Genome-wide transcriptome profiling revealed biological macromolecules respond to low temperature stress in *Brassica napus* L

Muhammad Azhar Hussain1†, Dan Luo1†, Liu Zeng1, Xiaoyu Ding1, Yong Cheng1, Xiling Zou1, Yan Lv1* and Guangyuan Lu2*

1Key Laboratory of Biology and Genetic Improvement of Oil Crops Research Institute, Oil Crops Research Institute, Chinese Academy of Agricultural Sciences (CAAS), Wuhan, China, 2School of Biology and Food Engineering, Guangdong University of Petrochemical Technology, Maoming, China

*These authors have contributed equally to this work

**KEYWORDS**

abiotic stress, low temperature stress, RNA sequencing, DEGs, transcription factors, photosynthesis, antioxidants

*Brassica napus* L. (*B. napus*) is a vital oilseed crop cultivated worldwide; low temperature (LT) is one of the major stress factors that limit its growth, development, distribution, and production. Even though processes have been developed to characterize LT-responsive genes, only limited studies have exploited the molecular response mechanisms in *B. napus*. Here the transcriptome data of an elite *B. napus* variety with LT adaptability was acquired and applied to investigate the gene expression profiles of *B. napus* in response to LT stress. The bioinformatics study revealed a total of 79,061 unigenes, of which 3,703 genes were differentially expressed genes (DEGs), with 2,129 upregulated and 1,574 downregulated. The Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis pinpointed that the DEGs were enriched in LT-stress-responsive biological functions and metabolic pathways, which included sugar metabolism, antioxidant defense system, plant hormone signal transduction, and photosynthesis. Moreover, a group of LT-stress-responsive transcription factors with divergent expression patterns under LT was summarized. A combined protein interaction suggested that a complex interconnected regulatory network existed in all detected pathways. RNA-seq data was verified using real-time quantitative polymerase chain reaction analysis. Based on these findings, we presented a hypothesis model illustrating valuable information for understanding the LT response mechanisms in *B. napus*.

**CITATION**

Hussain MA, Luo D, Zeng L, Ding X, Cheng Y, Zou X, Lv Y and Lu G (2022) Genome-wide transcriptome profiling revealed biological macromolecules respond to low temperature stress in *Brassica napus* L. *Front. Plant Sci.* 13:1050995. doi: 10.3389/fpls.2022.1050995

**COPYRIGHT**

© 2022 Hussain, Luo, Zeng, Ding, Cheng, Zou, Lv and Lu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.
Introduction

*Brassica napus* is extensively grown and distributed in the Yellow River basin in China (Ke et al., 2020). *B. napus* is the third most important oil crop in China (Li et al., 2021). To date, *B. napus* has become a research hotspot due to its commercial and ecological benefits (Jia et al., 2019). It has long been known that environmental concerns such as low temperature (LT) stress, including chilling (0–10°C) and freezing (-4°C), affect the germination, growth, development, production, and spatial distribution of crops. In recent years, winter- and semi-winter rapeseeds cultivated in the Yangtze River basin were sowed from the end of September to mid-October, which leads to threatened growth and production of rapeseed under LT stress conditions (Cong et al., 2019; Huang et al., 2020). Improvement in cold tolerance has been a major goal for the agricultural research of *B. napus* over the recent years; there is an urgent need to develop and cultivate early-maturing rapeseed varieties with cold resistance (Raza et al., 2021).

Over the years, available literature has reported LT-responsive genes and regulatory mechanisms in *Arabidopsis* (Du et al., 2017; Yu et al., 2020). The researchers focused on elucidating the LT response molecular regulatory mechanisms in different crop species such as tomato (Niu et al., 2022), apple (An et al., 2018), banana (Liu et al., 2018a), rice (Lv et al., 2017; Hang et al., 2018), rapeseed (Luo et al., 2019; Pu et al., 2019; Huang et al., 2020), and maize (Li et al., 2016b). These efforts revealed numerous genes, molecular regulators, and biological pathways to ameliorate plant LT stress effects (Shi et al., 2018; Ding et al., 2019). To cope with unfavorable environmental factors, increasing attention has been given to understanding the cold response mechanisms in oilseed crops, especially rapeseed—for example, next-generation sequencing (NGS) technology is widely applied to reveal the transcriptomic changes in rapeseed under different stresses (Xiong et al., 2022). Recently, the transcriptomic analysis identified various cold-responsive (COR) pathways that regulate photosynthesis, abscisic acid (ABA) homeostasis and transport, plant hormone signal transduction, ribosome biogenesis, MAPK signaling pathway, calcium signal transduction, and antioxidant defense systems in rapeseed (Ma et al., 2019). Different studies reported that the accumulation of different metabolites, including proline, soluble sugar, and protein contents, and antioxidant enzyme activity were rapidly increased under freezing stress (-2°C) in rapeseed (Yan et al., 2019). Dehydrins such as late embryogenesis abundant (LEA) proteins are accumulated in response to cold stress by a higher expression of LEA10 and LEA18, LEA90, and LEA104 genes in *B. napus* (Maryan et al., 2019). LT stress-inducible proteins are antifreeze proteins, cold-shock domain proteins, and heat shock proteins (HSPs). Under LT stress, various enzymes, such as oxidases and desaturases, are activated to scavenge the reactive oxygen species (Heidarvand and Maali, 2010; Su et al., 2021). Among different antioxidant enzymes, superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT) act as the first line of reactive oxygen species (ROS) scavenging to protect stress-induced oxidative damage in plants (Sharma et al., 2019; Su et al., 2021).

The phenotypic and RNA-seq profiling of transgenic *B. napus* ectopic overexpressing *Arabidopsis* C-repeat/DRE-binding factor (CBF) has suggested that the CBF cold response of *Arabidopsis* has been maintained in rapeseed (Jaglo et al., 2001). In the grape plant, CBL-interacting protein kinases 18 act as a positive regulator of the CBF cold signaling pathway by modulating ROS homeostasis (Yu et al., 2022b). Plant-hormone-mediated pathways are also critical in plant LT stress tolerance—for example, sly-miR156e-3p mediated the posttranscriptional regulation of transcription factor S1MYB15, which positively regulates ABA-mediated cold tolerance in the tomato (Zhang et al., 2022b). In the last decade, different efforts have been made to investigate the physiological and molecular perspectives of LT response of *B. napus* (Du et al., 2016; Huang et al., 2018; Xin et al., 2019; Ke et al., 2020). Despite these efforts, the complex molecular mechanisms of LT stress responses in *B. napus* need to be further explored. The NGS profoundly helped to dissect genome-wide novel and diverse molecular response mechanisms in plants. These NGS technologies provide simple, low-cost, sensitive, accurate, and fast tools for elucidating the genome-wide regulation of desired traits (Tyczewska et al., 2016; Sharma et al., 2018). The RNA-seq technology has many successful stories in profiling the genetic architecture of LT stress tolerance, including *B. rapa* (Ma et al., 2019), *B. oleracea* (Zhang et al., 2019c), *B. napus* (Du et al., 2016; Ke et al., 2020), *A. thaliana* (Kaplan et al., 2007), *Z. mays* (Li et al., 2016b; Li et al., 2019), *O. sativa* (Guan et al., 2019), and *E. japonica* Lindl (Zhang et al., 2022a).

Based on the abovementioned details, the molecular picture associated with cold response has become clearer. *B. napus* was capable of growth at LT conditions; however, the cold responses of the early-maturing variety in a short time remain to be characterized. In this study, we assessed the dynamic changes that occur at the molecular level to elucidate the LT stress responses through biochemical and transcriptome analysis in *B. napus*. Biochemical analysis helps to identify the activation of different kinds of phytohormones under LT stress. The transcriptome analysis helps explore various COR gene pathways and their regulatory mechanisms operating under LT conditions in *B. napus*. The data generated in this study would enhance the understanding of LT response and regulatory mechanism and identify substantial genetic resources that are useful for modifications and enhancing LT tolerance in *B. napus*.
Materials and methods

Plant materials, growth conditions, and treatments

Rapeseed Cultivar-18, an excellent inbred winter cultivar bred by Oil Crops Research Institute, Wuhan, China, that can be successfully cultivated under LT conditions, was used in this study. The seeds were germinated on moist filter paper in petri plates under soft tube white lights at a temperature range of 25 ± 1°C, humidity of 60%, and 16-h light/8 h dark photoperiod in a growth chamber. One-week-old seedlings were transferred to plastic pots filled with a 1:1:1 mixture of peat vermiculite/moss/perlite in a growth room (16 h light/8 h dark photoperiod), with a constant temperature of 25 ± 1°C for approximately 21 days. At a 4-leaves stage, healthy seedlings were selected and transferred to LT stress (4°C) conditions. Before LT treatment (0 d) and after LT conditions (1 d), leaves were sampled, immediately frozen in liquid nitrogen, and preserved at -80°C until further use. Seedlings were recovered under normal growth conditions for two days after LT stress treatment. Total RNA was extracted from a pooled sample of seedling leaves from each group.

RNA preparation and deep sequencing

RNA preparation and transcriptome analysis were performed as previously described (Raza et al., 2021). Briefly, total RNA was extracted from the leaves of LT stressed and non-stressed samples using the TRIzol kit (Invitrogen, USA). RNA concentration and quality were quantified using NanoDrop 2000 (Thermo). The RNA sample was prepared from 1 μg RNA per sample for sequencing. cDNA libraries were produced using NEBNext UltraTM RNA Library Prep Kit for Illumina (NEB, USA), following the manufacturer’s instructions at the Biomarker company (Beijing, China), and then sequenced on an Illumina platform employing paired-end technology. Clean data was further subjected to Q20, Q30, GC-content and subsequent analysis. Hisaat tools software was used to map clean reads with the reference genome sequence of *B. napus* ([https://www.genoscope.cns.fr/brassicane](https://www.genoscope.cns.fr/brassicane)) (Chalhoub et al., 2014). The sequence data is publicly accessible on the National Center for Biotechnology Information database (PRJNAS96550).

Differential expression analysis

Differential expression analysis of data under two conditions, LT stressed and non-stressed, was performed using the DESeq, which provided gene expression data based on the negative binomial distribution. Gene expression level was measured by the number of fragments per kilobase of exon in per million fragments mapped reads (Trapnell, 2010). Benjamini and Hochberg's method was used to control the false discovery rate (Benjamini and Hochberg, 1995). Genes with an adjusted P-value <0.001 were considered differentially expressed genes (DEGs). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using a hypergeometric test with a false discovery rate adjusted p-value <0.05.

Gene functional annotation

The functional annotations of all these obtained DEGs were explored using the BLASTx program to find out the NCBI non-redundant protein (NR) and *Arabidopsis* protein (TAIR10) databases. The DEGs were subjected to GO enrichment analysis using the GOseq R packages based on Wallenius non-central hyper-geometric distribution (Young et al., 2010). The Blast2GO program was used to categorize the GO terms into three major categories of molecular function, cellular component, and biological process (Conesa and Götz, 2008). The KEGG pathway annotation was performed using KOBASE software to elaborate the corresponding metabolic pathways (Mao et al., 2005).

Physiological and biochemical analysis

To explore the physiological and biochemical changes under LT stress conditions, the contents of hydrogen peroxide (H₂O₂), malondialdehyde (MDA), soluble sugar, and proline (Pro) were quantified using commercial kits purchased from SolarBio ([https://solarbio.com](https://solarbio.com)) following the manufacturer’s protocol. The antioxidant enzyme activities, including superoxide dismutase (EC 1.15.1.1), peroxidase (EC 1.11.1.7), catalase (EC 1.11.1.6), and ascorbate peroxidase (EC 1.11.1.11), were quantified following the SolarBio kits ([https://solarbio.com](https://solarbio.com)) guidelines. The photosynthesis parameter Fv/Fm was measured using a portable chlorophyll-fluorometer OS-30p+ (OPTI-SCIENCES, China). All physiological and biochemical parameters were measured using a spectrophotometer microplate reader (Epoch, BioTek, Instruments, USA) in triplicate biological replicates.

qRT-PCR validation

The RNA-seq data was validated through qRT-PCR analysis. The first-strand cDNA was reverse-transcribed from 1 μg DNA-free RNA using EasyScript® One-Step cDNA Synthesis SuperMix (Trans) according to the manufacturer’s instructions. Amplification was performed using a StepOnePlusReal-Time PCR System (Applied Biosystems) with a Power SYBR® Green PCR Master Mix according to the manufacturer’s instructions. The reaction protocol was as
follows: 95°C for 10 m, 42 cycles at 95°C for 15 s, and 60°C for 15 s, with 10 ul as the final volume following three technical replicates. Relative expression data were analyzed using the \( 2^{-\Delta\Delta Ct} \) algorithm using \( B. napus \) ACTIN as an internal control (Wang et al., 2014). The primers used for qRT-PCR are listed in Supplementary Table S1.

**Results**

**Physiological and biochemical response to LT stress**

LT stress has damaging effects, which impair the growth and productivity of \( B. napus \). After a short-term 4°C stress treatment, \( B. napus \) seedlings showed >99% survival rate after 2 days of recovery (Figures 1A, B). However, 4°C-LT-stress-treated seedlings exhibited significant differences in physiological and biochemical indices compared with CK conditions. Therefore, the maximum quantum efficiency of photosystem II (PSII) \( \text{Fv/Fm} \) is reduced by 29.22% under LT stress treatment in rapeseed (Figure 1C). However, LT stress leads to the induction of \( \text{H}_2\text{O}_2 \) and MDA contents in plants. \( \text{H}_2\text{O}_2 \) plays a dual role in response to stress conditions and acts as a ROS and signaling molecule to activate the stress response mechanism in plants. However, excessive \( \text{H}_2\text{O}_2 \) accumulation is damaging to cells. LT stress treatment significantly increased the \( \text{H}_2\text{O}_2 \) and MDA levels—specifically, \( \text{H}_2\text{O}_2 \) increased by 16.52% and MDA increased by 29.77% (Figures 1D, E). Similarly, osmoprotectants, such as soluble sugars and proline, increased by 19.14% and 42.97% compared with CK, respectively (Figures 1F, G). These results indicate that the accumulation of osmoprotectants has a protective role in LT-stressed \( B. napus \) seedlings. In response to higher ROS, the plant defense system is activated to ameliorate the effects of LT stress compared with CK. The built-in plant defense system is comprised of various kinds of
antioxidant enzymes. These antioxidant enzymes, including SOD, POD, CAT, and APX, equilibrate ROS production and are involved in the conversion to the least deleterious biochemicals within plant cells under LT stress. We observed that the LT stress significantly increased the SOD enzyme activity by 19.61%, POD enzyme activity by 15.82%, and APX enzyme activity by 19.69% compared with CK seedlings (Figures 1H–J). In contrast, CAT enzyme activity was reduced by 15.3% compared with CK (Figure 1K). These results indicate that, in B. napus seedlings, complex physiological and biochemical responses are simultaneously activated to protect them from the injurious effects of LT stress (Figure 1).

RNA-seq and data quality control analysis

The current study used B. napus plants as experimental material to reveal transcriptome changes under LT stress. At the four-leaves stage, healthy plants were subjected to 4°C for 1 day under 16/8-h photo/dark period for LT treatment. The morphological changes in the leaves of B. napus plants were recorded. The phenotypic changes indicated that B. napus plants were challenged by LT stress (Figure 1A). Subsequently, the leaves were harvested, and two kinds of cDNA libraries were constructed from LT-stressed and CK samples. The cDNA libraries were sequenced using Illumina technology with 10x depth. After trimming the low-quality reads, averages of 45,971,689 and 46,358,438 clean reads were obtained from the CK and LT samples, respectively. The Q30 for all sequenced libraries was greater than 95%, and the GC content of each sample was 47%. Averages of about 39,943,728 (86.84%) and 40,987,248 (88.46%) clean reads were successfully mapped to the reference genome from the CK and LT samples, respectively (Table 1). A total of 7,9061 unigenes were procured.

## TABLE 1 Summary statistics of RNA-seq generated and processed data.

| Samples | Replication | Total reads | Mapped reads | Unique mapped reads | Multiple mapped reads | Pair end mapped reads | Single mapped reads | Mapped reads on positive (+) strands | Mapped reads on negative (-) strands |
|---------|-------------|-------------|--------------|--------------------|----------------------|----------------------|-------------------|------------------------------------|------------------------------------|
| Control | Replication 1 | 4817818 | 42755427 | 40484781 | 2270646 | 40680430 | 2074997 | 20957374 | 21074499 |
|         | Replication 2 | 44924694 | 39383275 | 3743088 | 1950187 | 37046052 | 2337223 | 19368339 | 19441183 |
|         | Replication 3 | 44872556 | 37692481 | 34724129 | 2968352 | 35737272 | 1955209 | 18082341 | 18457390 |
|         | Average of three replications | 45971689 | 39943728 | 37547333 | 2396395 | 37821251 | 2122476 | 19460935 | 19657691 |
| Low temperature stress | Replication 1 | 40595778 | 36073778 | 34278941 | 1794837 | 34263206 | 1810572 | 17703661 | 17791797 |
|         | Replication 2 | 53939434 | 47416698 | 45002296 | 2414402 | 44647190 | 2769508 | 23266287 | 23358934 |
|         | Replication 3 | 44540102 | 3971267 | 37328725 | 2142542 | 37553730 | 1917537 | 19303117 | 19424016 |
|         | Average of three replications | 46358438 | 40987248 | 38869987 | 2117260 | 38821375 | 2165872 | 20091022 | 20191582 |

### Annotation of the B. napus transcriptome

B. napus unigenes were annotated using the BLAST function against the NR and TAIR freely available databases. B. napus best matched with B. napus in the NR database compared with B. oleracea, B. rapa, Raphanus sativus, and other related species, which demonstrated that it was immensely homologous with B. napus (Figure 2). GO analysis was performed to categorize the function of each unigene. In total, 79,061 unigenes were successfully annotated to 55 GO terms. These terms were categorized into 55 GO terms, including 20 groups in biological processes, 14 in molecular functions, and 12 in cellular components. The biological process terms were enriched in cellular processes, metabolic processes, single organism process, responses to stimulus, and biological regulation. The enriched terms in molecular functions were binding, catalytic activity, nucleic acid binding transcription factor activity, transporter activity, and structural molecule activity. Among the cellular component terms, the five most significant classifications were cell, cell part, organelle, membrane, and organelle part (Supplementary Figure S1; Supplementary Table S2). In addition to GO analysis, the KEGG pathway analysis revealed several essential pathways involved in LT stress response. In total, 18,993 unigenes were successfully assigned to 119 pathways using KOBASE software. The major KEGG pathways were enriched in ribosome biogenesis, plant hormone signal transduction, carbon metabolism, biosynthesis of amino acids, starch and sucrose metabolism, protein processing in the endoplasmic reticulum, spliceosome, and endocytosis (Supplementary Table S3).

### Identification and analysis of DEGs

In order to evaluate the response to LT stress in C18, differential expression analysis was performed between

---

**Frontiers in Plant Science**

[10.3389/fpls.2022.1050995](https://doi.org/10.3389/fpls.2022.1050995)
samples before cytokinin (CK) and after LT treatment (LT stress). In the comparison between two groups, a total of 3,703 were identified as significant DEGs in the threshold of log2(fold change) ≥ 1 or ≤ -1 (P-value ≤ 0.05) (Supplementary Table S4).

To characterize the biological functions of the DEGs, we performed GO biological process (GO-BP) enrichment analysis on all upregulated and downregulated DEGs (Supplementary Table S5). The results showed that upregulated DEGs were mostly involved in water deprivation response, pyrimidine ribonucleotide biosynthetic process, hyperosmotic salinity response, cold response, RNA methylation, heat acclimation, chitin response, defense response, abscisic acid response, protein import into the nucleus, and response to jasmonic acid. While the downregulated DEGs mainly participated in chitin response, glucosinolate biosynthetic process, carboxylic acid catabolic process, light stimulus, and respiratory burst involved in defense response were the most enriched terms. As shown above, diverse changes and adaptations in biological reactions may occur in *Brassica napus* in response to LT stress.

To further categorize the important LT-stress-related pathways, the KEGG pathway enrichment analysis was performed for upregulated and downregulated DEGs (Supplementary Table S6). The major KEGG pathways for upregulated DEGs were ribosome biogenesis and alphalinolenic acid metabolism, while downregulated DEGs were enriched in plant hormone signal transduction, glyoxylate and dicarboxylate metabolism, peroxisome, photosynthesis, and carbon metabolism, which suggested that LT stress has harmful effects on various biological processes, and *B. napus* simultaneously initiates its defense through complex biosynthesis and metabolic pathways (Figures 3A, B).
Transcription factors’ response to LT stress

Transcription factors (TFs) recognize and bind to cis DNA elements—small regulatory sequences typically composed of non-coding DNA sequences (Wittkopp and Kalay, 2012)—in the promoter region to regulate the expression of downstream genes that have crucial functions in various plant stress responses. In the current study, 176 DEGs belonging to eight TF families that respond to biotic or abiotic stresses were determined (Figure 4). Specifically, 52 AP2/ERF members were identified, including 35 upregulated genes (BnaC03g26480D, BnaC06g40040D, BnaA07g35130D, BnaA03g13620D, etc.) and 17 downregulated genes (BnaA01g34910D, BnaA07g23650D, BnaA10g05780D, etc.). In our data, the basic helix–loop–helix (bHLH) family was the second largest that consist of 25 members, including three upregulated genes (BnaA10g21700D, BnaC09g45980D, and BnaC05g30500D) and 22 downregulated genes. We detected 21 MYB family members, including 12 upregulated and nine downregulated genes that respond to LT stress in *B. napus*. In the current study, it is also evident that WRKY TFs play significant regulatory roles in *B. napus* under LT stress (Figure 4). We found that 23 WRKY TFs respond to LT stress; among them, 22 members have induced expression, and only one member, BnaA04g23480D, was repressed under LT stress. Similarly, a total of 22 NAC TFs were found in this study, including 19 upregulated members and two downregulated members (BnaC03g30500D and BnaA04g42740D). In addition, among all identified basic leucine zipper transcription factor (bZIP TF) family members, seven were upregulated, while 11 were downregulated. Thus, it is suggested that the strong activation or repression of transcription factors by LT stress may promote the defense response in *B. napus*.

Analysis of DEGs related to potential pathways

When plants were exposed to cold stress, dynamic changes in starch content occurred. Many genes involving starch and sucrose metabolism have been previously proven to respond to LT stress, such as ADP-glucose pyrophosphorylase, β-amylase, and sucrose synthases, and were detected (Sicher, 2011; Yu et al., 2022a). As summarized in the heat map (Figure 5), different combinations of starch-degrading enzymes operate under LT stresses, and most of them were upregulated—for instance, we found the activation of three genes encoding sucrose synthase 1-like (BnaA03g08110D, BnaC09g37040D, and BnaA10g14710D) that promotes the catalysis of sucrose to uridine di-phosphoglucose and fructose. Various genes involved in galactose metabolism were also upregulated, such as BnaA09g15290D, BnaA09g48480D, and BnaC04g56100D, which encode galactinol synthase 2, galactinol synthase 3, and galactinol/sucrose galactosyltransferase 5. These results are consistent with previous reports (Ciereszko et al., 2001; Baud et al., 2004). ROS such as hydrogen peroxide (H₂O₂), superoxide (O₂⁻), singlet oxygen (¹O₂), and hydroxyl radical (OH⁻) are quickly produced from multiple organelles under LT stress (Qi et al., 2018), known as toxic agents, and also perceived as the second messengers by plant cells and trigger rapid responses. Here almost all DEGs belonging to the ROS-scavenging enzyme system were upregulated, including six SOD members, one CAT member, nine POD members, and five glutathione S-transferase
(GST) members (Figure 5), except only one CAT gene (BnaAnng11640D) that was downregulated, suggesting that these enzymes were actively expressed to form a complex antioxidant defense under short-time LT stress. Phytohormones include auxins, ABA, gibberellins (GA), CK, ethylene (ET), salicylic acid, jasmonates (JA), brassinosteroids (BR), and strigolactones, and they play critical roles in helping the plants to adapt to temperature stresses (Verma et al., 2016). As shown in Figure 5, 14 DEGs involved in the ABA signal pathway have an induced expression in response to LT stress, followed by one gene that showed a repressed expression. In addition, seven ET signal pathway-related and 10 JA signal pathway-related DEGs were upregulated. Three DEGs belonging to the BR signal pathway were significantly downregulated; however, three BR biosynthesis pathway genes were upregulated. Therefore, the abovementioned results indicated that the ABA, ET, BR, and JA signaling pathways were activated in the LT stress response of B. napus.

Interaction network between B. napus LT-stress-related genes

To elaborate the genes’ role in regulating LT response at the protein level, the protein–protein interactions network was constructed using DEGs enriched in GO or KEGG terms, including sugar metabolism, hormone, antioxidant activity, transcription factors, and photosynthesis. The homologs of potential DEGs were referred to the Arabidopsis database and subjected to STRING database analysis. As the results demonstrated, the homologs of DEGs were divided into six expression patterns, and the interaction lines exhibited different types of association among genes. Specifically, PYR/PYL/RCAR-PP2C-SnRK2-ABF, known as the ABA-dependent pathway, was detected. Meanwhile, nearly half of the genes were associated with each other and regulated the CBFs and corresponding stress-related genes. Besides the abovementioned data, JAZ/TIFYs pathway genes were detected, which indicates that the jasmonic acid signal pathway may participate in the quick cold response of B. napus (Figure 6).

Validation of transcriptome data by qRT-PCR analysis

To validate the transcriptome data, 32 candidate genes were randomly selected and subjected to qRT-PCR analysis. These target genes belong to various functional categories of the KEGG pathways, including photosynthesis, sugar metabolism, plant hormone signal transduction, anti-oxidant defense systems, and transcription factors. Finally, we compared the RNA-seq expression level with the qRT-PCR results. The qRT-PCR results were consistent with the RNA-seq, confirming the reliability of the transcriptome results (Figure 7).

Discussion

To dissect the LT response mechanisms in B. napus, various LT-related candidate DEGs were explored from transcriptomic data, and their mRNA abundance was investigated in LT stress...
response processes. In this study, our primary focus was on those DEGs involved in sugar metabolism (starch, sucrose, and carbohydrate), photosynthesis, plant antioxidant defense system, plant hormone signaling networks, and TFs. The LT stress had adverse effects on photosynthesis. Under LT stress, the cellular respiration process of mitochondria and the photosynthesis process in chloroplast generate oxidative stress and lead to ROS stockpiling (Oelze et al., 2008; Janmohammadi et al., 2015). Oxidative stress due to higher levels of ROS accumulation causes damage to DNA, lipids, and proteins (Schieber and Chandel, 2014). In B. napus, photosynthesis-related genes were mostly downregulated and took part in the light reaction and the Calvin cycle. Decreased photosynthesis activity is directly linked with reduced plant productivity under LT stress conditions. In addition to downregulated genes, few genes were induced and might have played a significant role in protecting the photosynthesis system under LT stress in B. napus. Genes involved in phytohormone signaling networks, sugar metabolism, and antioxidants had variable expression levels, which showed the complexity of the molecular regulation network in response to LT stress (Bari and Jones, 2009; Li et al., 2016a; Ding et al., 2019).

Sugar metabolism, source of energy, and keeping osmotic homeostasis

Previous studies demonstrated that soluble sugars accumulate in response to stress; however, they may play multiple roles under stress. Sugar molecules act as biochemical components for cold acclimation and protect plant cells from damage (Gilmour et al., 2000). Sugars also function as stress signal molecules and trigger a series of signal transduction and defense reactions (Eveland and Jackson, 2012). Sucrose synthase and sucrose phosphate synthase are essential enzymes responsible for metabolic energy in the sugar metabolism pathway (Fugate et al., 2019; Stein and Granot, 2019). During LT stress, Pi accumulation decreased in the cytoplasm (Strand et al., 1999), and altered Pi level signals led to the activation of enzymes in the sucrose synthesis pathway (Hurry et al., 2000). Thus, we have focused on the starch and sucrose metabolism pathway in B. napus and several genes related to sugar metabolism surveyed under LT stress conditions (Figure 5) and verified their expression pattern through qRT-PCR (Figure 7). Different kinds of sugars accumulated in plants, such as sucrose, glucose, raffinose, and fructose, which are involved in LT tolerance (Gu et al., 2018). Sucrose synthase involved in sucrose catabolism and sucrose phosphate synthase involved in biosynthesis are important enzymes primarily responsible for metabolic energy in the sugar metabolism pathway. The sucrose catabolization process acts as a source of ATP or cell wall biosynthesis (Fugate et al., 2019). Therefore, sucrose synthase enzymes are mainly localized in cell walls, mitochondria, and vacuoles (Stein and Granot, 2019). Similarly, upregulated sucrose synthase genes were reported under LT stress in Arabidopsis (Ciereszko et al., 2001; Baud et al., 2004). Furthermore, one sucrose phosphate synthase gene (BnaA10g03060D) was upregulated, which describes the possible role of sucrose biosynthesis under LT stress in B. napus (Figure 5A). Similarly, our biochemical analysis revealed the higher accumulation of soluble sugar and proline under LT stress (Figures 1F, G). A previous study has reported that up to 50% of the activity of sucrose phosphate synthase gene was induced in wheatgrass under LT stress (Jalilkar et al., 2016). In rice, the interaction of calcium-dependent protein kinase OsCPK17 with sucrose phosphate synthase and plasma membrane intrinsic protein...
FIGURE 7
qRT-PCR validation of RNA-seq data: (A) Sugar metabolism, (B) Antioxidants, (C) Plant Hormone, (D) Transcription factors, (E) Photosynthesis.
is compulsory for LT stress response (Almadanim et al., 2017). Fructokinase plays an essential role in sugar metabolism, signaling, and growth and also has osmosis-protective effects in LT stress (Li et al., 2017b). A high expression of fructokinase genes has been proven to enhance LT tolerance in Arabidopsis (Su et al., 2018). In this study, two genes encoding fructokinase-1 (BnaCmng30740D and BnaA05g11350D) were upregulated in B. napus under LT stress (Figure 5A). Activation of these fructokinase genes suggested a critical role in LT stress tolerance in B. napus.

The overexpression of galactinol synthase (GolS) genes enhanced LT stress tolerance in A. hypopityanthus napus (Liu et al., 2016). GolS catalyzes the raffinose family oligosaccharide biosynthetic pathway (Salvi et al., 2016). In the current study, various genes involved in galactose metabolism were also upregulated (Figure 5A). Similarly, pentose phosphate pathway genes were induced, such as BnaA06g24370D and BnaC05g24530D which encode ribose-phosphate pyrophosphokinase 2, which was chloroplastic under LT stress (Figure 5A). It has been reported that pentose phosphate pathway genes are hub genes involved in rapid rapeseed seed germination under cold stress (Luo et al., 2019). We also observed multiple up- and downregulated genes, such as beta-D-xylosidase 4, involved in amino sugar and nucleotide sugar metabolism. Hence, when exposed to LT stress, numerous genes involved in sucrose–starch metabolism were induced or repressed, which may lead to the accumulation of osmoprotectants such as sucrose, glucose, and fructose, subsequently maintaining osmotic protection or providing necessary energy under LT stress conditions.

Antioxidant defense system: A cellular cushion against LT stress

A higher accumulation of ROS after LT stress always causes oxidative damage to cellular organs, such as nucleic acid, lipids, and proteins (Suzuki and Mittler, 2006; Schieber and Chandel, 2014). Accordingly, we observed higher H2O2 levels in LT-stressed B. napus seedlings (Figure 1D). In addition to oxidative damage, ROS-mediated activated enzymatic and non-enzymatic ROS scavenging systems detoxify and relieve the cellular oxidative stress level (Miller et al., 2010; Sies et al., 2017). In B. napus during LT stress, the ROS-scavenging enzymatic antioxidant system consists of POD, SOD, CAT, APX, GST, and glutathione peroxidase (GPX) (Yan et al., 2019). The non-enzymatic antioxidant system includes the reduced form of glutathione system, vitamin E/vitamin C system, and secondary metabolites or antioxidants, such as carotenoids, sterols, flavonoids, and anthocyanins. In B. napus, ROS homeostasis—by utilizing these antioxidant systems—protects cellular oxidative stress injury. SOD is known as the first line of defense for ROS-scavenging systems. SOD converts damaging free radicals to less harmful products (H2O2 and O2) in cells through the dismutation process (Zhang et al., 2019b). The induced expression of SOD genes upon cold exposure was reported in Medicago truncatula (Song et al., 2018) and Setaria italica (Wang et al., 2018b). In plants, SODs are mainly divided into three classes based on metal co-factors: copper- and zinc-containing superoxide dismutase (Cu/Zn-SODs), iron superoxide dismutase (Fe-SOD), and manganese superoxide dismutase (Mn-SOD). Different classes of SODs are generally considered eukaryotic enzymes mainly distributed in the cytosol and chloroplast (Kroll et al., 1995; Wang et al., 2018a). Our findings indicate that various SOD genes (BnaC08g16470D, BnaA10g11080D, BnaA06g11500D, BnaC09g40740D, BnaA06g11500D, and BnaC01g04330D) were induced under LT stress conditions, indicating that it may have LT response regulation in B. napus (Figure 5B). Similarly, we observed an induced SOD activity in LT-stressed seedlings through biochemical analysis, which indicates that SOD activity is genetically controlled (Figure 1H). POD and CAT catalyzed H2O2 into simple H2O in cells. Ascorbate peroxidase enzymes detoxify H2O2 in plant cells. APX is involved in the ascorbate–glutathione cycle to catalyze the H2O2 into H2O using ascorbate as a specific electron donor. APX enzymes are mainly located in subcellular compartments, such as chloroplasts, cytosol, mitochondria, and peroxisome (Caverzan et al., 2012). In our results, the higher activation of APX enzyme activity depicted a protective role from the accumulated H2O2 and MDA by converting them into less harmful components or exclusion from the cellular environment (Figures 1D, E). In this study, POD genes (BnaC05g27350D, BnaA02g23560D, BnaC03g18600D, BnaA08g06260D, BnaA04g19410D, BnaA09g12900D, BnaC06g27340D, BnaA05g10200D, and BnaA07g25540D) were significantly upregulated upon LT stress exposure in B. napus. Accordingly, our biochemical analysis revealed the induction of POD activity in LT-stressed B. napus seedlings, which indicates a close relationship between the expression of these POD genes and POD enzyme activity (Figures 1, 5B). One of the CAT genes’ expressions (BnaC07g15270D) was upregulated, while another (BnaA09g11640D) was repressed. POD and CAT genes have variable responses to the LT stress, which implicated that these genes may play divergent roles during LT stress response in B. napus (Figure 5B). We found that CAT enzyme activity was suppressed in LT stress seedlings (Figure 1K). These results also indicate that rapeseed A genome genes might play a critical role in the CAT enzyme activity compared with the B genome. Similarly, GST and GPX respond to environmental stress and act as scavengers of ROS and oxidizing radicals (Milla et al., 2003; Xu et al., 2015). In this study, the expression of GST genes (BnaC05g07890D, BnaC09g40740D, BnaA06g11500D, BnaC08g37940D, and BnaC04g13950D) was significantly enhanced, depicting their role in LT stress (Figure 5B). In one sentence, these findings elaborated various ROS signaling
pathways and provided insight into antioxidant genes, which formed a complex antioxidant defense system under LT stress conditions in *B. napus*.

**Plant hormone signal network to encounter LT stress**

Plant hormones were extensively studied under various environmental cues, including LT stress conditions in plants. Phytohormones have key functions in stress responses, such as to initiate a series of signal events and activate the expression of stress-responsive genes (Ku et al., 2018). Calcium sensors in cellular membranes respond to external stimuli to activate downstream signaling events and are influenced by ABA (Edel and Kudla, 2016). The plant responds to LT stress via ABA-dependent and ABA-independent pathways (Gusta et al., 2005; Ishitani et al., 1997). ABA accumulation mitigates the LT stress effects in rice (Zhao et al., 2015) and bermudagrass (Huang et al., 2017). Interestingly, exogenous ABA treatment reduced the H2O2, electrolyte leakage and MDA contents in bermudagrass compared with non-ABA-treated plants under LT stress conditions (Huang et al., 2017). In this study, we detected the ABA biosynthesis, catabolism, and signaling pathway genes as DEGs (Figure 8). Our findings indicated that genes involved in ABA catabolism, such as CYP707A1/2 (*BnaC07g35800D* and *BnaA03g43960D*), were activated in response to LT stress in *B. napus* (Figure 5C). CRY707A encodes cytochrome P450 monooxygenase that catalyzes the ABA into 8′-hydroxy ABA (Seiler et al., 2011). During the biosynthesis process, ABA repressed the expression of CYP707A genes but increased the expression of NCED. Variation in CYP707A expression level changed the cellular ABA levels and accordingly repressed or induced the NCED expression level. ABA catabolism and biosynthesis work antagonistically, which shows a strong feedback and feedforward loop mechanism to limit or enhance the ABA contents in cells. In addition to ABA accumulation, CYP707A works as a hub to coordinate with auxin, GA, and ABA signals (Liao et al., 2018). The variable expression level of CYP707A genes suggested that ABA catabolism and biosynthesis work side by side in response to LT stress in *B. napus* (Figure 4). The ABA signaling pathway is a double-negative regulatory system consisting of proteins SNF1-related protein kinase 2 (SnRK2), type 2C protein phosphatase (PP2C), and ABA receptors pyrabactin resistance 1 (PYR1)/PYR1-like (PYLs)/regulatory components of ABA receptors (RCAR) family (Hauser et al., 2011). In the absence of ABA, PP2C-mediated dephosphorylation repressed SnRK2 kinases to block signal transduction. In response to external stimuli, ABA-receptor PYR/PYL/RCAR protein inactivates PP2C, resulting in SnRK2 activation. Activated SnRK2 causes the upregulation of bZIP group TFs to increase the mRNA level of downstream ABA-responsive genes (Sirichandra et al., 2009; Li et al., 2011) (Figure 8). ABA-insensitive 5 (ABI5) is a bZIP TF that regulates ABA-dependent seed germination, growth, and development under environmental constraints. ABI regulates the expression of downstream ABA pathway genes that consist of the ABSCISIC ACID RESPONSE ELEMENT motif in the promoter region (Skubacz et al., 2016). The *Arabidopsis* mutant for ABI5 genes exhibited a reduced ABA level and seed dormancy, while complementation of ABI5 rescues the expression of ABA-responsive genes and dormancy. Similarly, the exogenous application of ABA reduces the cold sensitivity of the ABI mutant of *Arabidopsis* (Wu et al., 2015). Phospholipase D (PLD) involved in ABA signaling is a key regulator of plant growth, development, and abiotic stress in *B. napus* (Lu et al., 2019). In this study, PYR/PYL/RCAR (*BnaA04g21960D*) was significantly downregulated. Meanwhile, PP2C (*BnaA09g49440D*, *FIGURE 8 ABA pathway.*)
Brassinosteroids enhanced cold stress tolerance in plants. The phytosteroids or ethylene (ET), C2H4, is an important regulator of the cold stress response. It is debated whether the effect of ET response to LT stress is negative or positive in plants (Hu et al., 2016). ET negatively affects freezing tolerance as the repression of ET biosynthesis enhances freezing tolerance in Medicago truncatula seedlings (Zhao et al., 2014). On the other side, ET accumulation positively regulates cold tolerance in grapevine (Sun et al., 2016). These results indicate that ET accumulation imparts LT stress tolerance in species-specific manners. The mechanism of the ethylene signaling pathway from ethylene recognition at the cellular membrane to transcriptional regulation in the nucleus and the post-transcriptional level was well reported (Yang et al., 2015; Ku et al., 2018). CTR1 modulates the ethylene signaling pathway through a direct interaction with ET receptors. Active CTR1 can repress EIN2, but EIN2 repression is rescued in the presence of ET. The EIN2 translocates from the cell membrane and activates EIN3/EIL1 expression and EIN3/EIL1 levels in Arabidopsis (Mizoi et al., 2012). In a previous study, 132 AP2/ERF TFs were found to differentially express and respond to LT stress in rapeseed (Mizoi et al., 2012). AP2/ERF TFs can repress the expression of JAZ proteins (Katsir et al., 2008). The JAZ proteins bind to the promoter of jasmonate signaling genes and repress jasmonate signaling (Figure 6). Different TF families play an important role in enhancing LT stress tolerance, such as APETALA2/ethylene response factor (AP2/ERF), bZIP, WRKY, and MYB in plants. The AP2/ERF TF family members are important regulators in stress response (Mizoi et al., 2012). In a previous study, 132 AP2/ERF TFs were found to differentially express and respond to LT stress in rapeseed (Du et al., 2016). The bHLH TF family is the second largest protein family in plants that respond to multiple stresses including drought, salt, and especially cold stress (Sun et al., 2019). A significant induction of WRKY TFs demonstrated the prominent roles of this family in B. napus LT stress response (Figure 4). Similarly, NAC TFs engaged in multiple biological processes, including signal transduction, development, and abiotic and biotic stress responses. The plant cold stress response of the NAC TF family was evident in Prunus mume (Zhuo et al., 2018), pepper (Hou et al., 2020), and rice (Pradhan et al., 2019). In previous studies, the role of bZIP TFs in cold stress tolerance was described, such as in B. oleracea (Hwang et al., 2016), Magnolia wufengensis (Deng et al., 2019), and rice (Pradhan et al., 2019).
Besides the abovementioned details, two gene families like BnaA03g39650D and electron transport (BnaA03g39520D), while genes related to photosynthesis, and BnaA01g19110D. Among upregulated genes, genes were related to napus photosynthetic apparatus were induced during LT stress in B. napus. Nonetheless, we also observed that numerous genes in the photosynthesis pathway genes (Figure 1C). was reduced in LT-stressed seedlings, indicating a direct relationship with photosynthesis pathway genes (Figure 1C). Most of these genes are essential components of the light reaction and the Calvin cycle process of photosynthesis. During light reaction, sunlight was utilized by chlorophyll pigments to synthesize the high-energy compounds ATP and NADPH (Armbruster et al., 2017). The Calvin cycle is a light-independent redox reaction to fix carbon dioxide into the sugar glucose molecules and gaseous detoxification during photosynthesis (Sun et al., 2015; Nowicka and Kruk, 2018). Repression of genes involved in photosynthetic components were reported in tea (Shi et al., 2019), hibiscus (Paredes and Quiles, 2015), and rice (Liu et al., 2018b) under LT stress. Nonetheless, we also observed that numerous genes in the photosynthetic apparatus were induced during LT stress in B. napus. Among upregulated genes, genes were related to chlorophyll A–B-binding protein (BnaC01g22830D, BnaA01g19110D, and BnaA01g24440D), cytochrome P450, thylakoid membrane, photosynthetic reaction center protein (BnaC09g27520D), and ferredoxin I (BnaC07g49690D and BnaA03g39520D), while genes related to photosynthetic electron transport (BnaA03g39650D) were downregulated. Besides the abovementioned details, two gene families like chloroplastic chaperone protein dnaJ (BnaA01g29880D and BnaA02g15280D) and heat shock protein 70 (HSP70) members (BnaC03g61170D, BnaA03g59360D, and BnaA06g08870D) were induced. Under stress, DnaJ proteins function for protein homeostasis and protein complex stabilization. Transgenic tomato plants overexpressing DnaJ proteins exhibited maximum efficiency and stability of PSII and D1 protein complexes under cold stress conditions. During cold stress, HSP70 was discovered as the partner of DnaJ proteins (Kong et al., 2014). Ferredoxin molecules are sensitive to stress. Engineering of tobacco chloroplasts by an iso-functional protein of ferredoxin (a cyanobacterial flavodoxin) enhanced the tobacco plant tolerance to multiple stresses, including chilling (Zurbriggen et al., 2008). In our study, some genes related to glyceraldehyde-3-phosphate dehydrogenase (GAPDH)—such as BnaA05g33200D, BnaC05g47450D, and BnaC01g40210D—were induced (Table 2). GAPDH is involved in cellular metabolism and generates energy. GAPDH was induced in potato tubers under cold stress (Liu et al., 2017a). The ubiquitous enzyme adenylate kinase interacts with the chloroplast GAPDH, forming a stable complex that regulates the ATP/NADPH ratio inside the chloroplast to optimize the Calvin-Benson cycle in response to environmental stress (Zhang et al., 2018). Then, we performed qRT-PCR to verify the expression pattern of photosynthesis pathway-related DEGs, which further confirmed the reliability of the RNA-seq data (Figure 7). Thus, differential expression of photosynthetic system genes indicates that they may play vital roles in response to LT stress in B. napus. The exact mechanism of these factors remains unresolved and provides new directions for future LT stress studies.

Role of photosynthesis system in LT stress response

The chloroplast, a photosynthesis organ, is acutely sensitive to LT stress. Thus, LT stress halts the photosynthesis process in plants (Ma et al., 2018). Photosystem II (PSII) is a crucial component of a photosynthetic machinery that is actively inactivated under stress, and the accumulation of ROS halts the repairing mechanism of PSII in plants (Nishiyama and Murata, 2014). LT stress reduces the photosynthetic capacity by affecting the electron transfer in PSII and the efficiency of CO₂ fixation to photosynthates (Paredes and Quiles, 2015). The repair mechanism of PSII involves the replacement of damaged D1 protein by newly synthesized D1 protein at the expense of ATP in the degradation and synthesis of D1 (Murata and Nishiyama, 2018). In this study, primarily genes related to the photosynthesis pathway were repressed, which indicated that LT stress halts the photosynthesis system in B. napus (Table 2). Accordingly, we also found that the maximum quantum efficiency of PSII Fv/Fm was reduced in LT-stressed seedlings, indicating a direct relationship with photosynthesis pathway genes (Figure 1C). Protein interaction networks in LT stress response

Protein interaction networks regulate various cellular functions, such as signal transduction, metabolic pathways, organ formation, cell cycle regulation, and plant defense (Bray, 2006; Zhang et al., 2010). The protein–protein interactions of the sugar metabolism, hormone, antioxidant activity, transcription factors, and photosynthesis-related genes revealed that the protein domains physically interact through a complex network. Specifically, the SnRK2 family contains key regulators of cellular osmotic stress and ABA responses in plants. Here the SNI1-related protein kinase 2 (SnRK2), ABI, AOS, JAZ, and HSP families shared a maximum number of interaction lines (Figure 6). CYP707A1 encodes abscisic acid 8'-hydroxylases, which is indispensable for seed dormancy and germination in Arabidopsis (Okamoto et al., 2006). Similarly, ABI5 plays a significant role in the regulation of seed germination, seedling growth, development, and abiotic stresses (Zinsmeister et al., 2016). In the ABA signal pathway, the ABI5 protein directly interacts with CYP707A1, ABI2, PP2C phosphatases, PYR/PYL/RCAR receptors, and SnRK2 kinases. The interaction of these
| B. Process | Gene ID | Arabidopsis | Annotation | Regulation |
|------------|---------|-------------|------------|------------|
| **Light reaction** | **BnaC01g22830D** | AT4G14690.1 | Chlorophyll A–B binding protein | up |
| | **BnaC04g01380D** | AT2G22840.1 | Chlorophyll A–B binding protein | up |
| | **BnaC03g43640D** | ATCG00065.1 | Photosystem I psaA/psaB protein | down |
| | **BnaA02g31810D** | AT5G24260.1 | Cytochrome P450 | up |
| | **BnaA01g39110D** | AT4G14690.1 | Chlorophyll A–B binding protein | up |
| | **BnaC04g13570D** | ATCG00350.1 | Photosystem I psaA/psaB protein | up |
| | **BnaA09g07070D** | AT5G54270.1 | Chlorophyll A–B binding protein | down |
| | **BnaA02g27520D** | ATCG00270.1 | Photosystem II protein | up |
| | **BnaA03g36810D** | AT3G22840.1 | Chlorophyll A–B binding protein | up |
| | **BnaA05g29390D** | AT5G01530.1 | Chlorophyll A–B binding protein | down |
| | **BnaA01g19110D** | AT4G14690.1 | Chlorophyll A–B binding protein | up |
| | **BnaC04g15570D** | ATCG00350.1 | Photosystem I psaA/psaB protein | up |
| | **BnaA09g27520D** | AT3G22840.1 | Chlorophyll A–B binding protein | up |
| | **BnaA01g24440D** | AT3G22840.1 | Chlorophyll A–B binding protein | up |
| | **BnaA02g34030D** | AT5G64040.2 | Photosystem I reaction center subunit N | down |
| | **BnaA08g01590D** | AT1G52230.1 | Photosystem I reaction center subunit VI | down |
| | **BnaA03g09330D** | AT1G52230.1 | Photosystem I reaction center subunit VI | down |
| | **BnaC09g97600D** | AT1G30380.1 | Photosystem I psaA/psaB protein | down |
| | **BnaA09g06420D** | AT5G64040.2 | Photosystem I reaction center subunit N | down |
| | **BnaA01g12690D** | AT4G21280.2 | Oxygen evolving enhancer protein 3 | down |
| | **BnaA01g11910D** | AT4G21280.2 | Oxygen evolving enhancer protein 3 | down |
| | **BnaC05g0420D** | AT1G14150.1 | Oxygen evolving enhancer protein 3 | down |
| | **BnaC09g21360D** | AT3G01440.1 | Oxygen evolving enhancer protein 3 | down |
| | **BnaA06g08980D** | AT1G14150.1 | Oxygen evolving enhancer protein 3 | down |
| | **BnaA07g0940D** | AT5G49730.1 | Photosynthetic electron transport chain | down |
| | **BnaA07g2440D** | AT1G68010.2 | Photosynthetic electron transport chain | down |
| | **BnaA07g25770D** | AT1G68010.2 | Photosynthetic electron transport chain | down |
| | **BnaA03g36650D** | AT5G23060.1 | Photosynthetic electron transport chain | down |
| | **BnaA09g29160D** | AT1G68000.1 | Photosynthetic electron transport chain | down |
| | **BnaA09g26410D** | AT5G49730.1 | Photosynthetic electron transport chain | down |
| | **BnaA01g28110D** | AT3G16250.1 | Photosynthetic electron transport chain | down |
| | **BnaA05g23450D** | AT3G16250.1 | Photosynthetic electron transport chain | down |
| | **BnaC01g28660D** | AT1G68000.2 | Photosynthetic electron transport chain | down |
| | **BnaC06g26170D** | AT1G68010.2 | Photosynthetic electron transport chain | down |
| | **BnaC04g04790D** | AT5G23060.1 | Photosynthetic electron transport chain | down |
| | **BnaA01g21380D** | AT4G21280.2 | Oxygen evolving enhancer protein 3 | down |
| | **BnaA08g28940D** | AT4G26710.1 | ATP synthase subunit H | down |
| | **BnaA07g09690D** | AT5G23240.1 | Ferredoxin 1 | up |
| | **BnaA03g39520D** | AT5G23240.1 | Ferredoxin 1 | up |
| | **BnaA06g06760D** | AT1G10960.1 | Ferredoxin 2 | up |
| | **BnaA03g22350D** | AT5G27510.1 | Ferredoxin 3 | up |
| | **BnaA07g22910D** | AT3G25770.1 | Chloroplast thylakoid membrane | up |
| | **BnaA01g23830D** | AT4G14690.1 | Chloroplast thylakoid membrane | up |
| | **BnaA02g31810D** | AT5G42460.1 | Chloroplast thylakoid membrane | up |
| **Calvin cycle** | **BnaA07g14420D** | AT5G38430.1 | Ribulose-1,5-bisphosphate carboxylase small subunit | down |
| | **BnaA05g09840D** | AT2G39730.1 | Ribulose bisphosphate carboxylase/oxygenase activase | down |
| | **BnaA03g18710D** | AT2G39730.1 | Ribulose bisphosphate carboxylase/oxygenase activase | down |
| | **BnaA04g07070D** | AT2G39730.1 | Ribulose bisphosphate carboxylase/oxygenase activase | down |
| | **BnaA03g22220D** | AT2G39730.1 | Ribulose bisphosphate carboxylase/oxygenase activase | down |

(Continued)
proteins suggested that the expression of these genes is tightly associated with LT stress response. Based on these findings, ABI5 is a hub protein under LT response in *B. napus* which directly or indirectly interacts with antioxidants, TFs, ABA signaling, and regulation of LT-stress-related genes along with various other proteins. In *Arabidopsis*, alginate oligosaccharide (AOS) induced resistance to the pathogen by a salicylic-acid-mediated signaling pathway (Zhang et al., 2019a). Jasmonate and related signaling compounds have a significant role in plant immunity and development. WRKY1 negatively regulates the ABA signal pathway through JAZ1 and ABI1 in drought response (Luo et al., 2020). Moreover, AOS functions in fine-tuning JA formation and improving LT stress tolerance (Pi et al., 2009; Liu et al., 2017b). In this study, the JAZ protein directly interacts with WRKY, AOS, and other essential genes. These results showed that a complex interactive network at the protein level was activated in response to LT stress response in *B. napus*.

**Conclusion**

*B. napus* is the major oil crop with the largest planting area in China. However, its productivity and cultivation area are severely

| Gene ID | Arabidopsis | Annotation | Regulation |
|---------|-------------|------------|------------|
| BnaA07g14420D | AT5G38430.1 | Ribulose bisphosphate carboxylase/oxygenase activase | down |
| BnaA05g33200D | AT3G04120.1 | Glyceraldehyde 3-phosphate dehydrogenase, C-terminal domain | up |
| BnaC05g74540D | AT1G13440.1 | Glyceraldehyde 3-phosphate dehydrogenase, C-terminal domain | up |
| BnaA02g28150D | AT1G12800.1 | Glyceraldehyde 3-phosphate dehydrogenase, C-terminal domain | down |
| BnaC01g40210D | AT3G04120.1 | Glyceraldehyde 3-phosphate dehydrogenase, C-terminal domain | up |
| BnaC07g12360D | AT1G23040.1 | Glyceraldehyde 3-phosphate dehydrogenase, C-terminal domain | up |
| BnaA07g16660D | AT3G55800.1 | Fructose-1,6-bisphosphatase | down |
| BnaC06g42620D | AT3G55800.1 | Fructose-1,6-bisphosphatase | down |
| BnaC08g35820D | AT2G21330.1 | Fructose-bisphosphate aldolase class-I | down |
| BnaA03g33270D | AT3G14200.1 | DnaJ domain | down |
| BnaAnng20610D | AT1G56300.1 | DnaJ domain | up |
| BnaC09g96610D | AT2G17880.1 | DnaJ domain | up |
| BnaC01g72700D | AT1G56300.1 | DnaJ domain | down |
| BnaCnng20870D | AT4G13830.2 | DnaJ domain | up |
| BnaC03g02360D | AT5G06110.1 | DnaJ domain | down |
| BnaCnng44960D | AT4G13830.2 | DnaJ domain | down |
| BnaA01g29880D | AT3G13310.1 | DnaJ domain | up |
| BnaA02g15280D | AT1G71000.1 | DnaJ domain | up |
| BnaAnng08930D | AT4G13830.2 | DnaJ domain | down |
| BnaC06g19600D | AT1G08920.1 | DnaJ domain | down |
| BnaA07g20150D | AT1G08920.1 | DnaJ domain | down |
| BnaA04g60570D | AT4G13830.2 | DnaJ domain | down |
| BnaC06g40110D | AT1G08920.1 | DnaJ domain | down |
| BnaA05g22410D | AT3G17830.1 | DnaJ domain | up |
| BnaA09g09350D | AT2G17880.1 | DnaJ domain | down |
| BnaC06g35620D | AT2G21510.1 | DnaJ domain | up |
| BnaC03g61170D | AT4G37910.1 | Hsp70 protein | up |
| BnaA02g21330D | AT4G12400.2 | Hsp70 protein | down |
| BnaA07g35930D | AT4G37910.1 | Hsp70 protein | up |
| BnaA05g13150D | AT1G12270.1 | Hsp70 protein | up |
| BnaA06g00870D | AT1G79920.1 | Hsp70 protein | up |
| BnaA10g27060D | AT1G09080.1 | Hsp70 protein | up |
| BnaCnng03470D | AT3G12580.1 | Hsp70 protein | up |
| BnaA09g48560D | AT1G09080.1 | Hsp70 protein | up |
| BnaA09g12780D | AT1G62740.1 | Hsp70 protein | up |
affected by LT stress. In the current study, the transcriptomic study helped to identify various LT-stress-responsive pathways and molecular events. A set of potential DEGs that respond to LT stress was discovered. Different expressions of the key systems indicated that they might play vital roles in response to LT stress in *B. napus*. It is noticeable that the *B. napus* response to LT stress is multiplex, not only regulating the genetic architecture but also activating the antioxidant and osmoprotectant system to improve the LT stress tolerance (Figure 9). A deep understanding of these molecular events will provide new avenues for LT stress tolerance improvement in *B. napus*.

**Data availability statement**

The data presented in the study are deposited in the National Center for Biotechnology Information (NCBI) repository, accession number PRJN596550.

**Author contributions**

MH and YL conceived the idea and wrote the manuscript. MH, DL, and ZL performed the experiments. DL and XD helped in the literature searches and data analysis. YC, XZ, GL, and YL supervised the work, reviewed and edited the manuscript, and managed funding resources. All authors contributed to the article and approved the submitted version.

**Funding**

This work was supported by Key Research Program & Technology Innovation Program of the Chinese Academy of Agricultural Sciences (CAAS-ZDRW202109 and CAAS-ZDRW202105), the National Nature Science Foundation of China (32072131), the Key Research and Development projects in Hubei Province (2022BBA0038), and the Science and Technology Innovation Project of the Chinese Academy of Agricultural Sciences (CAAS), China, and Fundamental Research Funds for the Central Nonprofit Scientific Institution (OCRI, CAAS, 1610172018010).

**Acknowledgments**

MH is thankful to CSC for providing a Ph.D. scholarship and to OCRI-CAAS for research environment. MH is thankful to GYL for article language improvement and providing APC. We would also like to thank all the members of the Key Lab of Biology and Genetic Improvement of Oil Crops, OCRI-CAAS, Wuhan, China, for their support throughout the study.

**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher’s note**

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

**Supplementary material**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.1050995/full#supplementary-material
expression analysis in response to abiotic stress.

Katan, L., Xu, J., Leiser, A. L., Stawick, P. E., Hu, S. Y., and Howe, G. A. (2008). COH is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. Mol. Plant Microbe Interact. 21, 1055–1063. doi:10.1097/MBI.0b013e3181775c2e

Ke, L., Lei, W., Wang, W., Wang, J., Gao, J., Cheng, J., et al. (2020). Genome-wide identification of cold responsive transcription factors in Brassica napus L. BMC Plant Biochem. 20, 62. doi:10.1186/s12870-020-2253-5

Kong, F., Deng, Y., Zhou, B., Wang, G., Wang, Y., and Meng, Q. (2014). A chloroplast-targeted DnaJ protein contributes to maintenance of photosystem II under chilling stress. J. Exp. Bot. 65, 143–158. doi:10.1093/jxb/eru357

Kroll, J. S., Langford, P. R., Wilks, K. E., and Keil, A. D. (1995). Bacterial [Cu, Zn] superoxide dismutase: phylogenetically distinct from the eukaryotic enzyme, and not so rare after all! Microbiology 141, 2271–2281. doi:10.1099/13500872-141-9-2271

Ku, Y. S., Sintatha, M., Cheung, M. Y., and Lam, H. M. (2018). Plant hormone signaling crosstalk between biotic and abiotic stress responses. Int. J. Mol. Sci. 19, 1–35. doi:10.3390/ijms19103206

Liao, X., Li, M., Liu, B., Yan, M., Yu, X., Li, H., et al. (2018). Interlinked regulatory loops ofABA catabolism and biosynthesis coordinate fruit growth and ripening in woodland strawberry. Proc. Natl. Acad. Sci. U.S.A. 115, E11542–E11550. doi:10.1073/pnas.1802375115

Li, H., Dong, Y., Chang, J., He, J., Chen, H., Liu, Q., et al. (2016a). Genome-wide characterization of glutathione peroxidase (GPX) gene family in rice. Plant Physiol. Biochem. 103, 103990. doi:10.1016/j.plaphy.2018.10.003

Li, H., Dong, Y., Chang, J., He, J., Chen, H., Liu, Q., et al. (2016b). Transcriptome sequencing identified genes and gene ontologies associated with early freezing tolerance in maize. Front. Plant Sci. 7, 1477. doi:10.3389/fpls.2016.01477

Li, J., Jia, H., Chai, Y., and Shen, Y. (2011). Abscisic acid perception and signaling transduction in strawberry: a model for non-climacteric fruit ripening. Plant Signal. Behav. 6, 1950–1953. doi:10.4161/psb.6.11.18024

Li, Y., Li, D., Deng, W., Li, H., Shi, T., Yang, X., et al. (2020). Genome-wide identification of osmanthus fragrans bHLH transcription factors and their regulatory loops of ABA catabolism and biosynthesis coordinate fruit growth and ripening in woodland strawberry. Plant Physiol. Biochem. 103, 103990. doi:10.1016/j.plaphy.2018.10.003

Lv, Y., Yang, M., Hu, D., Yang, Z., Ma, S., Li, X., et al. (2017). The OsMYB30 transcription factor suppresses cold tolerance by interacting with a JAZ protein and suppressing β-amylase expression. Plant Physiol. 173, 1475–1491. doi:10.1104/pp.16.116725

Ma, X., Chen, C., Yang, M., Dong, X., Lv, W., and Meng, Q. (2018). Cold-regulated protein (SIC0R413M1) confers chilling stress tolerance in tomato plants. Plant Physiol. Biochem. 124, 29–39. doi:10.1016/j.plaphy.2018.01.003

Mao, X. Z., Cai, T., Olyarchuk, J. G., and Wei, L. P. (2005). Automated genome annotation and pathway identification using the KEGG orthology (KO) as a controlled vocabulary. Bioinformatics 21, 3787–3793. doi:10.1093/bioinformatics/bti340

Maryan, K. E., Lahiji, H. S., Farrokhli, N., and Komeilzadeh, H. H. (2019). Analysis of Brassica napus npr1-resistant, mediated cold and heat stress tolerance genotypes in relation to cold stress. Gene Expr Patterns 31, 7–17. doi:10.1016/j.gep.2018.10.002

Miller, G., Suzuki, N., Ciftci-Yilmaz, S., and Mittler, R. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ. 33, 455–467. doi:10.1111/j.1365-3040.2009.02041.x

Mizus, J., Shinohara, K., and Yamaguchi-Shinozaki, K. (2012). AP2/ERF family transcription factors in plant abiotic stress responses. Biochem. Biophys. Acta 1829, 86–96. doi:10.1016/j.bbagrm.2011.08.004

Murata, N., and Nishimura. Y. (2018). ATP is a driving force in the repair of photosystem II during photoinhibition. Plant Cell Environ. 41, 285–299. doi:10.1111/pce.13108

Nishimura, Y., and Murata, N. (2014). Revised scheme for the mechanism of photoinhibition and its application to enhance the abiotic stress tolerance of the photosynthetic machinery. Appl. Microbiol. Biotechnol. 98, 8777–8796. doi:10.1007/s00253-014-5952-y

Niu, X., Lu, H., Fan, Y., Wang, W., Yuan, Y., Hawkins, M., et al. (2022). Manipulation of the transcription factor SINAC1 for improved tolerance to abiotic stress in tomato. Plant Cell Environ. 1–14. doi:10.1111/pce.14437

Nowicka, B., and Kruk, J. (2018). [Genetic engineering as a method for the improvement of photosynthesis]. Postepy Biochem. 64, 13–20. doi:10.18388/pb.2018_100

Oezde, M. L., Kandhinder, A., and D. K. J. (2008). Redox regulation and over reduction control in the photosynthesizing cell: complexity in redox regulatory networks. Biologia 63, 1780, 1261–1272. doi:10.1515/biologia.2008.03.013

Okamoto, M., Kuwahara, A., Seo, M., Kushiro, T., Asami, T., Hirai, N., et al. (2006). CYP707A1 and CYP707A2, which encode abscisic acid 8'-hydroxylases, are potentially connected events to freezing stress. PloS One 10, e10509. doi:10.1371/journal.pone.010509

Pi, Y., Jiang, K., Cao, Y., Wang, Q., Huang, Z., Li, L., et al. (2009). Allene oxide synthase (AOS) is essential for chloroplast biogenesis in rice under cold stress. J. Exp. Bot. 60, 3949–3961. doi:10.1093/jxb/er124

Liu, W., Wang, H., Chen, Y., Zhu, S., Chen, M., Lan, X., et al. (2017b). Cold stress improves the production of artemisinin depending on the increase in endogenous jasmonate. Biotechnol. J. 12, 305–314. doi:10.1002/btj.1943

Liu, Y., Zhang, L., Chen, L., Ma, H., Ruan, Y., Xu, T., et al. (2016). Molecular cloning and expression of an encoding galactinol synthase gene (AtGOLS1) in seedling stage chilling stress tolerance in indica rice via RNA-seq analysis. BMC Plant Biol. 19, 352. doi:10.1186/s12870-019-1922-8

Pu, Y., Liu, L., Wu, J., Zhan, Y., Bai, J., Ma, L., et al. (2019). Transcriptome profile analysis of winter wapeseed (Brassica napus L.) in response to freezing stress, reveal potentially connected events to freezing stress. Int. J. Mol. Sci. 20, 1–24. doi:10.3390/ijms20113742

Qi, J., Song, C. P., Wang, B., Zhou, J., Kangasjärvi, J., Zha, J. K., et al. (2018). Reactive oxygen species signaling and stomatal movement in plant responses to drought stress and pathogen attack. Integr. Plant Biol. 60, 805–826. doi:10.1111/ipb.12654
Calvin cycle and in the sucrose-biosynthesis pathway. Increasing cytoplasmic volume accompanies increased activities of enzymes in the family in medicago truncatula. Front. Plant Sci. identiﬁcation and expression analysis of superoxide dismutase (SOD) genes in foxtail millet (Setaria italica l.). Biomolecules 27, 1–16. doi:10.3389/fbiochem.2009.09.0035

Sicher, R. (2011). Carbon partitioning and the impact of starch degradation and sucrose accumulation of lily bulbs after cold storage. Postharvest Biol. Technol. 64, 1–8. doi:10.1016/j.postharvbio.2012.01.002

Xiong, H., Wang, R., Jia, X., Sun, H., and Duan, R. (2022). Transcriptional analysis of rapeseed (Brassica napus l.) seed development in xiangride, qinghai jinhu hybridus ABSCISIC ACID INSENSITIVE 5 (GhABI5) is an important transcription factor targeted by sly-miR156e-3p positively regulates ABA-mediated cold signaling pathway in arabidopsis thaliana. Mol. Genet. Genomics 300, 851–868. doi:10.1007/s00438-018-1607-0

Yan, L., Kong, X., Huang, H., Wu, W., Park, J., Yun, D. J., et al. (2020). STCH4/homeostasis. Cell Rep. 30, 99–102. doi:10.1016/j.celrep.2020.07.023

Sun, X., Zhao, T., Gan, S., Ren, X., Fang, L., Karungo, S. K., et al. (2016). Ethylene positively regulates cold tolerance in grapevine by modulating the expression of ETHYLENE RESPONSE FACTOR 057. Sci. Rep. 6, 20466. doi:10.1038/srep20466

Su, W., Raza, A., Gao, A., Jia, Z., Zhang, Y., Hussain, M. A., et al. (2021). Genome-wide analysis and expression proﬁle of superoxide dismutase (SOD) gene family in rapeseed (Brassica napus l.) under stress conditions. Front. Plant Sci. 12, 643. doi:10.3389/fpls.2021.643

Tyczewska, A., Gracz, J., Kuczyński, J., and Twardowski, T. (2016). Deciphering the soybean molecular stress response via high-throughput approaches. Acta Biochim. Pol. 63, 631–643. doi:10.1088/abp.2016.1430

Verma, V., Ravindran, P., and Kumar, P. P. (2016). Plant hormone-mediated regulation of stress responses. BMC Plant Biol. 16, 86. doi:10.1186/s12870-016-0771-y

Wang, Z., Chen, Y., Fang, H. D., Shi, H. F., Chen, K. P., Zhang, Z. Y., et al. (2014). Selection of reference genes for quantitative reverse-transcription polymerase chain reaction normalization in brassica napus under various stress conditions. Mol. Genet. Genomics 289, 1023–1035. doi:10.1007/s00438-014-0853-1

Wang, L. B., Wang, L., Zhang, Z. E., Ma, M., Wang, R. Z., Qian, M., et al. (2018a). Genome-wide identiﬁcation and comparative analysis of the superoxide dismutase gene family in pear and their functions during fruit ripening. Postharvest Biol. Technol. 143, 66–77. doi:10.1016/j.postharvbio.2018.04.012

Wang, T., Song, H., Zhang, B., Lu, Q., Liu, Z., Zhang, S., et al. (2018b). Genome-wide identiﬁcation, characterization, and expression analysis of superoxide dismutase (SOD) genes in foxtail millet (Setaria italica l.). Biotechnol. 8, 486. doi:10.1007/s13205-018-1502-6

Wittkopp, P. J., and Kalay, G. (2012). cis-regulatory elements: molecular mechanisms and evolutionary processes underlying divergence. Nat. Rev. Genet. 13, 59–69. doi:10.1038/nrg31095

Wu, J., Seng, S., Sui, J., Vunapatris, E., Luo, X., Gong, B., et al. (2015). Gladiolus hybacinthiacus ARBICISIC ACID INSENSITIVE 1 (GhABICISIC) is a CO2-dependent transcription factor in ABA signaling that can enhance gladiolus dormancy and abscisic acid seed dormancy. Front. Plant Sci. 6, 960. doi:10.3389/fpls.2015.00960

Xin, H., Xianchao, N., Pan, X., Wei, L., Min, Y., Yu, K., et al. (2019). Comparative transcriptome analyses revealed conserved and novel responses to cold and freezing stress in brassica napus l. G3 (Bethesda). 9, 2723–2737. doi:10.1101/g3.jeng.14.4.110029

Yang, C., Lu, X., Ma, B., Chen, S. Y., and Zhang, J. S. (2015). Ethylene signaling in rice and abscisic acid: conserved and diverged aspects. Mol. Plant 8, 495–505. doi:10.1093/mp/51.5.1003

Yan, L., Shah, T., Cheng, Y., Lu, Y., Zhang, X. K., and Zou, X. L. (2019). Physiological and molecular responses to cold stress in rapeseed (Brassica napus l.). J. Integr. Agr. 18, 2742–2752. doi:10.1007/s13205-018-16247-1

Young, M. D., Wakefeld, M. J., and Smyth, G. K. (2010). Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biol. Evol. 11, 1–12. doi:10.1093/gbe/evq101

Yu, H., Kong, X., Huang, H., Wu, W., Park, J., Yun, D. J., et al. (2020). STCH4/EIL2 confers cold stress tolerance in arabidopsis by promoting RNA processing and CBF protein translation. Cell Rep. 30, 229–242. doi:10.1016/j.celrep.2021.03.003

Yu, J., Xu, S., Liu, X., Li, T., Zhang, D., Teng, N., et al. (2022a). Starch degradation and sucrose accumulation of lily bulbs after cold storage. Int. J. Mol. Sci. 23, 1–17. doi:10.3390/ijms230816247-1

Yu, Q., Zheng, Q., Shen, W., Li, J., Yao, W., and Xu, W. (2022b). Grape CIPK18 acts as a positive regulator of CBF cold signaling pathway by modulating ROS homeostasis. Environ. Exp. Bot. 203, 105063. doi:10.1016/j.envexpbot.2022.105063

Zhang, J., An, H., Zhang, X., Xu, F., and Zhou, B. (2022a). Transcriptional analysis reveals potential gene regulatory networks under cold stress of loquat (Eriobotrya japonica lindl.). Front. Plant Sci. 13, 944269. doi:10.3389/fpls.2022.944269

Zhang, Y., Gao, P., and Yuan, J. S. (2010). Plant protein-protein interaction network and interactome. Curr. Genomics 11, 40–46. doi:10.2174/138945010789218016

Zhang, C., Howlader, P., Liu, T., Sun, X., Jia, X., Zhao, X., et al. (2019a). Alginic algichloreharshia (AOS) induced resistance to Pst DC3000 via salicylic acid-mediated signaling pathway in arabidopsis thaliana. Carbohydr. Polym. 225, 115221. doi:10.1016/j.carbpol.2019.115221

Zhang, Y., Luany, H., Liu, F., Lebrun, R., and Gontier, B. (2018). Interaction between adenylate kinase 3 and glyceroldehyde-3-phosphate dehydrogenase from chlamydomonas reinhardtii. FEBS J. 285, 2495–2503. doi:10.1111/febs.14494

Zhang, J., Li X. M., Lin, H. X., and Chong, K. (2019). Crop improvement through temperature resilience. Annu. Rev. Plant Biol. 70, 753–780. doi:10.1146/annurev-arplant-050718-100016

Zhang, L., Song, J., Lin, R., Tang, M., Shao, S., Yu, J., et al. (2022b). The SlMYB15 transcription factor targeted by sly-miR156e-3p positively regulates ABA-mediated cold tolerance in tomato. J. Exp. Bot. erac370. doi:10.1093/jxb/erac370
Zhang, W., Wang, S., Yu, F., Tang, J., Shan, X., Bao, K., et al. (2019c). Genome-wide characterization and expression profiling of SWEET genes in cabbage (Brassica oleracea var. capitata l.) reveal their roles in chilling and clubroot disease responses. *BMC Genomics* 20, 93. doi: 10.1186/s12864-019-5454-2

Zhang, D., Ye, H., Guo, H., Johnson, A., Zhang, M., Lin, H., et al. (2014). Transcription factor HAT1 is phosphorylated by BIN2 kinase and mediates brassinosteroid repressed gene expression in arabidopsis. *Plant J.* 77, 59–70. doi: 10.1111/tpj.12368

Zhao, M., Liu, W., Xia, X., Wang, T., and Zhang, W. H. (2014). Cold acclimation-induced freezing tolerance of medicago truncatula seedlings is negatively regulated by ethylene. *Physiol. Plant* 152, 115–129. doi: 10.1111/ppl.12161

Zhao, J., Zhang, S., Yang, T., Zeng, Z., Huang, Z., Liu, Q., et al. (2015). Global transcriptional profiling of a cold-tolerant rice variety under moderate cold stress reveals different cold stress response mechanisms. *Physiol. Plant* 154, 381–394. doi: 10.1111/ppl.12291

Zheng, Y., Luo, L., Wei, J., Chen, Q., Yang, Y., Hu, X., et al. (2018). The glutamate receptors AGLR1.2 and AGLR1.3 increase cold tolerance by regulating jasmonate signaling in arabidopsis thaliana. *Biochem. Biophys. Res. Commun.* 506, 895–900. doi: 10.1016/j.bbrc.2018.10.153

Zhuo, X., Zheng, T., Zhang, Z., Zhang, Y., Jiang, L., Ahmad, S., et al. (2018). Genome-wide analysis of the NAC transcription factor gene family reveals differential expression patterns and cold-stress responses in the woody plant prunus mume. *Genes* 9, 1–22. doi: 10.3390/genes9100494

Zinsmeister, J., Lalanne, D., Terrasson, E., Chatelain, E., Vande casteele, C., Vu, B. L., et al. (2016). ABR1 is a regulator of seed maturation and longevity in legumes. *Plant Cell.* 28, 2735–2754. doi: 10.1105/tpc.16.00470

Zurbriggen, M. D., Tognetti, V. B., Fillat, M. F., Hajirezaei, M. R., Valle, E. M., and Carrillo, N. (2008). Combating stress with flavodoxin: A promising route for crop improvement. *Trends Biotechnol.* 26, 531–537. doi: 10.1016/ j.tibtech.2008.07.001