Transcriptome Analysis of Photosynthetic Characteristics was Induced by Low Temperature Stress in Brassica napus L.

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Abstract

Background RNA Sequencing (RNA-Seq) technique could be utilized to compare the transcription groups of two different cold-resistant rapeseed leaves responding to low temperature at the seedling stage, analyze the photosynthetic characteristics of rapeseed subjected to low temperature stress, and identify the related genes for low temperature induction in rapeseed leaves. Results Using cold-tolerant variety 17NS and sensitive variety NF24 as experimental materials, carrying out RNA-Seq analysis by photosynthetic parameter determination and Illumina HiSeqTM platform. and screen out the KEGG significant enrichment pathway related to photosynthetic characteristics under low temperature stress. Differential Expressed Genes (DEGs) were used for real-time PCR to verify the reliability of RNA-Seq results. The results showed that the response of Brassica napus L. to low temperature stress mainly was achieved by inhibiting photosynthesis, the cold-tolerant variety 17NS had a strong ability to maintain membrane system stability and structural integrity after 24 h of low temperature stress, while the sensitive variety NF24 photosynthesis was significantly inhibited. Two pathways of Photosynthesis and Photosynthesis-antennas, which were significantly correlated with photosynthetic characteristics and low temperature stress were screened by KEGG enrichment. The results of DEGs indicated that 64 differentially expressed genes in these two pathways were induced by low temperature stress, and 8 of them were up-regulated expression and 56 of them were down-regulated expression. The expression pattern of DEGs was consistent with the results of RNA-Seq analysis by qRT-PCR detection and confirmed the reliability of RNA-Seq results. Conclusion Our study analysis and identified 17 low-temperature-induced photosynthetic-related candidate genes in Brassica napus L., and the GO and KEGG metabolic pathways clarified the molecular function of differentially expressed genes.
Background

Brassica napus, as one of the main oilseed crops in China, is widely distributed and highly adaptable. With the northward movement of cabbage winter rape, the planting range of Brassica napus is also expanding day by day. The quality, yield and lodging resistance of the Brassica napus are better than those of the Brassica rapa, but the cold resistance is weak and is greatly affected by the low temperature stress [1]. Low temperature will cause the delay of sowing date and growth period of soybean, corn, rice and other crops [2], which is the main factor leading to reduce of crop yield loss [3, 4]. Rape as the overwintering crop second only to winter wheat, is also deeply hurt by low temperature [5]. Brassica napus L., one of China's major oil crops, is also deeply damaged by low temperature stress. The cold-tolerant ability of Brassica napus L. is far less than that of cabbage-type winter rape. The middle and lower reaches of Yangtze river, extreme low temperature was frequently occurrence during the flowering stage of Brassica napus L. in the spring, the photosynthesis was significantly inhibited and the supply of photosynthetic products was insufficient, resulting in the loss of flower and fruit, and the yield was seriously reduced [6]. Therefore, the low temperature-induced photosynthetic characteristics of Brassica napus L. was studied, and the potential genes in response to low temperature and regulating photosynthesis were explored. It is important to clarify the molecular functions and metabolic pathways of these genes. Previous studies have found that plants themselves can form a series of defense mechanisms during evolution in order to adapt to low temperature stress [7]. Low temperature mainly causes damage to plant leaves, roots, growth points and flowers [8], causing physiological and biochemical changes such as penetrating substances, photosynthetic parameters [9, 10], photosynthesis, respiration and antioxidant enzyme systems, which will cause plant development poor, metabolic and molecular dysfunction and even death [11-13]. Low
Temperature inhibits plant photosynthetic pigment content and physiological processes such as photosynthesis[14-17]. Photosynthesis is the main pathway for plant vegetative organs, which can use light energy to synthesize organic compounds from carbon dioxide and water, from light-dependent parts (photoreaction) and light independence part (dark reaction, carbon fixation) composition. Low temperature stress can cause a decrease in photosynthetic parameters such as agronomic traits, photosynthetic rate, transpiration rate, leaf stomatal conductance and intercellular CO$_2$ concentration[18, 19]. Chloroplasts, as the main organelles for photosynthesis of plants, are also the most sensitive target organs for stress such as low temperature[20]. After chilling stress, the morphological structure of chloroplasts changes significantly. The chlorophyll content is reduced to reduce the light-capturing ability of leaves [21], and the grainy lamella are reduced or blurring, the number of them were reduced, the number of hungry granules increased and the starch granule content decreases or even disappears[22-25]. In recent years, with the continuous improvement of molecular biotechnology, predecessors have successfully isolated many low temperature stress response genes[26-28] and antifreeze proteins[29, 30] through genomics research. At present, the research of omics analysis on Brassica napus L. is also relatively mature. Du Chunfang found that after treatment of Brassica napus L. at low temperature for 24h, transcriptomics analysis got 17824 significantly different genes by high-throughput sequencing analyzed, and 573 differential protein [31] were identified with proteomics analysis by iTRAQ sequencing. There are few studies on photosynthetic regulation mechanisms based on transcriptomics analysis of low temperature stress response. Therefore, based on high-throughput RNA-Seq technology, combined with leaf ultra-microstructure and photosynthetic gas parameters, the photosynthesis response genes of Brassica napus L. leaves under low temperature stress and the photosynthetic regulation mechanism of Brassica napus L. under low temperature
stress was revealed were analyzed.

Results

*Responses of plant morphology and leaf development to low temperature stress*

Observation on the morphological development of plants under low temperature stress

The Brassica napus L. was treated at low temperature for 24 h at 4 °C (Fig.1). The morphological changes of the plants showed that the leaves of 17NS and NF24 had wilting and sagging, of which the old leaves of sensitive NF24 were obviously wilted, the new leaves were slightly wilted, and the old leaves of cold tolerant 17NS were wilted while the new leaves barely changed. The two varieties after low temperature treatment were restored at room temperature for 24 hours. It was found that the old leaves of the sensitive variety NF24 all died, the new leaves returned to life, and the cold-tolerant variety 17NS recovered after 24 hours, the new leaves and the old leaves are green and revitalized. The results showed that the new leaves showed strong tolerance and adaptability in response to low temperature stress and the leaves of the cold-resistant variety 17NS have strong frost resistance under low temperature stress.

Observation chloroplast morphological under low temperature stress by transmission electron microscopy

Transmission electron microscopy observation of rapeseed leaves under low temperature stress showed that low temperature stress had a great influence on the structure and quantity of organelles in leaf cells, which were manifested in the morphological structure of chloroplasts, the number of hypersalotic particles and starch granules were changed.
The varieties with different cold resistance have different degrees of changes in organelles. After 24 hours of cold tolerance of 17NS cold-resistant varieties, the grum were dispersed, the chloroplasts shrank, the volume became smaller, and the starch granules became smaller (Fig.2-A1). Compared with the control (Fig.2-B1), the number of starch granules increased. There are up to 3 starch granules in the chloroplast, and the number of starvation granules increases (Fig.2-B2), the number and morphology of chloroplasts are not obvious (Fig.2-B3). In the sensitive variety NF24, the control (Fig. 2-C1) starch granules were faintly visible, disappeared after low temperature stress, and the thylakoid layer was significantly reduced (Fig.22-D1). The chloroplast changes from a spindle shape close to the cell wall to a paramecium shape far away from the cell wall, the number of mitochondria increased significantly (Fig.2-D2), and the number of chloroplasts in individual cells decreased (Fig.2-D3). It indicated that the varieties with different cold resistance had different responses to chloroplast photosynthesis and respiration after low temperature stress.

Response of Physiology and Biochemistry of Brassica napus L. to Low Temperature Stress

Response of cell membrane of Brassica napus L. to low temperature stress

When the plant tissue is damaged by the stress, the low temperature stress firstly damages the membrane system of the cell, the membrane function is impaired or the structure is destroyed, and the permeability is increased, the water-soluble substances in the cells, including the electrolyte, will have different degrees of extravasation. The relative conductivity measurement showed that the relative conductivity of the cold-tolerant variety 17NS and the sensitive variety NF24 was almost the same before the low
temperature stress. After the low temperature stress, the relative conductivity increased significantly (Fig.3). The relative conductivity of the sensitive variety NF24 increased sharply. It is 0.869 ms/cm$^3$, which is larger than the rising value of 17NS (0.760 ms/cm$^3$), indicating that the electrolyte of the cell membrane of Brassica napus L. has a large amount of extravasation after low temperature damaging. Sensitive varieties have large electrolyte exosmosis, poor cold tolerance and adaptability while cold resistant variety have strong ability to maintain the relative stability and structural integrity of the cell membrane system, and better resistance to low temperature damage.

Effects of low temperature stress on photosynthetic parameter

The photosynthetic parameters of 0~24h under low temperature stress showed that the low temperature significantly reduced the net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr), and increased the intercellular CO$_2$ concentration (Ci). Pn, Gs, Tr and Ci between different varieties were significantly different with the treatment time (Fig. 4). Before the low temperature stress, the Pn, Gs and Tr of the cold-tolerant variety 17NS were lower than the cold sensitive variety NF24, but the Ci was higher than the cold sensitive variety NF24. After 24 h of low temperature stress, the Tr, Gs and Pn of the cold-tolerant 17NS leaves were significantly lower than the control, with the reductions being 30%, 60% and 72%, respectively. During the low temperature stress of NF24, the Pn, Gs and Tr of the leaves increased first and then decreased, however, it was significantly higher than the control group after 24h of stress, Pn decreased by 66.13% compared with the control, and Tr and Gs increased by 205.01% and 280% compared with the control. The variation range of cold sensitive cultivars was
more unstable than that of cold resistant cultivars at the low temperature stress. The Ci of the cold-tolerant variety 17NS and the cold-sensitive variety NF24 were first decreased and then increased. After 24 hours, the Ci increased by 16.29% and 20.01%, respectively. Therefore, low temperature significantly affects photosynthesis and stomatal gas exchange, and has a more greater impact on Tr, Gs and Pn of cold sensitive varieties.

Effect of low temperature on chlorophyll content of Brassica napus L

It can be seen from Fig.5 that the chlorophyll a and chlorophyll b contents showed a significant downward trend after 24 h of low temperature stress in the functional leaves of different Brassica napus L., and the chlorophyll a and chlorophyll b contents of sensitive NF24 sharp fell compared with the cold resistant variety 17NS, which was 0.184 mg/ g FW and 0.387 mg/g FW. The carotenoid content increased with the decrease of temperature, and the cold resistant variety 17NS increased faster, reaching 0.337 mg/g FW. It can be seen that the content of chlorophyll a and chlorophyll b in Brassica napus L. decreased with low temperature stress, and the content of carotenoids increased, and the decrease of chlorophyll content of cold resistant varieties was smaller than that of sensitive varieties, indicating that the cold resistant varieties had stronger resistance after low temperature stress. The strong photosynthetic capacity of the cold resistant variety is conducive to maintaining the normal growth of seedlings for adversity injury.

*Screening of differentially expressed genes in Brassica napus L. under low temperature stress*
According to the transcriptome data of Brassica napus L. under low temperature stress, the difference gene expression was analyzed by using edgeR software. The differential gene was screened by FDR and log2FC. The screening criteria were FDR < 0.05 and |log2FC| > 1. Among the different cold cultivars, NF24t0-17NSt0 was paired to obtain 18978 genetic difference genes (Table 1.). NF24t-17NSt paired to obtain 22019 differential genes, and the genetic background difference was removed to obtain significant differences in low temperature induction. A total of 3041 genes were expressed (739 differential genes up-regulated expression and 2302 differential genes down-regulated expression).

KEGG pathway analysis

By KEGG Pathway analysis of differential genes, the pathway with Qvalue ≤ 0.05 was chosen as the pathway for differential gene enrichment. The results showed that in all differentially expressed genes, after 24 h of low temperature stress, 4229 differentially expressed genes of NF24t0-17NSt0 and 4675 differentially expressed genes of NF24t-17NSt were enriched in 131 and 132 pathways, respectively. Twenty of the most significant pathways were screened by P < 0.05 (Fig.6), and Ribosome and Metabolic pathway were the most significant pathways before and after low temperature stress, respectively.

Light and regulation related differential expression gene analysis

The analysis of pathways related to photosynthetic characteristics after low temperature
stress revealed that Photosynthesis and Photosynthesis-antenna proteins are significant difference in energy metabolism, of which Photosynthesis-antennas were ranked in the top 20 in the NF24 t-vs-17NS t comparison group (Fig.6). Using Venny online software (http://bioinfogp.cnb.csic.es/tools/venny/) to analyze the DEGs(Differentially Expressed Genes) of different varieties under low temperature stress (Fig. 7), it was found that the significantly DEGs of Photosynthesis have 50 and the significantly DEGs of Photosynthesis-antennas have 12 in the NF24t0-17NST0 comparison group, and the significantly DEGs of Photosynthesis have 69 and the significantly DEGs of Photosynthesis-antennas have 30 in the NF24t-17NST comparison group.

Analysis of photosynthesis regulation pathway induced by low temperature stress

After low temperature stress, in the photosynthesis and photosynthesis-antenna proteins related to photosynthetic regulation, DEGs repeated with NF24t0-vs-17NST0 were removed from the DEGs of NF24t-vs-17NST. 64 significant DEGs were obtained (As shown in the blue part of Fig.8). There were 38 DEGs in the Photosynthesis pathway, of which 7 DEGs up-regulated expression and 31 DEGs down-regulated expression, 26 DEGs in the Photosynthesis-antenna proteins pathway, of which one DEGs up-regulated expression and 25 DEGs down-regulated expression (Fig.9 and Fig.10).

Go function classification of specific expression genes

Through the function annotation (Fig.11) of Go function, it is found that 64 specific DEGs related to photosynthetic regulation in the differentially expressed genes under low temperature stress are mostly annotated into cell component processes and biological
processes, and the number of differentially expressed genes are least in molecular function. It indicated that low temperature stress affected the growth and development of Brassica napus L. Among them are mainly cellular processes, metabolic processes, cells, cellular components, organelles, binding, catalytic activity and transport activity.

qRT-PCR validation of specific expression genes

The 64 low-temperature-induced specific DEGs (Table 2), mainly with photosynthetic system I, photosynthetic system II, oxygen-releasing protein, iron Oxygen-reducing protein-NADP reductase, chlorophyll protein, and ATP synthase are related; most of the genes of sensitive NF24 and cold-tolerant cultivar 17NS were down-regulated after low temperature stress. From Photosynthesis pathway screening one up-regulated DEGs and 7 up-regulated DEGs, from Photosynthesis - antenna proteins pathway screening one gene up-regulated DEGs and 8 down-regulated performed with qRT-PCR. The results (Fig. 12) of qRT-PCR indicate that were consistent with the results of RNA-Seq analysis; the expression of 17 DEGs in qRT-PCR and RNA-Seq showed the same trend, confirming the reliable results of RNA-Seq analysis.

Low temperature stress inhibited the expression of ferredoxin-coenzyme ii reductase, photosystem I reaction, oxygen-releasing protein, ATP synthase, photosystem II repair protein PSB27-H1, photosystem I chlorophyll a/b binding protein, chlorophyll a-b binding protein and so on, and induces expression of ferredoxin and chlorophyll a-b binding protein CP26. It is indicated that the inhibition of photosynthesis by low temperature mainly occurs in chloroplasts, and photosynthesis is inhibited by inhibiting photosynthetic pigments and photoreaction systems.
Analysis of cold resistance of Brassica napus L

To promote the breeding process of Brassica napus L. were used, the existing with the aid of Brassica genome information, analysis identified 1173 flowering related genes of Brassica napus L.[34], Two QTLS related to the content of fatty glycosides in seeds [35] and Twenty abiotic drought-related genes[36], There were 1651 up-regulated genes and 405 down-regulated genes in tea trees after low temperature stress and are closely related to photosynthesis regulation[37, 38]. At present, there are many researches on the analytical mechanism of plant cold tolerance, for example, RbohB gene[26], COLD1[39] and chitinase gene (BnCHB4)[28], COR[40], ICE[41], HSF[42], CBF[43] and other antifreeze proteins. This study found that low temperature stress could cause to the chloroplast number of Brassica napus L. reduce were used, the volume contraction of the chloroplast, grana lamella fuzzy, addicted to hungry particle number increases, chlorophyll a, chlorophyll b content decreased significantly, the photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (Gs) and intercellular CO2 concentration (Ci) go down and then it goes up. It indicates that plants can alleviate the damage of low temperature by adding osmotic regulatory substances, and the varieties with stronger cold resistance are hindered in CO2 supply and CO2 assimilation capacity [44-46], which eventually leads to decreased photosynthetic capacity and affects photosynthetic response [47-49], which is consistent with previous research results[22, 24, 25, 50].

Transcriptome analysis under low temperature stress

Through the transcriptome analysis, get two significantly photosynthesis regulation pathways with Photosynthesis and Photosynthesis-antenna proteins. NF24t-v-17NST0 for
comparison, NF24t-v-17NSt for processing, 7 DEGs has up-regulated and 31 DEGs has
down-regulated in Photosynthesis pathway[]1 DEGs has up-regulated and 25 DEGs has
down-regulated in Photosynthesis-antenna proteins pathways, a total of 64 specific gene
expression. Analyzed found that the low temperature stress lead to PS I and PS II be
suppressed[51], was mainly related to PS I, PS II, increasing oxygen protein, Ferrix -NADP
reductase put oxygen reductase, phyllochlorin, ATP synthesis enzyme and so on, partially
restore ferredoxin is used for by PETH reduction NADP +, which is used for producing
NADPH carbon assimilation and other biosynthetic pathway, and can also be mediated
electron flow to protect leaf light system [38]. Genetic variations of top up or down
expression by qRT - PCR analysis, test and verify the reliability of the RNA - Seq results,
and access to 17 stable expression of photosynthetic regulation candidate genes,
expression of 15 genes, 2 raised expressed genes, confirmed that the low temperature
stress on the stress of Brassica napus L. were used mainly by inhibition of photosynthesis
and light reaction system. Further research is needed on the molecular mechanism of
candidate genes.

Conclusions

Through the analysis of the photosynthetic parameters of Brassica napus L. under low
temperature, the results showed that the photosynthesis was inhibition at low
temperature stress, and the strong cold resistance varieties is suppressed to a lesser
degree. By RNA - Seq analysis found that the response of low temperature stress
significantly photosynthesis pathway for Photosynthesis and Photosynthesis-antenna
proteins, got 64 significantly DEGs. By analyzing qPCR, verified the reliability of the
transcriptome and determine the 17 low temperature response of photosynthesis
regulation genes.
Methods

Materials

17 NS, a strong cold winter variety of Brassica napus strains and professor Liu of Gansu Agricultural University breeding from "Tiangan" of Brassica napus L. as the female parent and "Longyou 7" of Brassica rapa L.as the male parent to selected homozygous line, and the cold sensitive material NF24 is an inbred line of Brassica napus L. which both have been undertook the formal identification by professor Liu in my study.

The aim

In order to reveal the molecular mechanism of rapeseed's adaptation to low temperature stress, RNA Sequencing (RNA-seq) technique is utilized to compare the transcription groups of two different cold-resistant rapeseed leaves responding to low temperature at the seedling stage, analyze the photosynthetic characteristics of rapeseed subjected to low temperature stress, and identify the related genes for low temperature induction in rapeseed leaves, by the determination of photosynthetic parameters and the RNS-seq analysis of Illumina HiSeqTM platform, the KEGG enrichment pathway associated with photosynthetic characteristics was identified under low temperature stress, and the (DEGs) in the significant enrichment pathway were Expressed by real-time quantitative PCR, to verify the reliability of the RNA-Seq results.

The design

By pot experiments, the seeds with the same clean and uniform size were placed in a petri dish with two layers of filter paper as the germination bed, cultured in an artificial climate chamber (6000 lux light 25 ° C / 14 h and 20 ° C / 10 h) , and then sown on mix nutrient matrix and vermiculite in a 3:1 pot after seed germination, artificial light incubator
culture, light 25 °C / 14 h and 20 °C / 10 h, relative humidity 40%. When the seedlings grow to 5~6 leaf stage, the low temperature -4 °C light incubator starts low temperature treatment for 0h and 24h, two pots of which are used for photographing, two pots are used for photometry, and the other two are used for sampling. Wearing a mask and sterile enzyme-free gloves collects photographs and measures photosynthetic parameter, and liquid nitrogen quick freeze the newly-fully-expanded leaves after rinsing with sterilized up-water and stored in a -70°C ultra-low temperature freezer for determination of various indicators.

**Determination of photosynthesis index**

Six pieces leaves without shade, no damage, no pests, and good growth of the fourth functional leaf were selected for each material, and the small-label was placed. According to the method of Liu Zigang[32], the LI-6400 portable photosynthetic apparatus produced in the United States was used. The changes of photosynthetic gas exchange parameters (Gs, Ci, Tr, Pn) of new leaves that were fully developed at 0h, 12h and 24h after low temperature treatment were repeated three times, setting the light intensity to 1200μmol/m², and the indoor temperature of the leaves was 20°C.

**Determination of chlorophyll content and relative conductivit**

The chlorophyll content and relative conductivity were measured according to the scholar[33].

**Transmission electron microscope**

On the new leaves which were completely unfolded at 0 h and 24 h after low temperature
treatment, a rectangular blade of 2 mm² was cut out with a blade carefully, and placed in a fixing solution containing 2 ml of 3% glutaraldehyde. According to the scanning procedure of electron microscopy, PB was rinsed three times, 15 min/time, hungry acid was fixed at 4 °C for 2 h, and then rinsed with PB three times, 15 min/time, according to 50%, 70%, 80%, 90%, 100%, acetone I and Acetone II gradient dehydration for 10 min each time. Acetone and resin embedding agent were embedded in an oven at 35 °C for 12 h, and then transferred to an oven at 60 °C until the material was completely polymerized. With the ultramicrotome, cutting into slices and fixing on a copper grid with 2% uranyl acetate and lead citrate for double staining and photographs were taken with a Hitachi JEM-1230 projection electron microscope.

Analysis of transcriptome data

The transcriptome analysis was carried out using cold-tolerant variety 17NS and low-temperature sensitive variety NF24. The low temperature stress of -4 °C was used as the control, and the low temperature stress of -4 °C was used as the treatment for 24 hours. Two varieties were treated at two different times, a total of four samples. Total RNA was extracted from the sample by the company named GENE DENOVO, the mRNA was enriched with magnetic beads with Oligo (dT), and the fragmentation buffer was added to the obtained mRNA to make a fragment. As a short fragment, the first strand of cDNA was synthesized with random hexamers using the mRNA after fragmentation, and the second strand of cDNA was synthesized by adding buffer, dNTPs, RNase H and DNA polymerase I, after QiaQuick The PCR kit was purified and eluted with EB buffer, the end of the repair, base A was added, the sequencing linker was added, and the fragment of interest was recovered by agarose gel electrophoresis, and PCR amplification was performed to complete the whole library preparation work. A good library was sequenced using Illumina
HiSeq™. After filtering the off-data, the clean data is compared, the reads are aligned to the reference genome, and the transcripts are assembled using Cufflinks to obtain known transcripts and new transcripts. Then, the obtained gene was analyzed and statistically analyzed, and differential expression analysis and functional enrichment analysis were performed. In addition, structural analysis of genes, including genetic structure optimization, variable shear, and the like.

Total RNA extraction from leaves

RNA extraction The total RNA of 17NS-0h, 17NS-24h, NF24-0h and NF24-24h was extracted according to the instructions of Tiangen kit (DP419). The reverse transcription was reversed according to the TaKaRa cDNA First-strand Synthesis Kit (RR036A). Single-stranded cDNA was obtained by transcription, and the concentration was measured by Bio Mater 5 ultra-micro UV spectrophotometer, and then stored in a refrigerator at -80 °C for use.

Real time PCR

Using the PrimeScript RT reagent Kit (Dalian Bioengineering Co., Ltd., Dalian), reverse transcription kit to reverse transcribe the RNA extracted from the previous step to synthesize the first strand of cDNA. According to the transcriptome data, the reverse transcription product was diluted 100-fold as a template, primers were designed with Primer Premier 5.0(Table 3), the internal reference was Bnactin, and the primer was synthesized by Shanghai Biotech Co., Ltd., using SYBR Premix Ex Taq (Bao Bioengineering Co., Ltd., Dalian) Quantitative kit, two-step amplification, each reaction is set to 3 repeats, the reaction system and procedures are shown in Table 4, according to the
amplification efficiency $E$ of the gene, the correction $2^{\Delta \Delta \text{Ct}}$ is calculated as $(1+E)^{\Delta \Delta \text{Ct}}$
relative quantification As a result, statistical analysis was performed using Excel 2010 and SPSS 22.

Thermal cycle program 95°C 30 sec predegeneration

PCR amplification 95°C 5sec degeneration 60°C 30sec annealing Total 40 cycles,
solubility curve 95°C 15sec 60°C 30 sec 95°C 15sec

Abbreviations

DEGs: Differential Expressed Genes

Pn: Photosynthetic rate

Gs: Stomatal conductance

Tr: Transpiration rate

Ci: Increased the intercellular CO$_2$ concentration

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Author Contributions
JJ interpreted conceptualization, data curation, writing - original draft and editing. LZ made formal analysis, done funding acquisition and supervision. ZY, MW, XM, XC, and JJ participated in test design and operation. LX, SW, WJ, FY, and LL provided methodology and software. All authors read and approved the final manuscript.

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Tables

Table 1 All significant differentially expressed genes identified by RNA-Seq

| Paired comparison     | Significantly up-regulated differential genes | Significantly down-regulated differential genes | Significantly different number of genes |
|-----------------------|-----------------------------------------------|-------------------------------------------------|-----------------------------------------|
| NF24 0h-NF24 24h      | 12583                                         | 24414                                           | 36997                                   |
| 17NS 0h-17NS 24h      | 11710                                         | 23141                                           | 34851                                   |
| NF24 0h-17NS 0h       | 10708                                         | 8270                                            | 18978                                   |
| NF24 24h-17NS 24h     | 11447                                         | 10572                                           | 22019                                   |

Table 2 Genetic information of 17 differentially expressed genes

| Gene ID       | Description                                                                 |
|---------------|------------------------------------------------------------------------------|
| ncbi_106366341| ferredoxin--NADP reductase, leaf isozyme 1, chloroplastic-like               |
| ncbi_106360470| photosystem I reaction center subunit VI, chloroplastic-like                 |
| ncbi_106400646| oxygen-evolving enhancer protein 2, chloroplastic                            |
| ncbi_106430073| ATP synthase gamma chain 1, chloroplastic-like                               |
| ncbi_106381576| ATP synthase gamma chain 1, chloroplastic-like                               |
| ncbi_106405529| photosystem I reaction center subunit VI, chloroplastic-like                 |
| ncbi_106401683| photosystem II repair protein PSB27-H1, chloroplastic                        |
| ncbi_106350097| ferredoxin-1, chloroplastic                                                  |
| ncbi_106371874| photosystem I chlorophyll a/b-binding protein 5, chloroplastic-like         |
| ncbi_106382137| chlorophyll a-b binding protein 2.4, chloroplastic-like                     |
| ncbi_106439795| chlorophyll a-b binding protein 2.1, chloroplastic                          |
| ncbi_106349237| chlorophyll a-b binding protein 2.4, chloroplastic-like                     |
| ncbi_106396209| chlorophyll a-b binding protein 1, chloroplastic                            |
| ncbi_106447496| chlorophyll a-b binding protein 1, chloroplastic                            |
| ncbi_106447497| chlorophyll a-b binding protein 1, chloroplastic-like                       |
| ncbi_106421061| photosystem I chlorophyll a/b-binding protein 3-1, chloroplastic            |
| ncbi_106440450| chlorophyll a-b binding protein CP26, chloroplastic-like                    |
Table 3 qRT-PCR identification primers used in this study
| KEGG pathway       | Gene ID       | Sequence of primer (5’-3’)                          | Amplification length (bp) | Amplification efficiency |
|-------------------|--------------|------------------------------------------------------|---------------------------|--------------------------|
| Photosynthesis    | ncbi_106366341 | AGATCCTAATGCCACCACCATCATC (F)                       | 100                       | 101.1%                   |
|                   |              | GTAGTCCTCATGCTCCTCAAAAG (R)                         |                           |                          |
|                   | ncbi_106360470 | CTCCTTACAAACCACTTCCAGAG (F)                         | 100                       | 96.8%                    |
|                   |              | GAGCCACCTCAAGAATCAA (R)                             |                           |                          |
|                   | ncbi_106400646 | CTACCGACAAGAAGTCCATCAG (F)                          | 102                       | 88.2%                    |
|                   |              | AGGCAGTCTCACGCAAGTA (R)                             |                           |                          |
|                   | ncbi_106430073 | GCAGAGGCAAGGATCAAAAG (F)                            | 99                        | 93.1%                    |
|                   |              | GTAAGGACGACGGAAATTAAG (R)                           |                           |                          |
|                   | ncbi_106381576 | GCAGAGGCAAGGATCAAAAG (F)                            | 99                        | 100.1%                   |
|                   |              | GTAAGGACGACGGAAATTAAG (R)                           |                           |                          |
|                   | ncbi_106401683 | CCGAGATTTGCTTCTCCTATCC (F)                          | 119                       | 103.1%                   |
|                   |              | CCGACGTATCTTCTACGTAATCC (R)                         |                           |                          |
|                   | ncbi_106405529 | CTCTTACAACCACTTCAGAG (F)                            | 100                       | 95.0%                    |
|                   |              | GAGCCTCCTCAAGAATCAA (R)                             |                           |                          |
|                   | ncbi_106350097 | CATACAACAGATGGAGAAGG (F)                            | 149                       | 102.1%                   |
|                   |              | CAACCGAGGCCAGAGATTTT (R)                            |                           |                          |
| Photosynthesis-antenna proteins | ncbi_106371874 | TAGCTGGAGATTACGGGGT (F)                             | 143                       | 122.1%                   |
|                   |              | GGTACGAGTAGGCTGGHTAAG (R)                           |                           |                          |
|                   | ncbi_106382137 | CTCGGAAACCCTAACTTGAATCC (F)                         | 100                       | 101.1%                   |
|                   |              | CACCTCCTATCTGTCACCTT (R)                            |                           |                          |
|                   | ncbi_106439795 | AACCGTGAGCTCGAAGTAATCC (F)                          | 100                       | 98.0%                    |
|                   |              | CTCGAAATTGTGATCCGTTCTT (R)                          |                           |                          |
|                   | ncbi_106349237 | AACCGTGAGCTCGAAGTAATCC (F)                          | 131                       | 102.1%                   |
|                   |              | ATCTGAGAACCCTCGTTGAAC (R)                           |                           |                          |
|                   | ncbi_106396209 | GAAGGTTGGGCTCAGACTATTT (F)                          | 112                       | 94.2%                    |
|                   |              | CGACTCTGTAACCCTCAGAG (R)                            |                           |                          |
|                   | ncbi_106447496 | GAAGGAGGCCTGTCACTTTT (F)                            | 99                        | 103.1%                   |
|                   |              | CTCAACAGGCTCCATCAGAAT (R)                           |                           |                          |
|                   | ncbi_106447497 | AAACGGAAGTTGTCGATGT (F)                             | 96                        | 100.1%                   |
|                   |              | GCCAAATGGTCGACAGATTC (R)                            |                           |                          |
|                   | ncbi_106421061 | CTGTCAGCAAGGAGCAAAC (F)                             | 102                       | 97.3%                    |
|                   |              | CAAGAGGGTCGAATCCATAGTC (R)                          |                           |                          |
|                   | ncbi_106440450 | AACCTTTTCTCTGCTGTAGTTT (F)                          | 98                        | 93.5%                    |
|                   |              | GGGTGTCGCTTCCCTCAAA (R)                             |                           |                          |
| Reference gene    | Bnactin-F     | TCCATCCATCGTCCTCAACAG                                |                           | 106.1%                   |
|                   | Bnactin-R     | GCATCATCAAGCATCCTT                                  |                           |                          |
F: Former primer
R: Reverse primer.

### Table 4 qPCR reaction system

| Reagent                              | Volume |
|--------------------------------------|--------|
| SYBR® Premix Ex Taq TM II            | 10μl   |
| Forward primer                       | 0.8μl  |
| Reverse primer                       | 0.8μl  |
| ROX Reference Dye II                 | 0.4μl  |
| Template cDNA                        | 2μl    |
| ddH₂O                                | 6μl    |

### Figures

Figure 1

Morphological were observed of Brassica napus L. under low temperature stress.

(a)17NS: -4°C- Treatment-0h; (b)17NS: -4°C-Treatment-24h; (c)17NS: -4°C-Treatment-24h-Recovery-24h; (d)NF24: -4°C-Treatment-0h; (e)NF24: -4°C-Treatment-24h; (f)NF24: -4°C-Treatment-24h-Recovery-24h
Figure 2

Transmission electron microscopic view of Brassica napus L. under low temperature stress
Figure 3

The influence of low temperature to conductivity in rapeseed leaves. Note: The figure with $P<0.01$ as highly significant, marking "A, B or C", as the same below.
Figure 4

Changes in photosynthetic characteristics of different cold-tolerant varieties under low temperature stress. (a): Intercellular CO2 concentration (µmol/mol); (b): Transpiration rate (mol/m²/s); (c): Stomatal conductance (mmol/m²/s); (d): Photosynthetic rate (µmol/m²/s).
Figure 5

The influence of low temperature to Chlorophyll content in rapeseed leaves
Figure 6

Branch of differential gene expression in Brassica napus under low temperature stress (TOP20). Note: The larger the Rich Factor, the higher the degree of enrichment. Q-Value is the P-Value after multiple hypothesis test corrections, ranging from 0 to 1, the closer to zero, the more significant the enrichment. The figure is plotted using the Q-value from small to large to sort the top 20 paths.

(a): NF24t0-vs-17NS0t; (b): NF24t-vs-17NS0t.
Figure 7

Venny diagram of differentially expressed genes in photosynthetic regulatory pathways (a): Photosynthesis; (b): Photosynthesis - antenna proteins.

Figure 8

Photosynthesis – Differentially induced genes in the antenna proteins pathway
Figure 9

Heat map of specific expression genes in the photosynthetically related significant enrichment pathway of Brassica napus L.
Number of differentially expressed genes in photosynthetic pathways significantly enriched in Brassica napus under low temperature stress.
Figure 11

Go classification of differentially expressed genes in cold stress response of Brassica napus L. (a): Photosynthesis; (b): Photosynthesis - antenna proteins
Validaton of 17 differentially expressed genes by qRT-PCR