

RESEARCH ARTICLE

Insight into the effect of low temperature treatment on trichome density and related differentially expressed genes in Chinese cabbage

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Abstract

Trichome is important for help plant resist adversity and external damage. However, it often affects the appearance and taste of vegetables. In the present study, the trichome density of leaves from two Chinese cabbage cultivars with and without trichomes treated at low temperature are analyzed by biological microscope, and the differentially expressed genes related to trichomes formation were screened through transcriptome sequencing. The results showed that the number of leaves trichomes was reduced by 34.7% at low temperature compared with room temperature. A total of 661 differentially expression genes effecting trichomes formation were identified at the CT vs C, LCT vs LC, CT vs LCT. Several differentially expression genes from every comparison group were enriched in plant hormone signal transduction and amino acid biosynthesis pathway. Combined with the central genes obtained by WGCNA analysis, five candidate genes *Bra029778*, *Bra026393*, *Bra030270*, *Bra037264* and *Bra009655* were screened. qRT-PCR analysis verified that the gene expression differences were in line with the trend of transcriptome data. This study not only found possible new key genes and laid a foundation for revealing the molecular mechanism regulating the formation of trichome in Chinese cabbage, but also provided a new way to study plant surface trichomes.

Introduction

Trichomes are special structures on the surfaces of plants derived from epidermal cells [1, 2], and its density and size are determined by the stage and location of growth and development in plants [3]. Trichomes not only can secrete some lipids or secondary metabolites, such as changing the body and feces odor of feeding insect larvae to attract ants to prey on

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them [4, 5], but secrete some volatile organic compounds to attract parasitic wasps and other insect natural enemies to prey on pests. Furthermore, trichomes play a crucial role in defending against pests or bacteria [6]. It is often an important factor in response to drought, water, salt, high temperature and other abiotic stresses [7–9]. For example, the total glandular hair density on both sides of leaves of *schizonepeta tenuifoliabriq chinensis* was significantly increased in response to salt stress [10], and the reduction of steam pressure difference on leaf surface resulted in the decrease of glandular hair density on leaves of Birch [11]. In addition, the trichome secretions are used as raw materials for spices, medicines, food additives, resins and essential oils [12, 13], which have important ecological and economic value to human beings.

Chinese cabbage is an important vegetable in Cruciferae, rich in vitamins, crude fiber, carotene and other nutrients. It is the vegetable with the largest cultivation area, the most daily consumption and the most popular among consumers in China. Chinese cabbage can be divided into trichomes and without trichomes types. Although Chinese cabbage trichomes play an obvious role in increasing leaf thickness to reduce heat of epidermis, loss of water, and defense against insect pathogen invasion and mechanical injury [14, 15], it also affects the consumption quality, and sometimes even cause stabbing injury to human body. Therefore, how to remove trichomes or regulate the formation of trichomes is extremely important for fruit and vegetables breeding. It was found that simulated summer heating increased the number of glandular hairs of *Empetrum nigrum* [16]. The decrease of temperature reduced the density of glandular hairs in the leaves of *Origanum vulgare* [17]. Hormone treatment showed that GA₃ and MeJA could significantly increase the number and density of tomato (*Solanum lycopersicum*) and *Arabidopsis* trichome [18, 19], and ethylene treatment increased epidermal branching in cucumber [20], while SA treatment inhibited epidermal branching in *Arabidopsis thaliana*. It was found that jasmonate promoted the development of *Arabidopsis thaliana* epidermis by inhibiting the interaction between jasmonate ZIM protein and GL1 and EGL3/GL3 in the transcriptional regulatory complex [21–23]. The transcription factor MYB23 gene was involved in controlling trichome branching and leaf edge trichome initiation [24–26]. Moreover, gibberellin, cytokinin and ethylene controlled the formation of epidermal and root hair cells in *Arabidopsis thaliana* through GIS family and subfamily genes [27].

Li located *BraGL1* gene controlling leaf trichome in Chinese cabbage and found that the DNA-binding domain of *Brassica GL1* was highly homologous with *Arabidopsis thaliana* [28, 29]. A gene located on chromosome 6 of Chinese cabbage was found functionally complementary to the *ttgll* mutant of *Arabidopsis thaliana* and affected the formation of trichomes [30]. The major gene located on A09 through CAPS markers and RAD-seq, contributed 78% to the number of trichomes in Chinese cabbage leaves [31]. The *BraGL1* gene in Chinese cabbage and showed that *BraGL1* was the cause of the absence of trichomes in Chinese cabbage. Furthermore, 266 genes including *GL3*, *EGL1*, *SAD2* genes and *WRKY*, *MYB*, *NAC* transcription factors that might be related to the development of leaf trichomes were screened from the differential expression profiles of F₂ generation trichome leaves without trichomes and with trichomes [32]. The number of candidate genes that affect the density of Chinese cabbage trichomes is still limited, especially the mechanism of low temperature inhibiting the formation of Chinese cabbage trichomes remains elusive.

The purpose of this study is to investigate the phenotypic changes and molecular mechanisms of leaf trichomes in Chinese cabbage in response to low temperature, and to explore a new pathway for the regulation of plant trichomes.

Materials and methods

Materials

The materials used for phenotypic and transcriptomic determination were Chuntai Chinese cabbage (CT, trichome) (purchased from Guangdong Superior Seed Import Service Company) and Chaozhou Kuai Da Xia Huang Bai (C, without trichome) (purchased from Shantou Jinxuan Seed Industry Company). CT and C are treated at room temperature, while Low temperature treatment with trichomes is denoted as LCT, low temperature treatment without trichomes is denoted as LC.

Plant culture

The full seeds were selected and placed in a petri dish covered with two layers of filter paper. The filter paper was soaked with distilled water and germinated in a light incubator at $22\pm 1^\circ\text{C}$. After germinating and whitening, the seeds are moved to a 7×7 cm pot and placed in a light incubator simulated room temperature (temperature: $27/21^\circ\text{C}$) when growing to 4 true leaves, 20 seedlings with the same size and growth trend were selected and transferred to a low temperature ($15/9^\circ\text{C}$) incubator in advance for low temperature treatment. Same number of seedlings were treated at room temperature. All plants are placed under the condition of 16 h/8 h light cycle and 6000 Lx light intensity.

Sampling and data analysis

After 10 days of treatment at low and room temperature, 3 Chinese cabbage plants were taken from each treatment and 3 leaves were selected from top to bottom. 3 holes were drilled in each leaf with a 9 mm hole punch and 3 fields were selected for each hole. The data were observed and recorded under a 10×10 biological microscope. Excel 2016, GraphPad Prism 5, Adobe Photoshop were used for data collation and mapping, and IBM SPSS Statistics 20 was used for significance test (Duncan, $P < 0.05$). At the same time, 0.5 g of the expanded third leaf of each plant was collected for three biological repeats for transcriptome detection.

RNA extraction and library construction

Total RNA was extracted from Chinese cabbage leaves, and the purity and integrity of sample RNA were detected. Now, all Raw data obtained by sequencing have been submitted to NCBI, SRA: PRJNA842790. All clean data were calculated using Q20 and Q30, and clean reads were sequentially compared with the reference genome using HISAT2 V2.0.5 to obtain location information on the reference genome and sequence characteristic information of the sequenced samples. Only perfectly matched reads are used for further analysis and annotation.

Differentially expressed genes (DEGs)

Differential genes were identified using FPKM (Fragments per kilobase per million reads) and DESeq2 software (1.20.0) was used for differential expression analysis between the two comparison combinations. Adjust the P value (padj) | $\log^2\text{FoldChange}$ | threshold for significantly differentially expressed. In order to comprehensively obtain the functional information of differential genes, gene function annotations were carried out through the database, including GO and KEGG. GO and KEGG enrichment analysis of differentially expressed genes were realized by software.

Construction and visualization of gene network

The R package WGCNA was used to calculate the functional set of weighted association analysis, network construction, gene screening. The co-expression network was visualized using Cytoscape (v.3.7.1) software.

qRT-PCR was used to verify the differential genes

The extracted RNA was reverse transcribed using HiScript III All-in-one RT Super Mix Perfect for qPCR kit. qRT-PCR was performed using ChamQ Universal SYBR qPCR Master Mix kit and Quant Studio 3 Real-time Quantitative PCR System (ABI, USA) instrument. The relative expression level of genes was calculated by $2^{-\Delta\Delta C_t}$ method, and Actin was selected as an internal reference gene. Differential genes were selected and gene-specific primers are shown in Table 1, were designed using Primer V5.0, which were synthesized by Shenggong Bioengineering (Shanghai).

Results

Influence of low temperature treatment on trichome density of Chinese cabbage

The density of trichomes on the adaxial side, abaxial side and edge of leaves of the Chinese cabbage varieties after low-temperature treatment were significantly reduced, among which, the number of trichomes on the abaxial side of leaves was significantly reduced by 53.6%, and the total number of trichomes was 65.3% of room temperature treatment on the three fields (Fig 1A and 1C). The hairless variety C was still hairless before and after low temperature treatment (Fig 1A and 1B).

Quality control of sequencing data

Refer to Brapa_sequence_V1.0. fasta database in Ensemble Plants to generate raw data in FASTQ format for this sequencing. The mismatch rate of experimental materials is low. The data volume of bases is between 6.1 and 7.3 G, and the proportion of Q20 is above 96%. The base recognition accuracy of Q30 is above 91%, and the GC content is above 45% (Table 2).

The position of all reads aligned to the reference genome was statistically analyzed. In C, 78.86% of reads were aligned to exons, 1.83% to introns, and 19.32% to gene spacer sequences. In CT, 73.8% of reads were aligned to exons, 2.49% to introns, and 23.71% to gene intervals. In LC, 85.7% of reads were aligned to exons, 1.72% to introns, and 12.58% to gene intervals. In LCT, 85.58% of reads were aligned to exons, 1.45% to introns, and 12.96% to gene intervals (Fig 2).

Table 1. qRT-PCR primer.

Gene ID	Forward (5' to 3')	Reverse (5' to 3')
<i>Bra023654</i>	GCAACAAGCAAAAAGAGCAACC	GCTTGAAATGAAGGTGGCTC
<i>Bra039762</i>	TCAAGTCATACCCGACCAAGAT	CGACTTTCCTCATCGCACC
<i>Bra003253</i>	GTGGACAAACTACCGTTGGAAT	TCCTCAACTTACGCTTACGAACT
<i>Bra029311</i>	GGCGAGTTTAGTTCAGAGGAGG	TGCGTGGTGGTGACAGTAGG
<i>Bra035148</i>	TCCGAGATGCTAACTTTGATGTG	CAATAACCGCTGAACTAAGTCT
<i>Bra018529</i>	GCGACGGTTCCTTGATTGT	GAACTTGGCGAAAGAAGCAGC
<i>Bra000809</i>	CGGTTCCCTTTCCTCGTTCA	CCAAAGGCAGCAAAGTGAA
<i>Bra025589</i>	TCCTCGGCAACAGATGGTC	ATCGTCGTCCGTAGTGTCAA
<i>Bra023486</i>	AGGTGGTCCTTGATTGCTAAA	CAATATCACCATTACTCGGCTTG
<i>Actin</i>	ATCTACGAGGGTTATGCT	CCACTGAGGACGATGTTT

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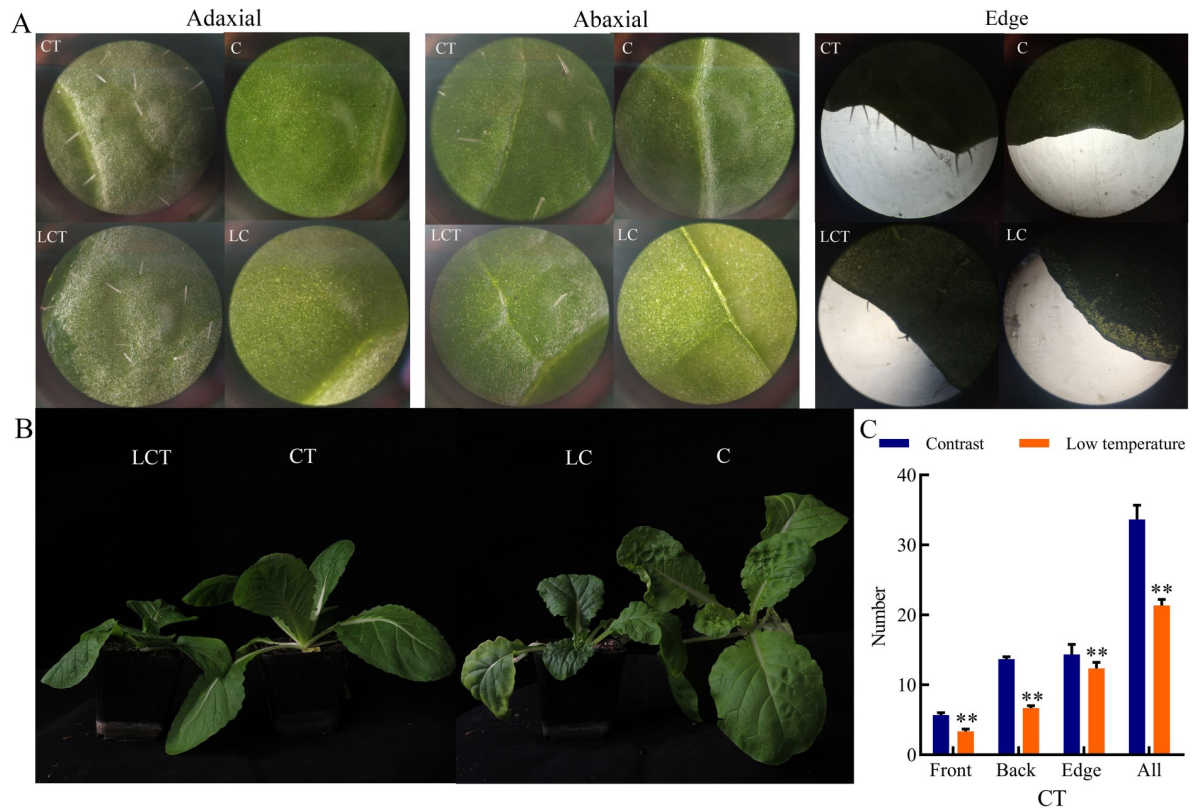


Fig 1. Effect of low temperature treatment on trichomes. A, Three visual fields (adaxial, abaxial, and edge) at room temperature (CT, C) and low temperature (LCT, LC); B, Plants at room temperature (CT, C) and low temperature (LCT, LC); C, Density of trichomes at room temperature (CT) and low temperature (LCT).

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Analysis of related expression levels

DESeq2 software was used to analyze the expression levels of each sample of Chinese cabbage treated at low temperature. It has been seen from the correlation heat map of each treatment and repetition that the sample has good correlation, which can be further analyzed (Fig 3).

Table 2. Statistical analysis of RNA-seq reading segments.

Sample	Raw reads	Clean reads	Mapped rate (%)	GC-count (%)	Q20 (%)	Q30 (%)
C1	50397258	48687520	88.6	46.62	98.13	94.64
C2	44962240	43493470	89.07	47.1	98.07	94.56
C3	46282198	44951976	86.1	46.85	98.2	94.77
CT1	44359098	41517946	88.12	45.65	97.85	94.11
CT2	47881796	47568590	87.52	47.86	96.83	91.8
CT3	46095274	44365122	88.51	45.96	98.07	94.51
LC1	42294282	40674090	90.68	47.27	98.24	94.78
LC2	44381250	42900888	90.56	47.61	98.2	94.67
LC3	47910268	47367088	88.4	47.27	97	91.91
LCT1	48549544	48119596	89.64	47.79	96.76	91.62
LCT2	43172328	41278792	89.94	46.43	98.14	94.78
LCT3	43261326	41226126	91.36	46.48	98.27	94.99

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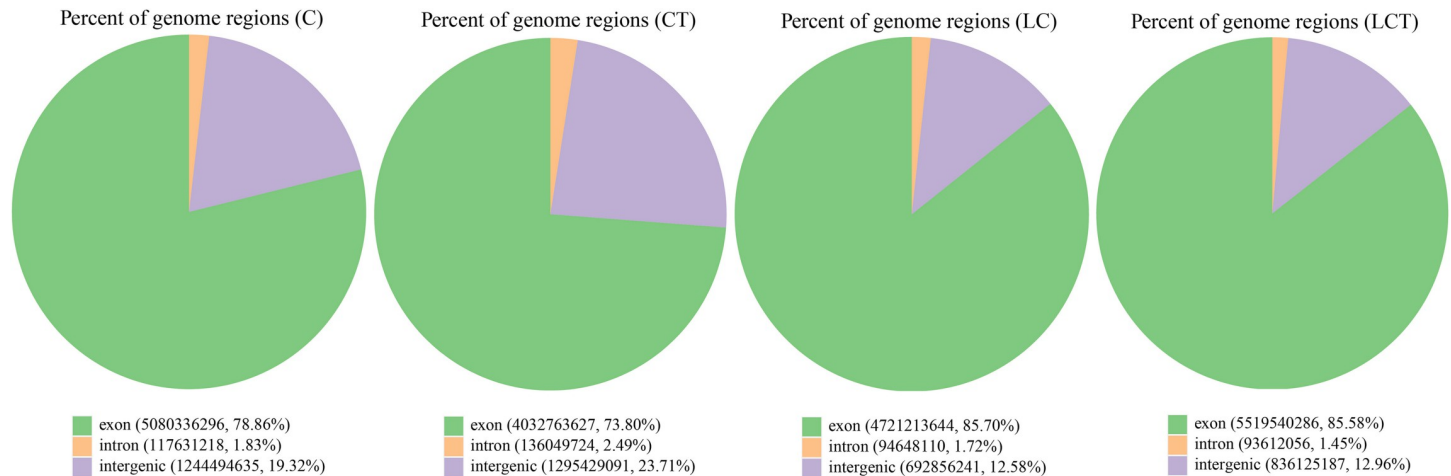


Fig 2. Distribution of reads in different regions of the reference genome.

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Analysis of differentially expressed genes

5684, 5814, 4944, 5310, 5806 and 9831 differentially expressed genes (DEGs) were obtained in CT vs C, LCT vs C, LCT vs CT, LCT vs LC, LC vs C, and LC vs CT respectively (Fig 4). There were 3149 up-regulated and 2535 down-regulated genes in CT vs C, and 2584 up-regulated and 2726 down-regulated genes in LCT vs LC, with 2175 common DEGs between CT vs C and LCT vs LC. 1979 up-regulated and 2965 down-regulated genes were identified in LCT vs CT, and 2761 up-regulated and 3045 down-regulated genes were screened in LC vs C, with 2039 common DEGs between LCT vs CT and LC vs C. Furthermore, it is speculated that 661 shared DEGs in CT vs C, LCT vs LC and CT vs LCT may be the candidate genes for the effect of low temperature on Chinese cabbage trichomes (Fig 5).

GO function analysis

The DEGs were annotated into 1041 GO terms which mainly distributed in structural constituent of ribosome, structural molecule activity and RNA binding in CT vs C. The DEGs screened from LCT vs LC were annotated into 1083 GO terms and mainly distributed in Structural constituent of ribosome, structural molecule activity and hydrolase activity, acting on glycosyl bonds. Furthermore, 1018 GO terms were annotated in CT vs LCT DEGs and also mainly distributed in structural constituent of ribosome, structural molecule activity and RNA binding.

The 661 DEGs screened out between CT vs C, LCT vs LC and CT vs LCT were annotated into 416 GO terms. Among them, the three most widely distributed differential genes in molecular function annotation are ribosomal biosynthesis, riboprotein complex and RNA modification. In the annotation of cell components, the differential genes were mainly distributed in ribosome and riboprotein complex. In the notes of biological processes, they are mainly distributed in structural components of ribosomes, structural molecular activity and RNA binding (Fig 6).

Enrichment analysis of KEGG metabolic pathway

The enrichment results demonstrated that DEGs found in “CT vs C” were enriched in 119 metabolic pathways, among which more DEGs were enriched in plant hormone signal transduction, carbon metabolism, biosynthesis of amino acids, MAPK signaling pathway-plant

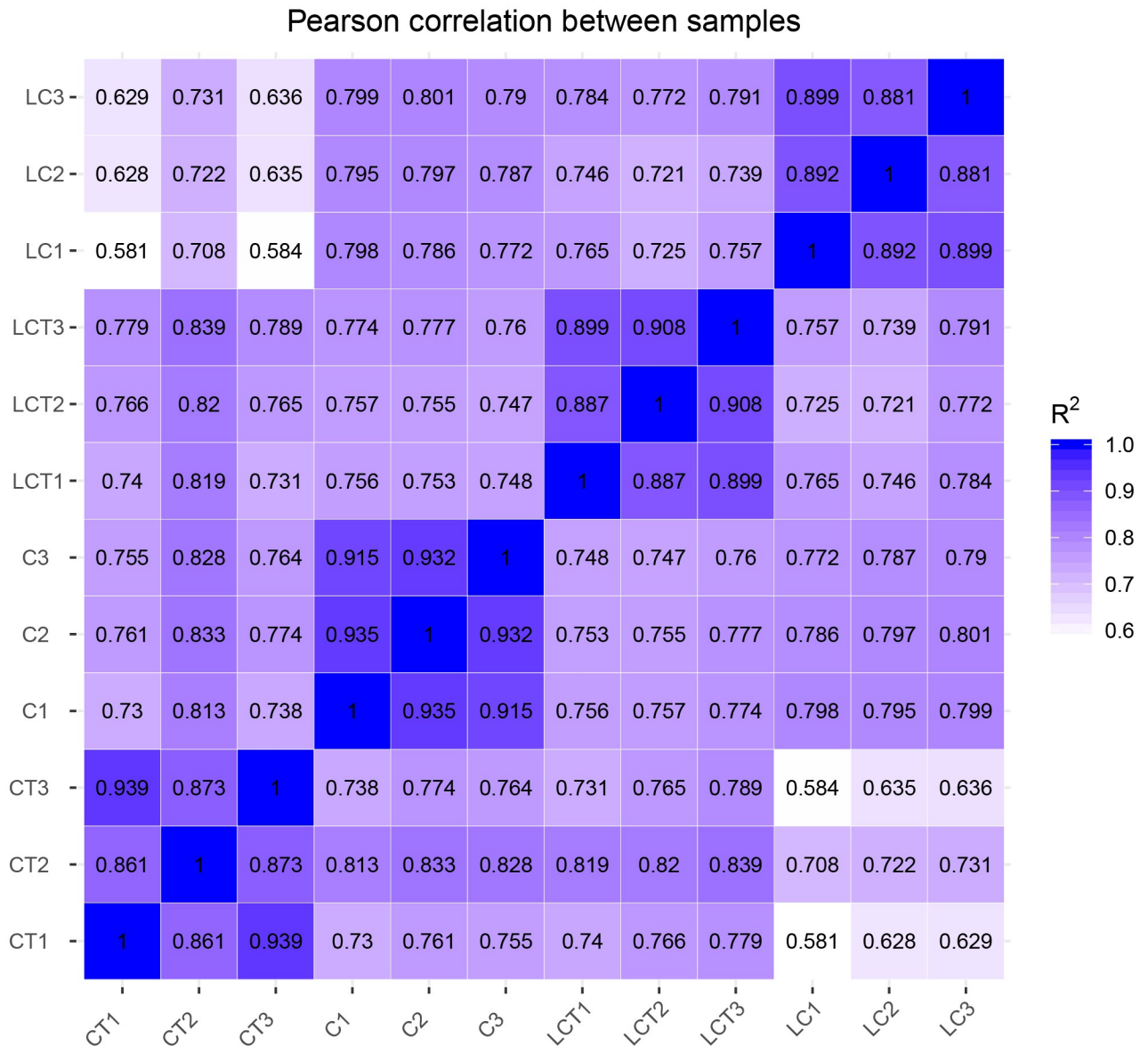


Fig 3. Correlation heat map between samples.

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pathways. And the ribosomal biogenesis and purine metabolism pathways in eukaryotes were significantly enriched. DEGs found in “LCT vs LC” were enriched in 119 metabolic pathways, with more DEGs enriched in biosynthesis of amino acid, carbon metabolism, plant hormone signal transduction, plant-pathogen interaction, 2-oxo-carbonyl acid metabolism pathways. DEGs found in “CT vs LCT” are enriched in 117 metabolic pathways, among which carbon metabolism, RNA transport, plant hormone signal transduction, ribosomal biogenesis in eukaryotes, biosynthesis of amino acid and other pathways are more enriched in DEGs. The ribosomal biogenesis in eukaryotes and photosynthesis—antenna protein pathway was significantly enriched. KEGG analysis indicated that 661 DEGs were enriched in 74 metabolic pathways, among which biosynthesis of amino acid, RNA degradation, ribosomal biogenesis in eukaryotes, carbon metabolism, purine metabolism and other pathways were more enriched.

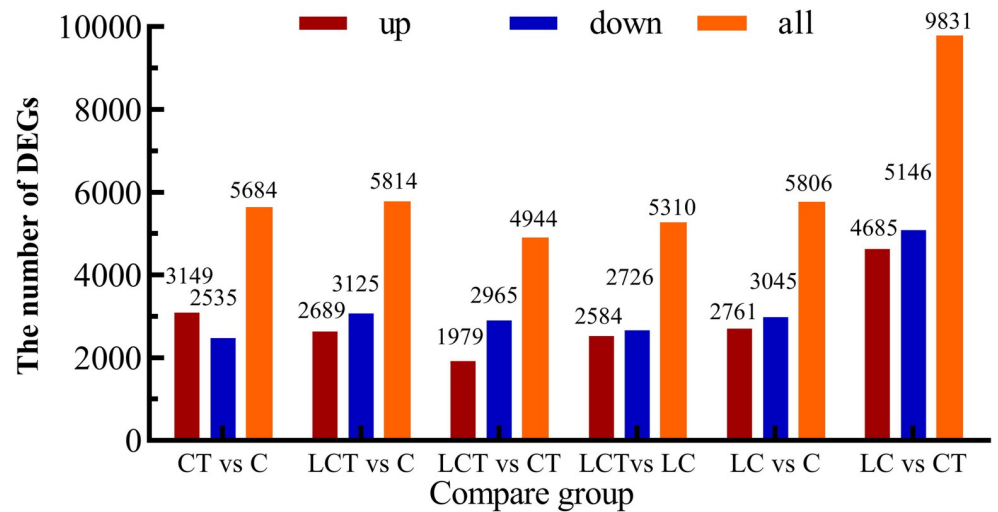


Fig 4. Statistical histogram of the number of genes in different combinations.

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Moreover, ribosomal biogenesis in eukaryotes metabolic pathways were significantly enriched (Fig 7).

Differential gene analysis of plant hormone signal transduction pathways and transcription factors

According to comparison group CT vs C, LCT vs LC, CT vs LCT, KEGG metabolic pathway enrichment analysis and previous studies, the plant hormone signal transduction pathway and some significantly different transcription factors are involved in the response to the trichomes. Among the more significant difference genes, more attention was given to auxin, jasmonic acid, ethylene, gibberellin signal transduction pathway. The results showed that, 7 auxin signal transduction pathways genes including *Bra027504*, *Bra001900*, *Bra011559*, *Bra002120*, *Bra003044*, *Bra006435* and *Bra023654* were screened out of DEGs (Fig 8A), while one gene *Bra007937* appeared in jasmonic acid pathway (Fig 8B), 3 genes *Bra014295*, *Bra036542*, *Bra022115* in ethylene pathway (Fig 8C), 5 genes *Bra039762*, *Bra024875*, *Bra017443*, *Bra000283* and *Bra007430* in gibberellin pathways (Fig 8D). Furthermore, ten differentially expressed new transcription factors including *Bra001588*, *Bra015882*, *Bra029778*, *Bra029311*, *Bra035148*, *Bra018529*, *Bra000809*, *Bra038033*, *Bra004679* and *Bra012910* were obtained in 661 DEGs (Fig 8F).

Analysis of gene weighted co-expression network among different varieties under low temperature treatment

Weighted gene co-expression network analysis (WGCNA) using non-redundant DEGs was performed to investigate the regulatory network of genes related to cold treatment and trichome and 32 modules were performed (Fig 9A). This is a heat map of the correlations between modules for all genes in different modules (Fig 9B). The correlation analysis of module-sample relationship, showed that it was high correlation between modules and modules, among which cyan module is correlated with CT module and pink module is correlated with LCT module (Fig 9C). It was found that seven central hub genes *Bra029778*, *Bra037264*, *Bra002294*, *Bra030270*, *Bra012403*, *Bra026393* and *Bra015780* were noteworthy in cyan module (Figs 9D and 10A). Among the 7 highly connected central genes *Bra011511*, *Bra009655*,

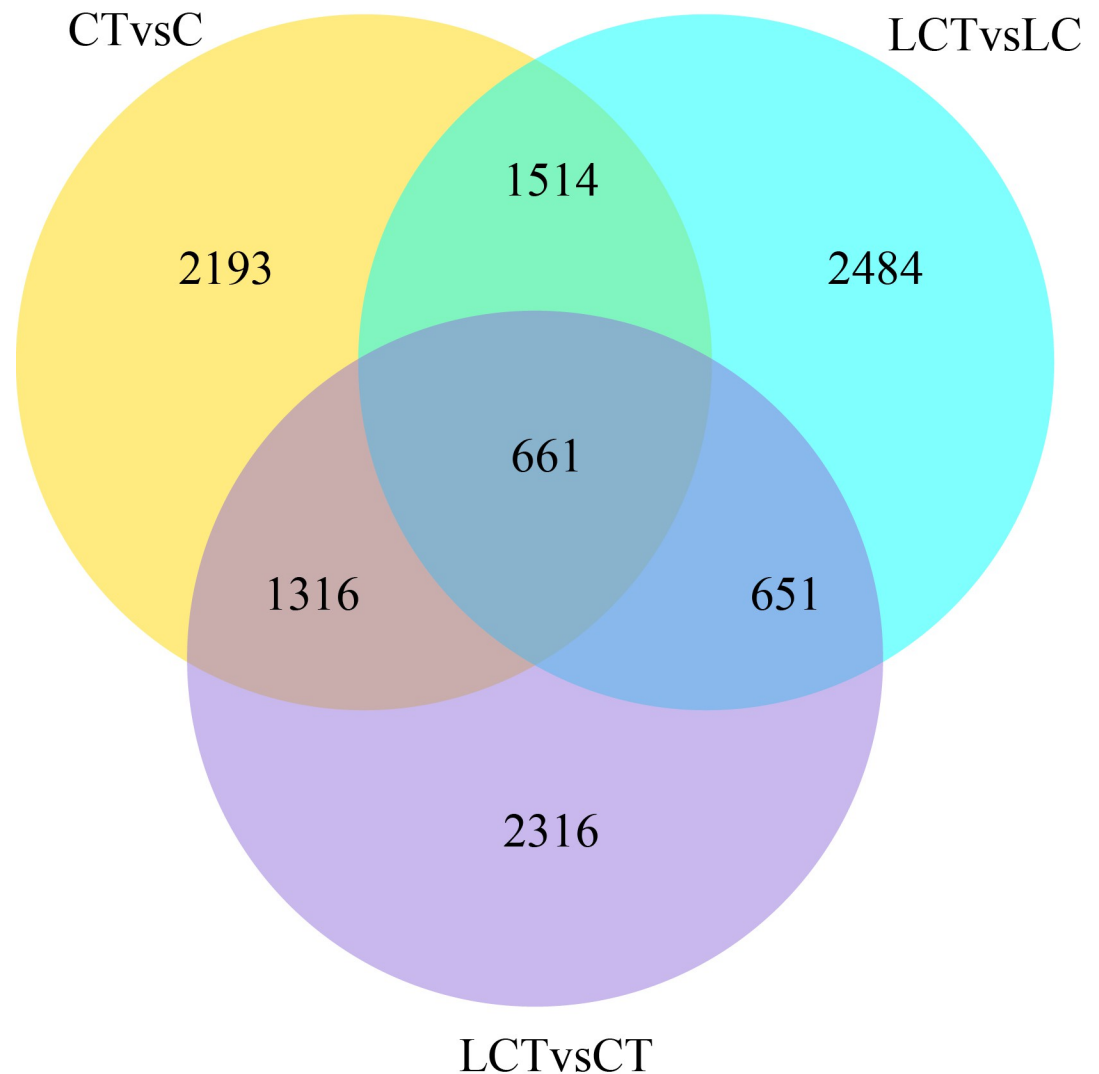


Fig 5. Venn diagram of differentially expressed genes.

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Bra002871, *Bra024095*, *Bra027347*, *Bra035988* and *Bra030635* in pink module, a related network was constructed for analysis (Figs 9E and 10B). It may speculate that these genes regulate the formation of trichomes at low temperature.

qRT-PCR analysis of differentially expressed genes

Nine DEGs closely related to the effect of low temperature on the trichomes were selected for real-time fluorescence quantitative PCR (qRT-PCR) detection (Fig 11). The qRT-PCR expression pattern is consistent with the RNA-seq trend.

Discussion

The density of glandular hairs at young leaves, stem tips and flower buds of many plants was higher than that of mature leaves, stems and flowers [11, 33], and the glandular hair density of the same leaf at different positions of front and back, edge and center was also different, especially represented by tomato and *Artemisia annua*. The external environment has a certain

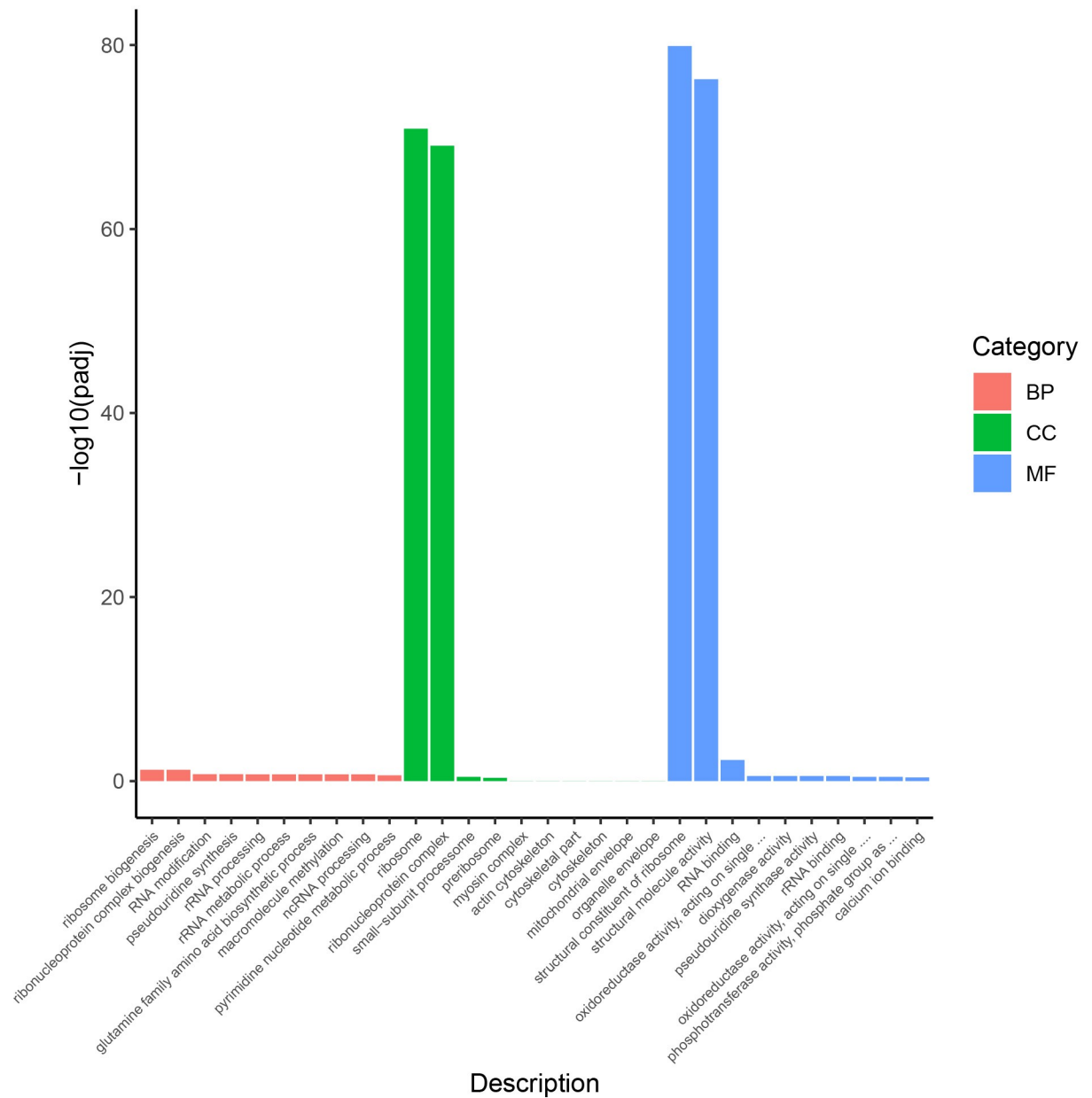


Fig 6. Histogram of differential gene GO enrichment.

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influence on the trichomes of Chinese cabbage. Temperature controls the phenotype by regulating the balance of various metabolic pathways in plants. Previous study reported that the differential DEGs of trichomes were mainly enriched in primary and secondary metabolic biosynthesis pathways [32]. In this study, we found that the number of trichomes on the adaxial, abaxial and edge of leaves was different, there were more trichomes on the abaxial and edge of leaves, which were 2.2 and 2.6 times of that on the adaxial, respectively. Compared with low temperature treatment, the DEGs from three comparison groups were mainly enriched in plant hormone signal transduction and biosynthesis of amino acids pathways. Furthermore, the differential genes were significantly up or down-regulated, and these genes can be involved in trichome formation.

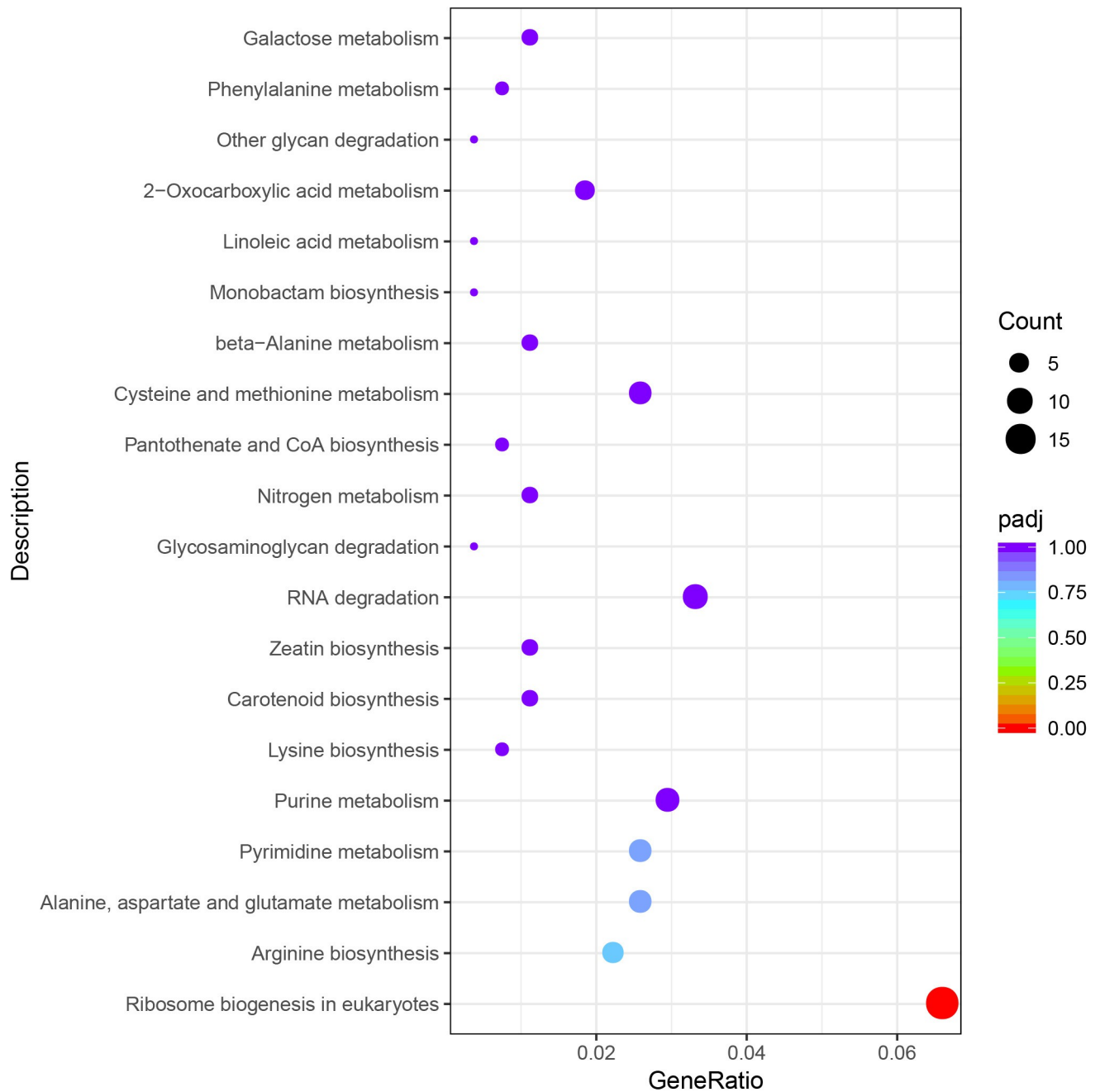


Fig 7. Enrichment bubble diagram of differential gene KEGG.

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Biosynthesis and signal transduction pathways of plant hormones are involved in the development of trichome [34]. Transcriptome analysis showed that auxin, ethylene and cytokinin regulate a set of similar root hair specific genes that control root hair elongation in *Arabidopsis thaliana*. Gibberellin signal transduction controls the accumulation and expression of gibberellin biosynthesis genes to regulate positive regulation of tobacco glandular hair initiation [35]. Spraying gibberellin on the surface of flue-cured tobacco increases the density of leaf glandular hairs [36]. Methyl jasmonate is a signal transduction molecule in plants, and exogenous

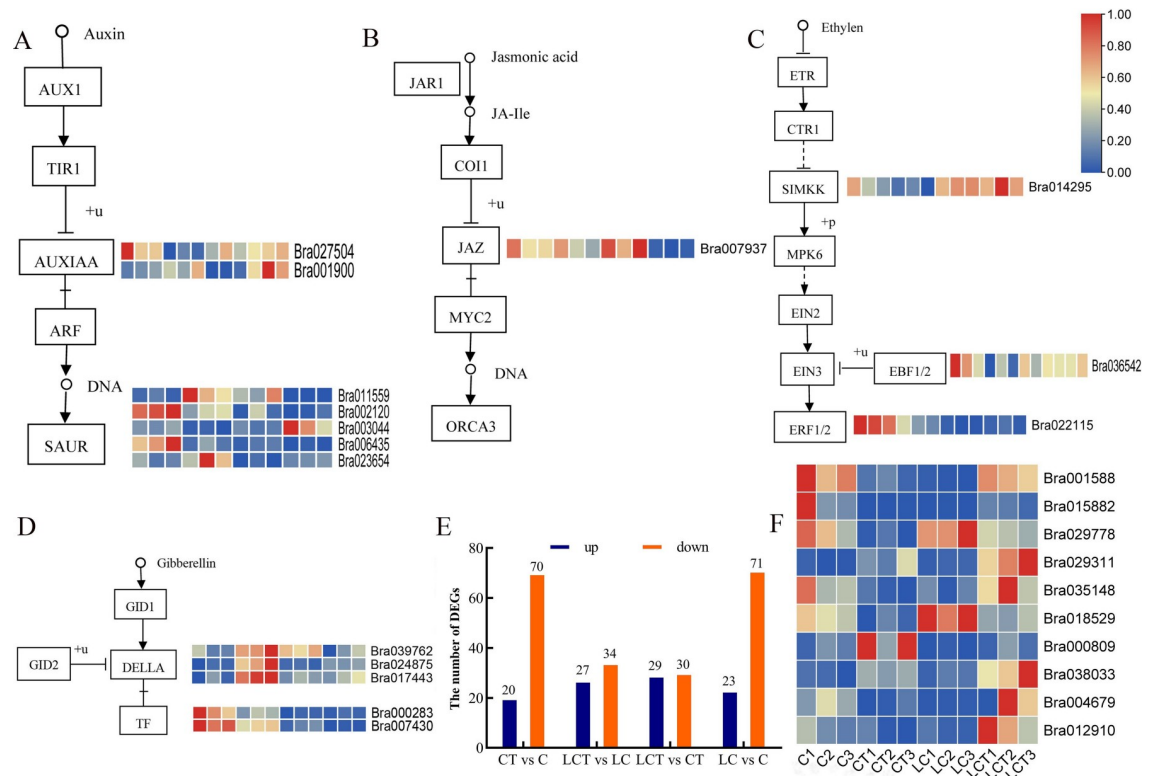


Fig 8. Hormone metabolic pathway and heat map of related transcription factors. A, B, C, D, Schematic diagram of gene expression related to hormone signal transduction pathway; E, Statistical histogram of 661 differential genes; F, Differential transcription factor heatmap.

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application significantly improved the transcription level of key genes in *Camptotheca acuminata* coat synthesis and promoted the significant increase of *Camptotheca acuminata* coat density [37]. However, it has been reported that plant hormones are not directly involved in regulating trichome development, but indirectly regulate by the expression levels of positive or negative regulatory factors [38]. We identified the hormone metabolism pathways were enriched before and after low temperature treatment. At room temperature, 90 DEGs were enriched in the hormone signal transduction pathway of Chinese cabbage with or without trichomes, including 20 up-regulated DEGs and 70 down-regulated DEGs. After low temperature treatment, 105 DEGs were enriched in plant hormone signal transduction pathway, among which 28 DEGs were up-regulated and 77 DEGs were down-regulated. Compared with the trichomes before and after treatment, 59 DEGs were enriched in plant hormone signal transduction pathways, among which 29 DEGs were up-regulated and 30 DEGs were down-regulated. Therefore, this experiment further proved the relationship between burrs and hormones, and screened the hormone genes *Bra039762*, *Bra007937*, *Bra017443*, *Bra24875*, *Bra027504*, *Bra023654* that may affect the formation of trichomes, laying a foundation for improving the molecular mechanism of hormone regulation of trichomes.

Transcription factors are important regulatory factors widely existing, and play a significant role in the regulation of trichome development. R2R3-MYB transcription factor and bHLH-like transcription factor can jointly form a trimer complex activator MYB-bHLH-WD40 [39, 40], directly acting on *GL2/TTG2*, positively regulates the development of *Arabidopsis* trichome [41, 42]. It has been found that R2R3-MYB transcription factor genes, bHLH

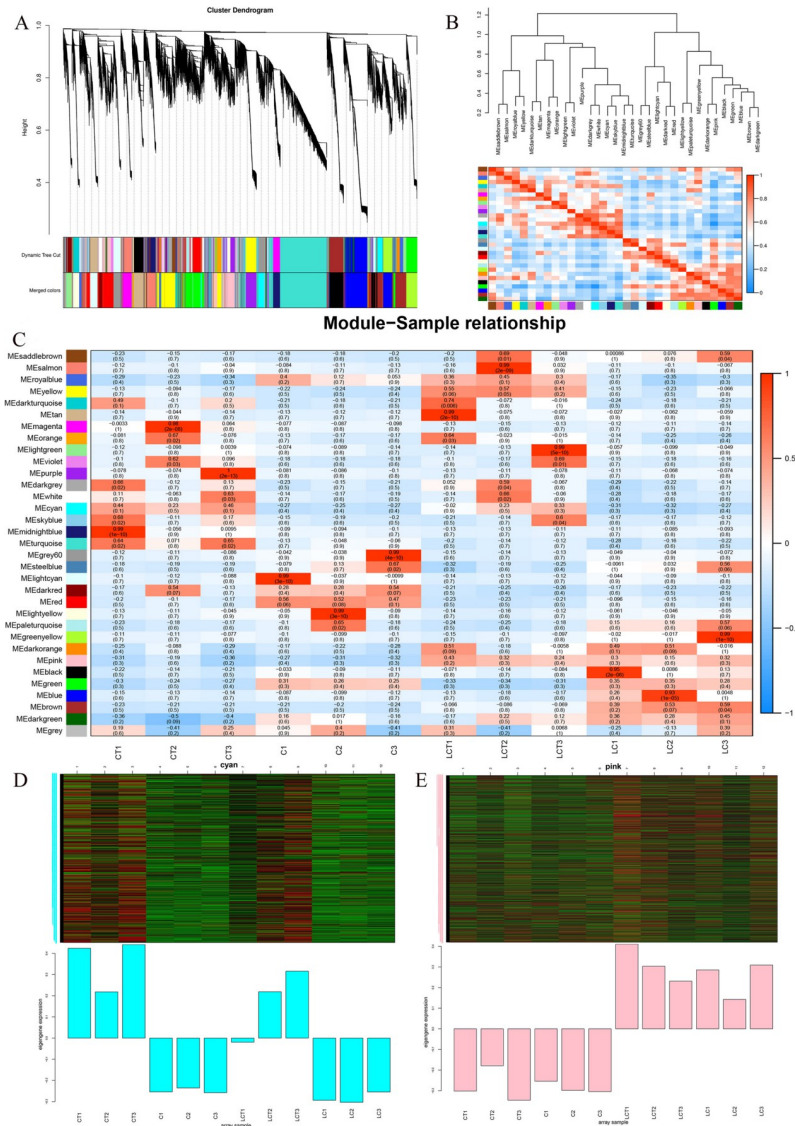


Fig 9. WGCNA analysis. A, Module hierarchy cluster tree diagram; B, Heat map of correlation between modules; C, Sample traits and module-to-module correlation heat map. The horizontal coordinates are samples, the ordinates are modules; D, E, Calorimetric map of gene expression in the module.

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transcription factors and HD-ZIP IV transcription factor genes are involved in the regulation of plant surface trichome development [43–49]. In addition, the AP2/ERF transcription factor OsHL6 protein interacts with OsWOX3B, a key regulator of rice surface coat initiation, to promote surface coat initiation and elongation [50]. Some WRKY, ERF and bZIP transcription factors can be specifically expressed in glandular hairs, and different types interact with each other. *Bra001588*, *Bra015882*, *Bra029778*, *Bra029311*, *Bra035148*, *Bra018529*, *Bra000809*, *Bra038033*, *Bra004679* and *Bra012910* were screened according to expression level and CT vs C, LCT vs LC and CT vs LCT comparison group. The 10 transcription factors are related to the effect of low temperature on the trichomes. Among them, *Bra029778* gene was also screened in the co-expression network, which is the central hub gene and can be verified for subsequent function. It can be seen that the regulation of transcriptional interaction induced by low temperature in plant trichome formation needs to be further explored.

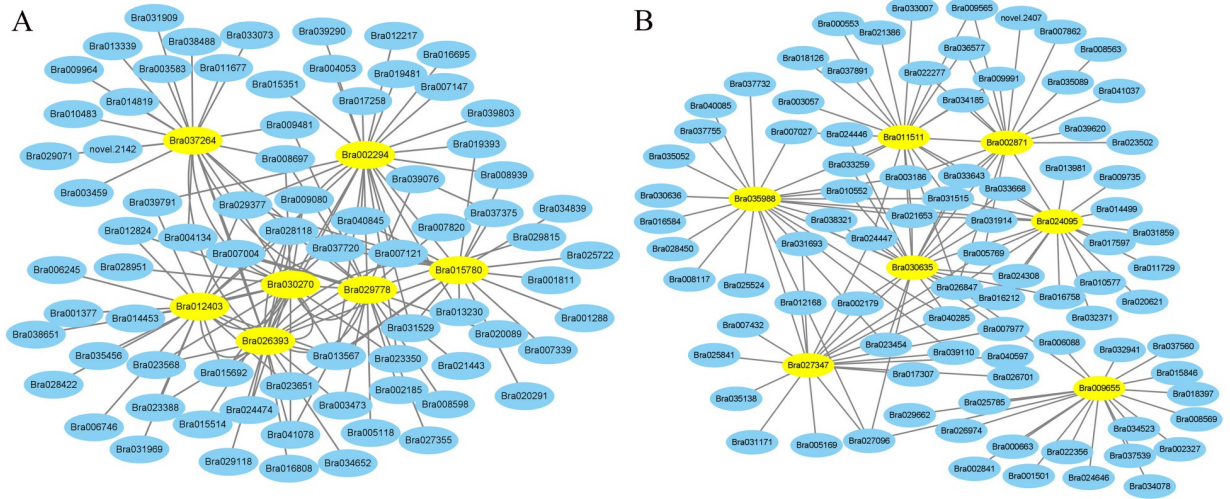


Fig 10. Analysis of hub gene co-expression network.

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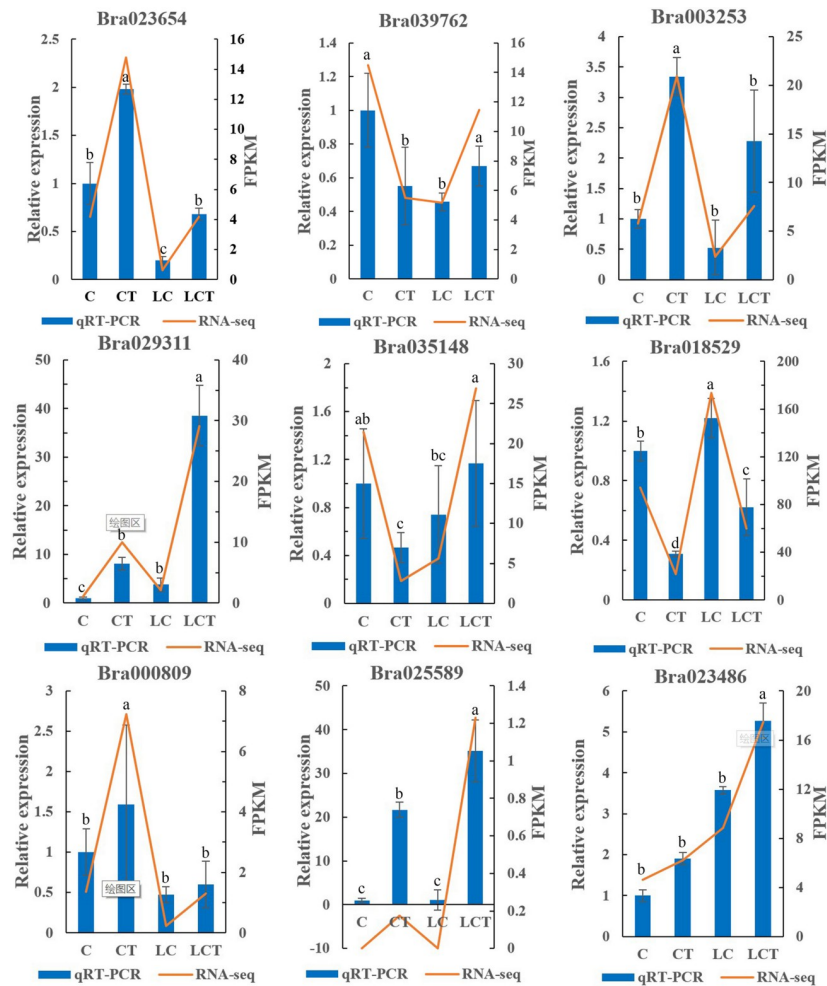


Fig 11. qRT-PCR verification of differential genes.

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Plant trichomes often secrete and synthesize different types of defense substances, such as terpenoids, amino acids, phenylpropanes, lipid derivatives. These secondary metabolites can protect plants from biological and abiotic stress and play an important role in defense. Phenylalanine, tryptophan and tyrosine are all aromatic amino acids, among which tryptophan is the precursor of auxin, alkaloids, indoline and plant antitoxin, while tyrosine is the precursor of isoquinoline alkaloids, betaine and quinones [51, 52], phenylalanine is the precursor of phenylcyclopropane pathway, and p-coumaroyl coenzyme A is the intermediate of phenyl-propane metabolic pathway, as well as the precursor of various substances such as phenyl-propylene and flavonoids [53]. The amino acid biosynthesis pathway of Chinese cabbage with or without trichomes was enriched in 88 DEGs, including 59 up-regulated DEGs and 29 down-regulated DEGs. After low temperature treatment, 76 DEGs were enriched in amino acid biosynthesis pathway, among which 30 DEGs were up-regulated and 46 DEGs were down-regulated. Compared with trichomes before and after treatment, 78 DEGs were enriched in amino acid biosynthesis pathway, 13 DEGs were up-regulated and 65 DEGs were down-regulated. *Bra026393*, *Bra030270*, *Bra037264*, *Bra009655*, *Bra019206*, *Bra021682*, *Bra020605*, *Bra040146*, *Bra016680* may be related to the effect of low temperature on trichomes. *Bra026393*, *Bra030270*, *Bra037264* and *Bra009655* were also selected in the gene co-expression network as the central hub genes.

Hormone signal transduction pathway and amino acid biosynthesis pathway were obtained by KEGG enrichment, and 661 differential genes were enriched in the three comparison groups. The differential genes were screened for qRT-PCR analysis. *Bra029311*, *Bra035148*, *Bra018529*, *Bra000809*, *Bra003253*, *Bra025589* and *Bra023486* were selected transcription factors in 661 genes. *Bra039762* and *Bra023654* were screened genes in plant hormone signal transduction pathway. The results were consistent with the transcriptome trend, which proved the reliability of transcriptome data. The expression levels of 9 genes were significantly different between trichome Chinese cabbage CT and without trichome Chinese cabbage C. After low temperature treatment, the expression level increased or decreased, and the difference was significant. It may be related to the effect of low temperature on the trichomes. The genes enriched in related pathway and comparison group were combined with WGCNA for analysis, and five genes *Bra029778*, *Bra026393*, *Bra030270*, *Bra037264* and *Bra009655* were screened together. The reliability was strong, and the subsequent functional verification could be carried out. In order to study the phenotypic changes and molecular mechanism of Chinese cabbage leaf trichomes in response to low temperature, a new path was explored.

Author Contributions

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