbf expression-C-repeat binding factor/DEB3 binding factor1 cascade and freezing tolerance in Arabidopsis. Plant Cell. 25(8):2907–2924.

Jin Y, Zhai S, Wang W, Ding X, Guo Z, Bai L, Wang S. 2018. Identification of genes from the ICE-CBF-COR pathway under cold stress in agelops-triticum composite group and the evolution analysis with those from trioeces. Physiol Mol Biol Plants. 24(2):211–229.

Ke L, Lei W, Yang W, Jiang G, Guo J, Cheng J, Sun Y, Fan Z, Yu D. 2020. Genome-wide identification of cold response transcription factors in Brassica napus L. BMC Plant Biol. (1):62–74.

Lenka SK, Muthusamy SK, Chinnusamy V, Bansal KC. 2018. Ectopic expression of rice PYL3 enhances cold and drought tolerance in Arabidopsis thaliana. Mol Biotechnol. 60(5):350–361.

Li X, Ma D, Lu SX, Hu X, Huang R, Liang T, Xu T, Tobin EM, Liu H. 2016. Blue light- and low temperature-regulated COR27 and COR28 play roles in the Arabidopsis circadian clock. Plant Cell. 28:2755–2769.

Liu Y, Tabata D, Imai R. 2016. A cold-inducible DEAD-Box RNA helicase from Arabidopsis thaliana regulates plant growth and development under Low temperature. PLoS One. 11(4):e0154040–0154060.

Miura K, Jin JB, Lee J, Yoo CY, Stirm V, Miura T, Ashworth EN, Bressan RA, Yun DJ, Hasegawa PM. 2007. SIZ1-mediated stomatal closure of CBF1 controls CBF3/DREB1A expression and freezing tolerance in Arabidopsis. Plant Cell. 19:1403–1414.

Niu C, Jiang M, Li N, Cao J, Hou M, Ni DA, Chu Z. 2019. Integrated bioinformatics analysis of As, Au, Cd, Pb and Cu heavy metal responsive marker genes through Arabidopsis thaliana GEO datasets. PeerJ. 7:e6495–6519.

Olate E, Jiménez-Gómez JM, Holouigue L, Salinas J. 2018. NTR1 mediates a novel regulatory pathway in cold acclimation by interacting with HSF1A factors. Nat Plants. 4(10):811–823.

Rizhky L, Davletova S, Liang H, Mittler R. 2004. The zinc finger protein Zat12 is required for cytosolic ascorbate peroxidase 1 expression during oxidative stress in arabidopsis. J Biol Chem. 279(12):11736–11743.

Shi Y, Ding Y, Yang S. 2018. Molecular regulation of CBF signaling in cold acclimation. Trends Plant Sci. 23(7):623–637.

Wang L, Ren W, Wang J, Xiang D, Wang Q, Zang Y, Wang Z, Huang S, Li X, Data R, et al. 2019. Arabidopsis UBC13 differentially regulates two programmed cell death pathways in responses to pathogen and low-temperature stress. New Phytol. 221(2):934–939.

Wu Z, Han S, Zhou H, Tung ZK, Wang Y, Jin Y, Shi H, Yang W. 2019. Cold stress activates disease resistance in Arabidopsis thaliana through a salicylic acid dependent pathway. Plant Cell Environ. 42(9):2645–2663.

Yuan P, Yang T, Poovaliah BW. 2018. Calcium signaling-mediated plant response to cold stress. Int J Mol Sci. 19(12):3896–3906.

Zhang X, Liu S, Takano T. 2008. Two cysteine proteinase inhibitors from Arabidopsis thaliana, AtCYSa and AtCYSb, increasing the salt, drought, oxidation and cold tolerance. Plant Mol Biol. 68(1-2):131–143.

Zheng Y, Luo L, Wei J, Chen Q, Yang Y, Hu X, Kong X. 2018. The glutamate receptors AtGLR1.2 and AtGLR1.3 increase cold tolerance by regulating jasmonate signaling in Arabidopsis thaliana. Biochem Biophys Res Commun. 506(4):895–900.

JOURNAL OF PLANT INTERACTIONS 353