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Analysis of Transcriptome Response to Low Temperature Stress in *Mesembryanthemum crystallinum* Linn.

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Abstract The second generation of high throughput sequencing technology was used to sequence ice plant under low temperature stress and to construct transcriptome database. 24.13 Gb of valid data and 24 045 annotations of Unigene were obtained. DEGs of 1 902 (T0 vs T1) and 2 134 (T0 vs T2) were obtained. Two groups of DEGs were classified into 40 and 41 functional small categories respectively by GO database and were obtained respectively 20 and 24 functional categories by KOG database and were annotated 55 and 272 genes by comparing DEGs with KEGG database, which were enriched in 74 and 105 metabolic pathways respectively. DEGs of T0 vs T1 group mainly annotated to 4 metabolic pathways; DEGs of T0 vs T2 group mainly annotated to 11 metabolic pathways. It was showed that there were the metabolic pathways of positive correlation: acetone alcohol biosynthesis, purine metabolism, glutathione metabolism, fatty acid metabolism, brass metabolism, amino acid biosynthesis and other metabolic pathways, and the metabolic pathways of negative correlation: plant-pathogen interaction, plant hormone signal transduction, starch and sucrose metabolism. The key genes of starch and sucrose metabolism pathway were analyzed: the expression of 5 key genes, such as trehalose 6-phosphate synthase, trehalose-6-phosphatase, beta-amylase, glucose-1-adenosine phosphate transferase and glycogen phosphorylase, were up-regulated, but down-regulated gene was not observed during T1 (1h) of low-temperature stress; Three key genes of trehalose-6-phosphosynthase, hexokinase and beta-amylase, were up-regulated and the glucolactone-1,3-beta-glucosidase gene was down-regulated under low temperature stress at T2(36h). Eight DEGs in starch and sucrose metabolism pathway were selected and analyzed by Quantitative Real-time PCR. The relative expression level of 8 DEGs was consistent with the transcriptome expression profile analysis.
Keywords Ice plant; Transcriptome; DEGs; Starch and sucrose metabolism

Ice plant (*Mesembryanthemum crystallinum* Linn.) is an annual or biennial herb of the genus *Mesembryanthemum* L. in the Aizoaceae family. Its stem is prostrate and its leaf is fleshy, which has high edible value (Wang et al., 2016). Ice plant originated in southern Africa, has been introduced and cultivated all over the world, and introduced to China in recent years. As an emerging vegetable, ice plant has the characteristics of drought resistance, salinity, high sunshine, cold and waterlogging, etc. The most suitable temperature for growth is 5℃~25℃, below 5℃ or higher than 30℃ will wither. Studies have shown that low temperature stress has a significant impact on both yield and quality of vegetables (Qian et al., 2019). As a new special resource, it is of great significance to study the effects of low temperature stress on the growth and development of ice plant.

Low temperature stress is one of the important environmental stress factors suffered by crops in the process of growth, and its response is a multi-factor collaborative process, mainly reflected in the cell membrane system, enzyme activity, cytoplasmic water loss, etc., which will lead to cell metabolism imbalance and cell decline (Liu, 2018). Carbohydrate is an important material basis for plant cell construction and energy consumption. Through interaction with a variety of hormones and growth factors, carbohydrate has the activities of regulating osmotic potential, enhancing cell water retention, and protecting biofilm and biomacromolecules (Hincha et al., 2002). Thus, regulating plant growth, development and stress resistance (He et al., 2016). In recent years, it has been found that sugars can also act as signal molecules and participate in physiological processes such as the regulation

of low temperature stress in plants. For example, under low temperature stress, carbohydrate metabolism in plants is strengthened, and soluble sugars such as starch, sucrose, fructose, glucose, fructan and trehalose increase (Sun et al., 2015). Trehalose is a signal molecule involved in plant stress response and regulation of growth and development (Matthew et al., 2008). In addition, under low temperature stress, the content and types of sugars are different among species (Shen et al., 2019; Diao et al., 2019). Therefore, it is necessary to study the physiological and molecular mechanism of soluble sugar metabolism under low temperature stress.

In recent years, the facility cultivation of ice plant has become more and more widespread in China, which is of great significance to the study of transcriptome level expression and metabolic regulation mechanism of ice plant under low temperature stress. Illumina Hi-seq 2500 high-throughput sequencing technology was used to study the response mechanism of cold stress and the changes of genes related to soluble sugar metabolism in ice plant by treating them for 1 h and 36 h at 4℃. Screening cryogenic tolerance genes and analyzing the expression mechanism of key genes in ice plant can not only explain the response mechanism of ice plant to low temperature stress, but also help to excavate the cryogenic variety resources and accumulate experience for cryogenic tolerance breeding of ice plant.

1 Results and Analysis

1.1 Data quality evaluation and analysis

After transcriptome sequencing, 24.13 Gb Clean data were obtained, among which T0 Clean data was 8.47 Gb, T02 Clean data was 7.69 Gb and T03 Clean data was 8.00 Gb, with Q30 exceeding 92.0% (Table 1). The sequencing data were good for correlation analysis. According to data assembly analysis (Table 2), 153 971 transcriptional sequences were assembled from Transcipt, with an average length of 1 384.0 bp and N50 length of 2 397 bp. 76 907 sequences were assembled from Unigene with 751.4 bp in length and 1 215 bp in N50.

Table 1 Statistics of clean data

Samples	Read	Base	GC Content (%)	Q30 (%)
T0	28 360 950	8 466 724 784	47.10%	92.72%
T1	25 735 464	7 689 769 894	46.98%	92.73%
T2	26 684 607	7 974 101 406	46.72%	92.95%

Table 2 Statistics of assembled results

Length range (bp)	Transcript number	Unigene number
200~300	31 005 (20.14%)	27 377(35.60%)
300~500	24 991 (16.23%)	19 389(25.21%)
500~1000	25 523 (16.58%)	14 189(18.45%)
1 000~2 000	33 023 (21.45%)	9 226(12.00%)
2000+	39 429 (25.61%)	6 726(8.75%)
Total Number	153 971	76 907
Total Length	213 095 569	57 787 754
N50 Length	2 397	1 355
Mean Length	1384 00	751 40

1.2 Unigene annotation

After comparison with COG, GO, KOG, KEGG, Pfam, Swiss-Prot, eggNOG and NR databases, statistics were made on the functional annotation of Unigene (Table 3). Among 76 907 sequences, 24 045 Unigene obtained annotation results, accounting for 31.26%. Compared with COG database, 7 627 annotations were obtained, accounting for 31.72% of the total annotated genes. Compared with KOG database, 12 913 annotations were obtained, accounting for 53.70% of the total annotations. Compared with GO database, 13 771 annotations were

obtained, accounting for 57.27% of the total annotations. Compared with KEGG database, 8 625 annotations were obtained, accounting for 35.87% of the total annotations. Compared with Pfam database, 16 296 annotations were obtained, accounting for 67.77% of the total annotations. Compared with Swiss-Prot database, 15 787 annotations were obtained, accounting for 65.66% of the total annotations. Compared with eggNOG database, 21 585 annotations were obtained, accounting for 89.77% of the total annotations. Compared with NR database, 23 510 annotations were obtained, accounting for 89.77% of the total annotated genes.

Table 3 Unigene functions annotated

Bioinformation database	Annotated unigene number	Unigene length (300≤length<1000)	Unigene length (length > 1000)	Percentage (%)
COG	7 627	1 144	5 299	31.72
KOG	12 913	2 962	7 812	53.70
GO	13 771	3 232	7 929	57.27
KEGG	8 625	1 927	5 266	35.87
Pfam	16 296	3 464	10 892	67.77
Swiss-Prot	15 787	3 822	9 735	65.66
eggNOG	21 585	5 406	12 465	89.77
NR	23 510	6 445	12 805	97.78
All annotated unigenes sequence number	24 045	6 563	12 823	100

1.3 Screening comparison and annotation analysis of DEGs

1.3.1 Comparison of DEGs between the two groups

The results showed that 1 902 DEGs (T0vsT1) and 2 134 DEGs (T0vsT2) were detected under low temperature stress, among which 1 317 genes were up-regulated and 585 down-regulated in T0vsT1 group. There were 1 184 up-regulated genes and 950 down-regulated genes in T0vsT2 group (Table 4). DEGs Venn diagram analysis of the two groups (T0vsT1 and T0vsT2) (Figure 1) showed 1 392 non-co-expressed genes, including 1 048 up-regulated genes and 344 down-regulated genes, under low temperature stress for 1 h. There were 1 624 non-co-expressed genes, including 915 up-regulated genes and 709 down-regulated genes, under low temperature stress for 36 h. A total of 510 genes were expressed at 1 h and 36 h under low temperature stress, including 269 up-regulated genes and 241 down-regulated genes. The results showed that the non-co-expressed genes of T1 increased by 163.67% compared with T2, in which the up-regulated genes decreased by 12.69% and down-regulated genes increased by 106.10%. Among the co-expressed genes of T1 and T2, the number of up-regulated genes (269) and down-regulated genes (241) were basically the same.

DEGs of T0 vs T1 and T0 vs T2 were annotated into 8 databases such as NR, and 1 089 and 1 512 genes were obtained, including 340 and 575 genes in COG database, 625 and 897 genes in GO database, 317 and 501 genes in KEGG database, respectively. There were 457 and 744 KOG databases, 868 and 1 220 Pfam databases, 825 and 1 176 Swiss-Prot databases, and 1 021 and 1 425 eggNOG databases, respectively. There were 1 091 NR databases and 1 500 NR databases respectively (Table 5), among which the annotation ratio of NR databases was the highest, both exceeding 99%.

Table 4 Expression of differential genes

Group	All genes	Up-regulation gene	Down-regulation gene
T0vsT1	1902	1317	585
T0vsT2	2134	1184	950
Co-expression gene	510	269	241

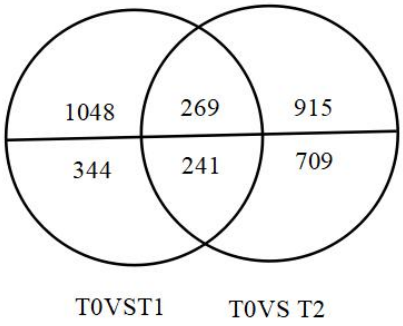

Figure 1 Differentially expressed genes Venn diagram
Note: The above and below number represented up and down genes respectively

Table 5 Statistics of annotated DEGs

DEG_Set	Annotated	COG	GO	KEGG	KOG	Pfam	Swiss-Prot	eggNOG	NR
T0vsT1	1098	340	625	317	457	868	825	1021	1091
T0vsT2	1512	575	897	501	744	1220	1176	1425	1500

1.3.2 GO database annotations

GO database consists of biological process, cellular component and molecular function. DEGs of ice plants under low temperature stress at different treatment time T1 and T2 were counted, and the results showed that DEGs of the two groups were classified into 40 and 41 functional subclasses respectively (Table 6). Biological processes: DEGs mainly concentrated in "metabolic process", "cell process", "single biological process", "biological regulation", "location", "stimulus response" and "cell component or biosynthesis", accounting for a high proportion of six functional subcategories. In terms of the difference of DEGs ratio between the two groups, the ratio of "reproduction", "reproductive process" and "cell composition and biosynthesis" was larger, all exceeding 2.0 times. It is concluded that the "cell composition and biosynthesis" process has important influence on the biological process of ice plant under low temperature stress. In terms of cell component process, DEGs mainly focuses on "membrane structure", "membrane component", "cell", "cell component", "organelle" and "organelle component" functional subcategories; The DEGs ratio difference between the two groups showed that the ratio of "organelle component" and "organelle" was 2.96 and 2.07 times, respectively. The results showed that cold stress had significant effect on the cellular constituent process "organelle components" and "organelle" functional subsets. Molecular functional processes: DEGs mainly focus on the functional subclasses of "catalytic activity", "binding activity" and "transport activity". In terms of the difference in DEGs ratio between the two groups, the ratio of "molecular function regulation" and "electronic carrier activity" was 3.00 times and 2.00 times, and the ratio of "nucleic acid binding transcription factor activity" was 0.5 times. It was preliminarily concluded that low temperature stress had an important effect on "molecular function regulation", "activity of electron carrier" and "activity of nucleic acid binding transcription factor" at the transcriptome level of ice plant.

1.3.3 KOG annotation

Two groups of DEGs, T0vsT1 and T0vsT2, were annotated into KOG database. By analyzing the homology of annotation results, 20 and 24 functional categories were obtained, respectively. Among them, 83 and 151 annotations were obtained for R (general function prediction), accounting for 18.17% and 20.29%, respectively. T (signal transduction mechanism) received 72 and 77 annotations, accounting for 15.09% and 10.35%, respectively. O (post-translational modification, protein conversion, chaperone), 46 and 94 annotations were obtained, accounting for 10.06% and 12.63%, respectively. Q (biosynthesis, transport and metabolism of secondary metabolites) received 40 and 68 annotations, accounting for 8.75% and 9.14%, respectively. G (carbohydrate transport and metabolism) received 28 and 68 annotations, accounting for 6.13% and 9.14%; I (Lipid transport and metabolism) received 21 and 50 annotations, accounting for 4.59% and 6.72%. K (transcription) obtained 31 and 38 annotation results, accounting for 6.78% and 5.11%. The difference of KOG function annotation between

the two groups was analyzed, R (general function prediction), and the difference was 68. O (post-translational modification, protein conversion, chaperone), the difference was 48; G (carbohydrate transport and metabolism), the difference was 40; I (lipid transport and metabolism), the difference was 29; The difference between Q (biosynthesis, transport and metabolism of secondary metabolites) and C (energy generation and transformation) was 28 (Table 7).

Table 6 Comparisons of DEGs GO annotation by low temperature stress treatment

Go term	Go classify	T0 vs T1	T0 vs T02	Ratio of DEGs (T0vsT02)/(T0vsT2)
Biological process	Metabolic process	247	429	1.74
	Cellular process	239	356	1.49
	Single-organism process	171	301	1.76
	Biological regulation	98	110	1.12
	Localization	58	97	1.67
	Response to stimulus	76	98	1.29
	Cellular component organization or biogenesis	23	51	2.22
	Developmental process	23	34	1.48
	Multicellular organismal process	16	25	1.56
	Reproduction	10	25	2.50
	Reproductive process	10	25	2.50
Cellular component	Cell	166	298	1.80
	Cell part	160	290	1.81
	Membrane	226	329	1.46
	Organelle	88	182	2.07
	Membrane part	177	266	1.50
	Organelle part	24	71	2.96
	Macromolecular complex	19	37	1.95
	Extracellular region	22	37	1.68
	Cell junction	10	18	1.80
Molecular function	Catalytic activity	289	502	1.74
	Binding	254	414	1.63
	Transporter activity	42	61	1.45
	Nucleic acid binding transcription factor activity	24	12	0.50
	Molecular function regulator	4	12	3.00
	Antioxidant activity	9	13	1.44
	Electron carrier activity	6	12	2.00

1.3.4 KEGG annotation

By comparing the two groups of DEGs with KEGG database (Table 8), 155 and 272 genes were annotated for T0vsT1 and T0vT2, which were enriched in 74 and 105 metabolic pathways, respectively. DEGs in T0vsT1 group mainly annotated four metabolic pathways including plant signal transduction (27), starch and sucrose metabolism (16), plant-pathogen interaction (16) and phenylpropanoid biosynthesis (12). DEGs in T0vT2 group were mainly described as phenylpropanoid biosynthesis (24), plant signal transduction (18), glutathione metabolism (13), starch and sucrose metabolism (12), purine metabolism (12), amino acid biosynthesis (12), carbon metabolism (11), amino sugar and nucleotide sugar metabolism (11), arginine and proline metabolism (10), fatty acid metabolism (10), flavonoid metabolism (9).

The difference analysis of DEGs between T0 vs T1 and T0 v T2 groups showed that the metabolic pathways under different low temperature stress (T1 and T2) were positively affected by acetone alcohol biosynthesis, purine metabolism, glutathione metabolism, fatty acid metabolism, flavonoid metabolism, amino acid biosynthesis, etc. Negative metabolic pathways include plant-pathogen interaction, plant hormone signal transduction, starch and sucrose metabolism. This study focused on the selection of starch and sucrose metabolic pathways to find the key genes for the occurrence of metabolic differences, so as to provide a theoretical basis for the study of starch and sucrose metabolism mechanisms under low temperature stress of ice plant.

Table 7 Comparisons of DEGs KOG annotation by low temperature stress treatment

KOG	Function classification	T0vsT1	T0vsT2	Differences of DEGs (T0vsT2)-(T0vsT1)
A	RNA processing and modification	6	16	10
C	Energy production and conversion	13	41	28
D	Cell cycle control, cell division, chromosome	10	24	14
E	Amino acid transport and metabolism	22	40	18
G	Carbohydrate transport and metabolism	28	68	40
I	Lipid transport and metabolism	21	50	29
K	Transcription	31	38	7
L	Replication, recombination and repair	5	21	16
M	Cell wall/membrane/envelope biogenesis	9	15	6
O	Posttranslational modification, protein turnover, chaperones	46	94	48
Q	Secondary metabolites biosynthesis, transport and catabolism	40	68	28
R	General function prediction only	83	151	68
S	Function unknown	15	29	14
T	Signal transduction mechanisms	72	77	5
U	Intracellular trafficking, secretion, and vesicular transport	12	19	7
V	Defense mechanisms	5	16	11
Z	Cytoskeleton	11	25	14

Table 8 Comparisons of DEGs KEGG function annotation by low temperature stress treatment

Number	KEGG function annotation	T0vsT1	T0vsT2	Differences (T0vsT2)-(T0vsT1)
ko00940	Phenylpropanoid biosynthesis	12	24	12
ko00230	Purine metabolism	0	12	12
ko00480	Glutathione metabolism	3	13	10
ko01212	Fatty acid metabolism	1	10	9
ko00941	Flavonoid biosynthesis	0	9	9
ko01230	Biosynthesis of amino acids	4	12	8
ko00330	Arginine and proline metabolism	3	10	7
ko00071	Fatty acid degradation	1	8	7
ko00053	Ascorbate and aldarate metabolism	0	6	6
ko01200	Carbon metabolism	5	11	6
ko00500	Starch and sucrose metabolism	16	12	-4
ko04075	Plant hormone signal transduction	27	18	-9
ko04626	Plant-pathogen interaction	16	6	-10

1.4 Effects of low temperature stress on starch and sucrose metabolism pathways in ice plant

Low temperature stress in the 1 h KEGG of ice plant starch and sucrose metabolic pathway of trehalose-6-phosphate synthase (TPS), trehalose 6-phosphate phosphatase (TPP), β-amylase (BAL), glucose-1-phosphate adenylyl transferase and glycogen phosphorylase were up-regulated, but no down-regulated genes were found. In the KEGG metabolic pathway (Ko0500), D-Glucose and UDP-Glucose were used as catalytic substrates to significantly up-regulate the expression of TPS and promote the accumulation of Trehalose-6P products. At the same time, extracellular Trehalose generates Trehalose 6-phosphate through phosphotransferase system (PTS) gene, which can transport and phosphorylate sugar. TPP catalyzes Trehalose 6-phosphate from two sources to generate intracellular Trehalose. The accumulation of intracellular algal carbohydrate was thought to be the response mechanism of low temperature stress in the early stage of ice plant. In addition, maltodextrin, starch and glycogen can be catalyzed to produce maltodextrin and dextrin. Starch and glycogen can produce ADP-glucose under the catalysis of glycogen phosphorylase (PYG) and glucose-1-phosphate adenyl transferase. Three key

genes including TPS, hexokinase (HK) and BAL were found to be up-regulated in starch and sucrose metabolism pathways in KEGG under low temperature stress for 36 h. Glucan endo-1,3-beta-glucosidase (GLGC) gene was down-regulated. In addition, TPS and BAL were up-regulated in multiple copies at 1 h and 36 h under low temperature stress (Table 9).

The accumulation of maltose and dextrin in ice plant leaves were further increased with the duration of low temperature stress, indicating the positive response mechanism of maltose and dextrin to low temperature stress in ice plant leaves. In this experiment, the differences between T1 and T2 of ice plant under low temperature stress were as follows: With the prolonged period of low temperature stress, the expression of TPS was up-regulated, while the relative expression of TPP was down-regulated, resulting in the massive accumulation of trehalose 6-phosphate. At T1 stage, starch and glycogen catalyzed by PYG and GLGC generated ADP-glucose strongly, while at T2 stage, ADP-glucose accumulation was weak and recovered to T0 level. In addition, hexokinase (HK) is up-regulated under T2 conditions, promoting the phosphorylation of D-glucose and D-fructose into D-glucose-6-phosphate (D-glucose-6-P) and D-fructose 6-phosphate (D-fructose 6-P). Down-regulated expression of Glucan endo-1, 3-beta-glucosidase inhibits the conversion of 1,3-β-glucan to D-glucose and indirectly accumulates 1,3-β-glucan. Finally, the expression levels of some β-d-glucosidase family genes were firstly down-regulated and then down-regulated with the prolongation of cold stress time. Some β-glucosidase family genes were normal at first, then down-regulated or up-regulated, and some β-glucosidase family genes were up-regulated at first, then normal. The response of β-glucosidase to different low temperature stress was not clear.

1.5 Verification analysis of key differential genes in starch and sucrose metabolic pathways in ice plant

Part of DEGs was quantified in real time by fluorescence. Eight differentially expressed genes were TPS (c22439), TPS (c37455), TPS (c31293), TPP (c21588), HK (c30615), BAL (c36048), BAL (c36010) and PYG (c36980). Actin gene (c38220) of ice plant was used as an internal reference for qRT-PCR validation (Table 9). The relative expression level of DEGs in qRT-PCR was consistent with the transcriptome expression level (Figure 2), indicating that the results of transcriptome level analysis were credible. Among them, the expression levels of BAL (c36010) genes T0 and T1 and T0 and T2 were significantly different, which provided a research basis for cloning key genes of starch and sucrose metabolic pathways responding to low temperature stress in ice plant.

Table 9 Expression of the key genes of starch and sucrose metabolism under low-temperature stress

Enzyme	Name	Abbreviation	FPKM			(T0 T1) Genes expression	(T0 T2) Genes expression	Gene ID
			T0	T1	T2			
EC:2.4.1.15	Trehalose 6-phosphate synthase	TPS	30.24	70.41	103.89	UP	UP	c22439
EC:2.4.1.15	Trehalose 6-phosphate synthase	TPS	19.23	39.55	78.8	UP	UP	c37455
EC:2.4.1.15	Trehalose 6-phosphate synthase	TPS	2.84	9.42	28.39	UP	UP	c31293
EC:3.1.3.12	Trehalose 6-phosphate phosphatase	TPP	3.58	9.11	3.94	UP	_	c21588
EC:2.7.1.1	Hexokinase	HK	1.25	2.8	6.64	_	UP	c30615
EC:3.2.1.2	beta-amylase	BAL	69.65	448.56	754.81	UP	UP	c36048
EC:3.2.1.2	beta-amylase	BAL	34.08	147.57	1050.72	UP	UP	c36010
EC:2.7.7.27	Glucose-1-phosphate adenylyltransferase	/	26.23	172.87	18.53	UP	_	c16507
EC:2.4.1.1	Glycogen phosphorylase	PYG	40.18	101.63	55.08	UP	_	c36980
EC:3.2.1.39	Glucan endo-1, 3-beta-glucosidase	/	28.6	14.69	6.94	_	DOWN	c20139

Note: "-": No up-regulation or down-regulation expression

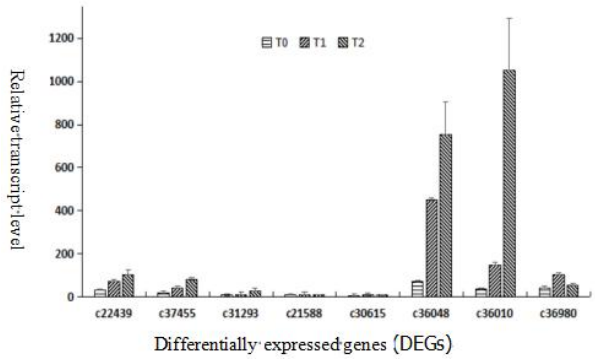

Figure 2 The relative expression levels of DEGS by qRT-PCR

2 Discussion

Under low temperature stress, starch hydrolysis in leaves can increase intracellular soluble sugar content, which is considered to be an important way for plants to respond to low temperature stress (Benina et al., 2013). Currently, the role of glucose metabolism in low temperature stress in plants has been widely concerned and confirmed (Janska et al., 2011). In starch metabolism, β-amylase is a key enzyme involved in starch hydrolysis in plants, and plays an important role in tea plants' response to low temperature stress (Yue, 2015). In this study, low temperature stress T1 and T2 of ice plant were consistent with their research results. Trehalose metabolism in plants is mainly synthesized by the TPS/TPP pathway. TPS and TPP are key enzymes in the regulation of trehalose synthesis (Ding et al., 2019), and most of its members can participate in the regulation of the physiological process of plant stress tolerance (Jiang et al., 2014). When crops suffer from low temperature, plant trehalose can be rapidly synthesized to protect the body (Wang et al., 2019). Key genes for trehalose synthesis were significantly expressed at T1 and T2, leading to rapid synthesis of trehalose and trehalose-6-phosphate, which had a good protective effect on ice plant leaves under low temperature conditions (Xu et al., 2017). In addition, the HK promoter contains low docile expression elements, which are susceptible to low temperature induction and lead to accumulation of glucose-6-phosphate and D-fructose-6-phosphate (Zhao et al., 2015). By analyzing the metabolic pathways of starch and sucrose in chilled vegetables under low temperature stress, DEGs analysis showed that trehalose, maltose, dextrin and ADP-glucose soluble sugars were accumulated in ice plant leaves at the early stage of low temperature stress T1. In the T2 stage of low temperature stress, trehalose 6-phosphate, maltose, dextrin, d-glucose-6-phosphate, d-fructose-6-phosphate and 1,3-β-glucan were further accumulated in cabbage leaves, which was similar to the accumulation of carbohydrate components in the process of low temperature relief of *Lilium pumilum* (Liu et al., 2016). By real-time qRT-PCR analysis, the relative expression level of DEGs was consistent with the transcriptome expression level.

Using high-throughput transcriptome sequencing technology, the early stage of the project team a preliminary analysis of ice plant salt stress related metabolic and key gene mining (Lian et al., 2019). On this basis, continue to study the changes of sucrose and starch metabolism and the accumulation of related sugars under low temperature stress, and preliminary elucidate the molecular mechanism. It will provide reference for further studies on transcription factors, key functional genes and signal transduction pathways that regulate the process of low temperature stress. The above study on the molecular mechanism of ice plant under salt stress and low temperature stress is helpful to the improvement and cultivation of new varieties of ice plant under stress, and has important significance in guiding the production practice of ice plant.

3 Materials and Methods

3.1 Test materials

Test materials: ice plant; Planting location: Experimental Farm, Institute of Subtropical Agriculture, Fujian Academy of Agricultural Sciences; Planting and treatment time: Seedlings were raised on December 11, 2019, transplanted on January 10, 2019, and placed in 4℃ cold storage for low temperature stress treatment on February 15. Light filling mode: fluorescent lamp is used to simulate outdoor lighting (9 h light treatment, 13h dark

treatment); Treatment time: T0: 0; T1: 1 h; T2: 36 h. Sample collection: 1 replicate for each basin and 3 replicates were set; The young stems and leaves of ice plant were sampled at 0, 1 and 36 h, respectively. The samples were quick-frozen with liquid nitrogen, and then placed in a refrigerator at -80℃ for use.

3.2 Test method

Total RNA was extracted from tender stems and leaves of ice plant, and the cDNA library was constructed by detecting the purity, concentration and integrity of the RNA samples, and then the quality of the library was detected. After qualified, the cDNA library was sequenced using Illumina Hiseq 2500 high-throughput sequencing platform, and a large amount of high-quality Raw Data was produced, and high-quality Clean Data was obtained through filtration. Sequence assembly of high-quality Clean Data using Trinity software. In this experiment, three ice plant sequencing samples were assembled by combination assembly method. Clean Data sequenced from ice plant transcriptomes were compared with the assembled Transcript or Unigene library (Table 3). Mapped Reads were used for subsequent analysis. The sequences were compared with the Unigene database, and the expression abundance of corresponding Unigene was indicated by FPKM values (Fragments per kilobase of Transcript per million mapped reads). Differential expression analysis was performed using EBSeq.

The differentially expressed genes of T0 and T1 and T0 and T2 were obtained under low temperature (4℃). DEGs can be divided into up-regulated genes and down-regulated genes according to the relative expression levels between samples. Then the two groups (T0 vs T1; T0 vs T2) data DEGs were analyzed by Venn diagram. Unigene was compared with 8 large databases such as NR, and KEGG Orthology annotation results were obtained in KEGG. The metabolic pathways and functions of gene products were systematically analyzed, and the corresponding DEGs were compared to the KEGG database to analyze the metabolic pathways related to DEGs.

3.3 Effects of low temperature stress on starch and sucrose metabolism pathways in ice plant

The transcriptome database was searched to analyze the expression of key genes in starch and sucrose metabolism pathway DEGs (key enzyme genes) under low temperature stress, and to study the effects of different low temperature stress duration on the intermediate products related to starch and sucrose metabolism.

3.4 Verification of key differential genes in starch and sucrose metabolic pathways in ice plant

1 μg total RNA of ice plant leaf was extracted and reversely transcribed into cDNA using reverse transcription kit. Primers were designed and the following primer sequences (5'-3') were synthesized (Table 10). RT-PCR was used to detect key differentially expressed genes in starch and sucrose metabolism pathway of ice plant treated by T0, T1 and T2 respectively. Ct value of target gene (including 1 reference gene) in the sample to be tested, and its relative expression level was calculated.

Table 10 Primers for quantitative real-time PCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
c38220 (Internal reference)	GAGAGAGAGAGAGAGAGAGAATGG	TCACCATAGAAGAAAATTCATTAATT
c28853	TGGTGGTGGTGGTGGTGG	TTCTC TCATCACAAT CAAGCC
c26482	GGTGGTGGTGGTGGTGGTGCCCCCATTGA	TTACTACCAC AAACAGCA
c31251	CTCAACTTAAGAGCGTCAGTAGAAACAC	CGAGGTTTCATGTTGGAAGG
c32701	AGGGAGCTAGCTAATGGGTG	AATGG ACCCTTTAAT CGCTC
c36305	CAGTTACAGAGGAACAATTCCATGTG	GTGCTCTGTT TCCGGTTGTG
c38701	GCCTACAATTTGCAAGCTCAG	CTAGAATGG ACCCTTTAAT CGCTC
c41466	GTCATTGCTCACAAGCTAGC	GGTGGCGTACGCCC

Authors' contributions

LZF is the experimental design and executor of this study. YYF completed data analysis and wrote the first draft of the paper; LDM and LBZ participated in experimental design and analysis of experimental results; HJJ is the architect and principal of the project, directing experimental design, data analysis, paper writing and revision. All authors read and approved the final manuscript.

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