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Combined transcriptomic and proteomic analysis of cold stress induced sugar accumulation and heat shock proteins expression during postharvest potato tuber storage

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ABSTRACT

Plant species differ greatly in their ability to acclimatise to and survive, cold stress. Normally, potato tubers are stored at low temperatures (below 10 °C) to delay sprouting. In this research, combined transcriptomic and proteomic analysis was conducted on potato tubers stored at 15 °C, 4 °C and 0 °C to investigate the mechanism of cold responses during postharvest storage. Results showed that soluble sugars were accumulated under low temperatures, regulating by granule-bound starch synthase 1, beta-amylase, invertase inhibitor and fructokinase. In addition, fifteen heat shock proteins (Hsps), including three Hsp70s, two Hsp80s, one Hsp90, one Hsp100 and eight small Hsps, were induced by low temperatures, which may act individually or synergistically to prevent physiological or cellular damage from cold stress in postharvest potato tubers. This research provided general information of sugar accumulation and defense response in potato tuber under cold storage.

1. Introduction

Cold stress from low temperatures limits yield and quality of crops and horticultural products during growth, and also limits the postharvest storage of fruits and vegetables (Liu, Yang, Zhu, & Wang, 2016; Wang et al., 2017; Zhang et al., 2016). A series of physiological changes, including electrolyte leakage, photosynthetic capacity and respiration rates, have been observed when cold-sensitive crops and fruits are exposed to low temperatures (Khan et al., 2019; Mustárdy, Vu, & Faludi-Dániel, 1982).

Harvested potato tubers exhibit various physiological and biochemical responses when stored at low temperature to inhibit sprouting and decay (Bagnaresi et al., 2008; Folgado et al., 2014). Sugars accumulate rapidly in potato tubers under low temperature storage, less than 10 °C (Hou et al., 2017; Lin et al., 2017; Xiao et al., 2018). It has also been reported that cold storage increases the total phenolics content and antioxidant activity of selected potato clones (Külen, Stushnoff, & Holm, 2013). Stress tolerance is acquired from cold stress to prevent cellular damage and re-build cellular homeostasis in plant. Heat shock proteins (Hsps) are key components contributing to cellular homeostasis in cells under cold stress, which are responsible for protein folding, assembly, translocation and degradation in abroad array of normal cellular processes (Wang, Vinocur, Shoseyov, & Altman, 2004). However, the molecular mechanism of potato tubers in response to cold stress during postharvest storage remains unclear.

Recent advances in high-throughput technologies enable quantitative monitoring of the abundance of various biological molecules and allow determination of their variation between biological states on a genomic scale. Two popular platforms are transcriptomics that measure messenger RNA transcript levels, and proteomics that quantify protein abundance. Transcriptomic profiling of potatoes under cold stress has been studied in both plants and tubers (Bagnaresi et al., 2008; Oufir et al., 2008). Previously, proteomic analysis has been used to investigate the cold responses in plants such as rice (Cen et al., 2018), Arabidopsis thaliana (Amme, Matros, Schlesier, & Mock, 2006), and grapevines (Yang et al., 2017). The combination of transcriptome and proteome is useful to understand the regulation mechanisms in stress responses. For now, transcriptomic combined proteomic analysis was

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conducted on potato plants under cold and salt stresses (Evers et al., 2012), but no such research has been conducted on potato tubers, particularly during postharvest storage.

In the present research, the potato tubers (*Solanum tuberosum* L.) were stored at 15 °C, 4 °C and 0 °C for 20 days and used for transcriptomic combined proteomic analysis. The objective was to investigate the mechanism of sugar accumulation and defense response towards cold stress in potato tubers during postharvest storage.

2. Materials and methods

2.1. Plant material and treatments

'Atlantic' potato tubers with uniform size were harvested from a farm in Zhangjiakou, Beijing, China. After curing for seven days, the potato tubers were stored at 15 °C, 4 °C and 0 °C with 85–95% relative humidity and sampled at 0 d, 3 d, 10 d, 20 d and 30 d after storage. Each sample consisted of twelve potatoes, which were divided into three replicates. The peeled flesh of potatoes was stored at −80 °C until analysis.

2.2. Measurements of sugars

The measurements of sugars were carried out using a method described previously (Lin et al., 2017). Dry potato tuber powder (200 mg) was mixed with 4 mL of ethanol (80%) and extracted for 30 min with ultrasonic. After centrifuging at 10,000 rpm for 10 min, the upper phase was dried and dissolved in ddH_2O. The samples were filtered and injected into an ion chromatograph (ICS-3000, Dionex, USA) equipped with a Carbo PacTMPA20 column (3 mm × 150 mm). The column temperature was kept at 35 °C, and the flow rate maintained at 0.5 mL min^{-1}. Equal gradient of 92.5% A (ddH_2O) and 7.5% B (0.25 mol L^{-1} NaOH) were used for elution. The contents of individual sugars were analyzed using standard curves.

2.3. RNA-Seq analysis

The work was conducted by Majorbio Co., Ltd (Beijing, China) on an Illumina HiSeq™ platform. Total RNA from potato tubers stored at 15 °C, 4 °C and 0 °C for 20 d was used for cDNA library construction. The clean reads were mapped to potato genome (http://solanaceae.plantbiology.msu.edu/cgi-bin/gbrowse/potato/). The read numbers were transformed to FPKM (Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced) value for gene expression quantification. The differentially expressed genes (DEGs) was analyzed using edgeR (http://www.bioconductor.org/packages/2.12/bioc/html/edgeR.html) with following criteria: False discovery rate (FDR) < 0.05 & $|\log_2 FC| >= 1$. Three biological replicates were used in each sample.

2.4. First strand cDNA synthesis and real-time quantitative PCR (Q-PCR)

The Q-PCR was carried out by previously described method with modifications (Lin et al., 2017). First strand cDNA was synthesized using iScriptTM cDNA Synthesis Kit (Bio-Rad). Q-PCR was performed on an ABI 7500 instrument (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). The reaction system of Q-PCR was as follows: 1 μL template, 0.4 μL forward primer (10 μM), 0.4 μL reverse primer (10 μM), 10 μL 2 × TransStart® Top Green qPCR SuperMix (+Dye II) (TransGen Biotech, Beijing, China), and 8.2 μL ddH_2O. The relative expression of genes was normalized with the reference gene *EF1a*. The program was initiated at 95°Cfor 10 min, at 95 °C for 15 s and at 60 °C for 1 min, after which, there were 40 cycles of 95 °C for 15 s and 60 °C for 1 min, followed by a melting curve procedure. The primers used in this research were shown in Supplementary Table 1. Three biological replicates consisting of fifteen tubers were used for gene expression analysis in each sample.

2.5. iTRAQ based proteomic analysis

The total protein content from potato tubers stored at 15 °C, 4 °C and 0 °C for 20 d was analyzed using Bradford protein assay kit. Tryptic digests of 15 °C stored potatoes were labeled with 116, 117 and 118 iTRAQ8 reagents while 4 °C stored potatoes with 117, 118 and 119 iTRAQ8 reagents, and 0 °C stored potatoes with 118, 119 and 120 iTRAQ8 reagents. The peptides were desalted and used for LC-MS/MS analysis. MS/MS spectra were searched with Protein Discoverer™ Software 2.1 against UniProt database. The best match for the peptide mass was used to determine the parent proteins. The searching parameters were up to two missed cleavages of tryptic digestion, carbamido methylation of cysteines and the iTRAQ of N terminus and lysine side chains of peptides as fixed modification, and methionine oxidation and protein N-terminal acetylation as variable modifications. Peptide spectral matches were confirmed based on the q-values at 1% FDR. The work was conducted by Majorbio Co., Ltd (Beijing, China).

2.6. Statistical analysis

Figures in this research were drawn by Origin 8.6 software and Multi Experiment Viewer software (MeV v4.8.1). Least significant differences (LSD) were calculated at 0.05 level by SPSS Statistics 22 Software.

3. Results

3.1. Effects of different temperatures on sugar accumulation in potato tuber during postharvest storage

Four soluble sugars, including sucrose, glucose, fructose, and galactose, were measured in the present research, and sucrose was considered as the predominant soluble sugar in potato tuber (Fig. 1). All the three sugar contents decreased during the whole storage period in potatoes under 15 °C storage, while sugar content increased in potatoes under 4 °C and 0 °C during the whole storage period. Compared to the potatoes stored at 4 °C, the contents of sucrose increased significantly in potatoes stored at 0 °C from 20 d of storage. No significant difference was observed in all the sugar contents in potatoes at 3 d of storage under different temperatures. To better investigate the mechanism of sugar metabolism under cold stress, the potatoes stored at 15 °C, 4 °C and 0 °C for 20 d were used for further research.

3.2. Analysis of differently expressed genes and proteins in potato tubers under different temperature storage

Potato tubers stored at 15 °C, 4 °C and 0 °C for 20 d were used for RNA-Seq and iTRAQ based proteomic analysis. For the RNA-Seq analysis, more than six clean gigabases with a GC percentage above 44.67%, a Q20 percentage above 97.27% and a Q30 percentage above 93.43% were obtained after removing adapters, low-quality regions, and possible contamination. The proportion of total reads in the potato transcriptome libraries that mapped to the potato reference genome ranged from 79.41% to 88.75% (Table 1). The gene expressions were validated by Q-PCR with a correlation coefficient above 0.92 (Supplementary Fig. 1).

The gene and protein expressions among different samples were compared as shown in Figs. 2 and 3,055 DEGs, including 1685 up-regulated and 1370 down-regulated DEGs, were found in the cluster of 4 °C vs. 15 °C, where 51 differently expressed proteins (DEPs) were found, including 41 up-regulated and 10 down-regulated DEPs. In the cluster of 0 °C vs. 15 °C, 7546 DEGs were investigated, including 4293 up-regulated and 3253 down-regulated genes, while 146, including 140 up-regulated and 36 down-regulated, DEPs were found. In the cluster of 0 °C vs. 4 °C, 7432 DEGs were investigated, including 3566 up-regulated and 3866 down-regulated genes, while 64, including 44 up-regulated

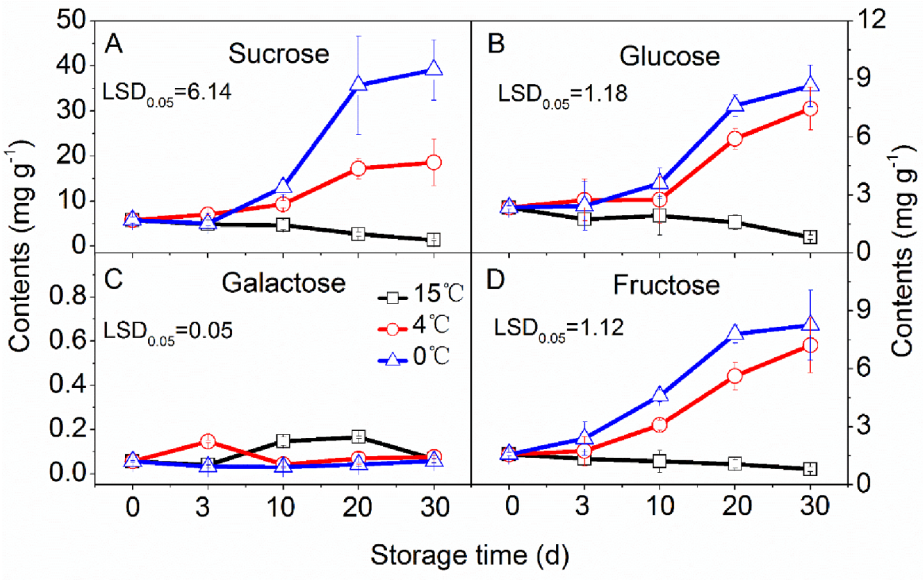

Fig. 1. Effects of different temperature on sugar contents variation in potato during postharvest storage. Sucrose (A), glucose (B), fructose (C), and galactose (D) were detected in the analysis. Error bars represent standard errors calculated from three independent biological replicates. LSDs represent least significant differences at the 0.05 level.

and 20 down-regulated, DEPs were found.

Fig. 3 shows that 10,671 DEGs and 167 DEPs were overlapped among clusters of 4 °C vs. 15 °C, 0 °C vs. 15 °C and 0 °C vs. 4 °C (Fig. 3A). When combining the DEGs and DEPs, 122 factors were significantly expressed in both RNA and protein levels, while 10,549 genes and 45 proteins were not overlapped in the analysis. Thus, the 122 factors that expressed in both RNA and protein levels were exhibited in heatmap, and classified into five sub-clusters according to their expressions (Fig. 3B; Supplementary Table 2). Seven factors were identified in cluster 1, which showed up-regulation in the RNA level and down-regulation in the protein level as storage temperature decreasing. Thirteen factors were identified in cluster 3, which showed opposite expression from cluster 1. The factors in cluster 2, cluster 4 and cluster 5 showed consistent expressions in the RNA and protein levels, with down-regulation in cluster 2 and up-regulation in cluster 4 and cluster 5 as storage temperature decreasing.

3.3. Sugar metabolism in response to cold temperature in potato tubers during storage

The enzymes involved in starch and sugar metabolism in potato tubers were investigated in the research (Table 2). Nine enzymes were significantly expressed in both RNA and protein levels, including granule-bound starch synthase 1 (PGSC0003DMG400012111), beta-amylase (PGSC0003DMG400001549), invertase inhibitor (PGSC0003DMG400004616), sucrose synthase 2 (PGSC0003DMG400013546), UDP-glucose: glucosyltransferase (PGSC0003DMG400019882), pyruvate kinase (PGSC0003DMG400006590), pyruvate decarboxylase (PGSC0003DMG400022953), beta-glucan-binding protein 4 (PGSC003DMG400044116) and fructokinase (PGSC0003DMG400024246). The expression of granule-bound starch synthase 1 (PGSC0003DMG400012111) decreased while the expression of beta-amylase (PGSC0003DMG400001549) increased in both RNA and protein levels as the storage temperature decreased, which indicated that the starch degradation pathway was promoted by low temperatures during storage. Compared to 15 °C and 4 °C stored potatoes, sucrose synthase 2 (PGSC0003DMG400013546) showed up-regulation in both the RNA and protein level at 0 °C storage, but no significant difference was observed between 15 °C and 4 °C stored potatoes. The expression of invertase inhibitor (PGSC0003DMG400004616) was down-regulated by low temperatures in both RNA and protein level, indicating the conversion of sucrose to glucose and fructose under low temperatures. The expression of fructokinase (PGSC0003DMG400024246) was up-regulated by low temperatures in both RNA and protein level, indicating the phosphorylation of fructose in potato tubers under low temperature. Other enzymes showed opposite or irregular expression in RNA and protein level, and will not considered for further analysis in this research.

3.4. Heat shock proteins in response to cold stress in potato tuber during postharvest storage

The Hsps in response to low temperatures in potatoes were investigated and are shown in Table 3. It can be seen that 21 members of Hsps were significantly expressed in potato under low temperatures. Six members showed opposite or inconsistent expression in the RNA and protein levels, including a small Hsp (PGSC0003DMG400003219), four members of Hsp70 (PGSC0003DMG400019208, PGSC0003DMG400008917, GSC0003DMG400000398, PGSC0003DMG400030405) and a Hsp90 (PGSC0003DMG400029787). The other fifteen members,

Table 1
Quality assessment of RNA-Seq data.

Groups	Samples	Total reads	Error (%)	Q20 (%)	Q30 (%)	GC (%)	Total mapped
I	15°C_A	61,722,788	0.0128	98.03	94.45	46.58	79.41%
	15°C_B	74,819,558	0.0127	98.07	94.52	48.67	80.59%
	15°C_C	70,984,330	0.0136	97.75	93.68	45.4	82.81%
II	4°C_A	70,329,580	0.0135	97.81	93.81	44.67	87.29%
	4°C_B	67,378,820	0.0134	97.86	93.94	44.93	87.51%
	4°C_C	73,534,476	0.0134	97.84	93.88	45.44	88.33%
III	0°C_A	73,691,570	0.0135	97.81	93.82	45.27	88.75%
	0°C_B	79,322,524	0.0137	97.67	93.43	45.29	88.03%
	0 °C_C	80,643,766	0.0135	97.74	93.61	44.9	88.72%

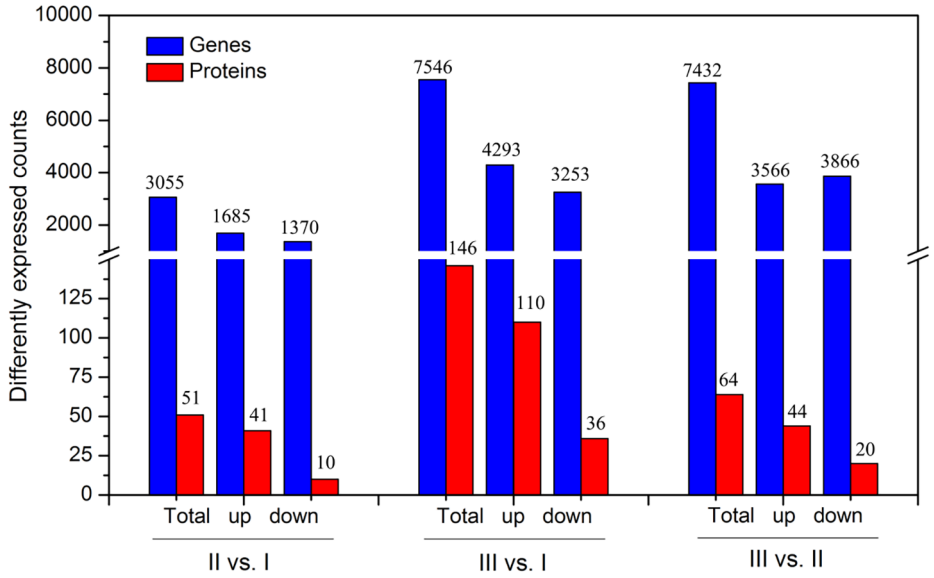

Fig. 2. Differently expressed genes and proteins in potato under different temperature storage. Group I: Potatoes stored at 15 °C for 20 days; Group II: Potatoes stored at 4 °C for 20 days; Group III: Potatoes stored at 0 °C for 20 days.

including three members of Hsp70 family (PGSC0003DMG400000444, PGSC0003DMG400010677, PGSC0003DMG400027750), two members of Hsp80 family (PGSC0003DMG400009509, PGSC0003DMG40 1028907), one member of Hsp90 family (PGSC0003DMG402028907), one members of Hsp100 family (PGSC0003DMG400024644) and eight members of small Hsp family (PGSC0003DMG400028624, PGSC0003DMG400009255, GSC0003DMG400004808, PGSC0003DMG 400011631, GSC0003DMG400030339, PGSC0003DMG400030426, PGSC0003DMG400030341, PGSC0003DMG400039484), were consistently up-regulated by low temperatures in both RNA and protein levels.

4. Discussion

Plant species differ greatly in their ability to acclimate and survive from cold stress. Sugars will accumulate in leaves, bark, fruits and tubers when suffer from cold during development or postharvest storage (Antikainen & Pihakaski, 1994; Jia et al., 2019; Lin et al., 2017; Sauter & Kloth, 1987; Zhang et al., 2018). Soluble sugars are working as osmoprotectants and nutrients, and can interact with the lipid bilayer to protect plant cells from cold stress (Tarkowski & Ende, 2015). In addition, sugars are reported to be primary messengers in signal transduction pathways (Hc & Van, 2017). Increased sugar contents can promote leaf senescence, indicating that the accumulation of soluble sugars might impact negatively on plants during cold stress (Wingler, Purdy, Maclean, & Pourtau, 2006). From our research, the contents of soluble sugars such as sucrose, glucose and fructose increased significantly under low temperatures in potato tuber during storage. The lower the temperature, the higher contents of soluble sugars detected, indicating that the soluble sugars were highly correlated with cold stress in potato tubers during postharvest storage.

Starch is converted to sugars during cold-induced sweetening in potato tubers (Hou et al., 2017). The enzymes of granule-bound starch synthase 1, beta-amylase, sucrose synthase 2, invertase inhibitor, and fructokinase play crucial roles in this process. Starch is degraded mainly through the phosphorolytic pathway, catalyzed by the phosphorylase and amylolytic enzymes in potato tuber, and compared with the rates of respiration, sugar metabolism, and the energetic requirements of different pathways (Isherwood, 1973). From our results, the granule-bound starch synthase 1 was down-regulated while beta-amylase was up-regulated, indicating the starch degradation was promoted by low temperatures. Reports have shown that vacuolar invertase can catalyze sucrose into fructose and glucose, resulting in reducing sugars accumulation, which could be regulated negatively by invertase inhibitor (Lin et al., 2015). From our results, invertase inhibitor was significantly down-regulated in low temperatures, suggesting the importance of invertase inhibitor in regulating reducing sugar accumulation of potato in response to cold stress. The transfer of a phosphate group from ATP to fructose is the initial step in its utilization and specifically catalyzed by fructokinase. The up-regulation of fructokinase under low temperatures indicated rapid utilization of fructose in potato tubers under cold stress.

Stress tolerance is acquired from various stresses to prevent cellular damage and re-build cellular homeostasis in plant. From our results, fifteen Hsps, including Hsp70, Hsp80, Hsp90, Hsp100 and small Hsp family, were significantly induced by low temperature in potato tubers during postharvest storage. Heat shock proteins are important defense induced factors, which have been reported to be in responsive to various stresses (Chen, Feder, & Kang, 2018). The expression of Hsp70 genes is consistent with the degree of thermotolerance, and it also plays important roles in water, salt, and heat stress in plants (Usman et al., 2017). The Hsp90 family is reported to respond to cold, heat, heavy metals, salt stress in Arabidopsis (Krishna & Gloor, 2001; Milioni & Hatzopoulos, 1997). Hsp100 family is induced by many environmental factors, including cold, dehydration, heat, and dark-induced etiolation (Keeler et al., 2000; Queitsch, Hong, Vierling, & Lindquist, 2000). The abundance of small Hsps in plants and their characteristics indicate that they play vital roles in stress tolerance in plants (Sun, Van, & Verbruggen, 2002).

Individual members from different Hsp family have special functions, but the co-operation among them is more important. The various Hsps play complementary or overlapped roles in protecting proteins under stress. Research has reported that small Hsps prevent non-native proteins aggregation by binding to them, thus providing substrates for followed refolding by Hsp70 orHsp100 family (Axel et al., 2003; Axel, Elke, Sonja, Elizabeth, & Bernd, 2010; Veinger, Diamant, Buchner, & Goloubinoff, 1998). Others also proposed that Hsp100s can efficiently resolubilize the protein aggregates, which are then refolded by the Hsp70s; the solubilized proteins might be refolded to form proteins with the assistance of Hsp60s (Ben-Zvi & Goloubinoff, 2001). Similar observations also have been reported in plants (Axel et al., 2003; Lee & Vierling, 2000).

In conclusion, the harvest potato tubers were usually stored at low

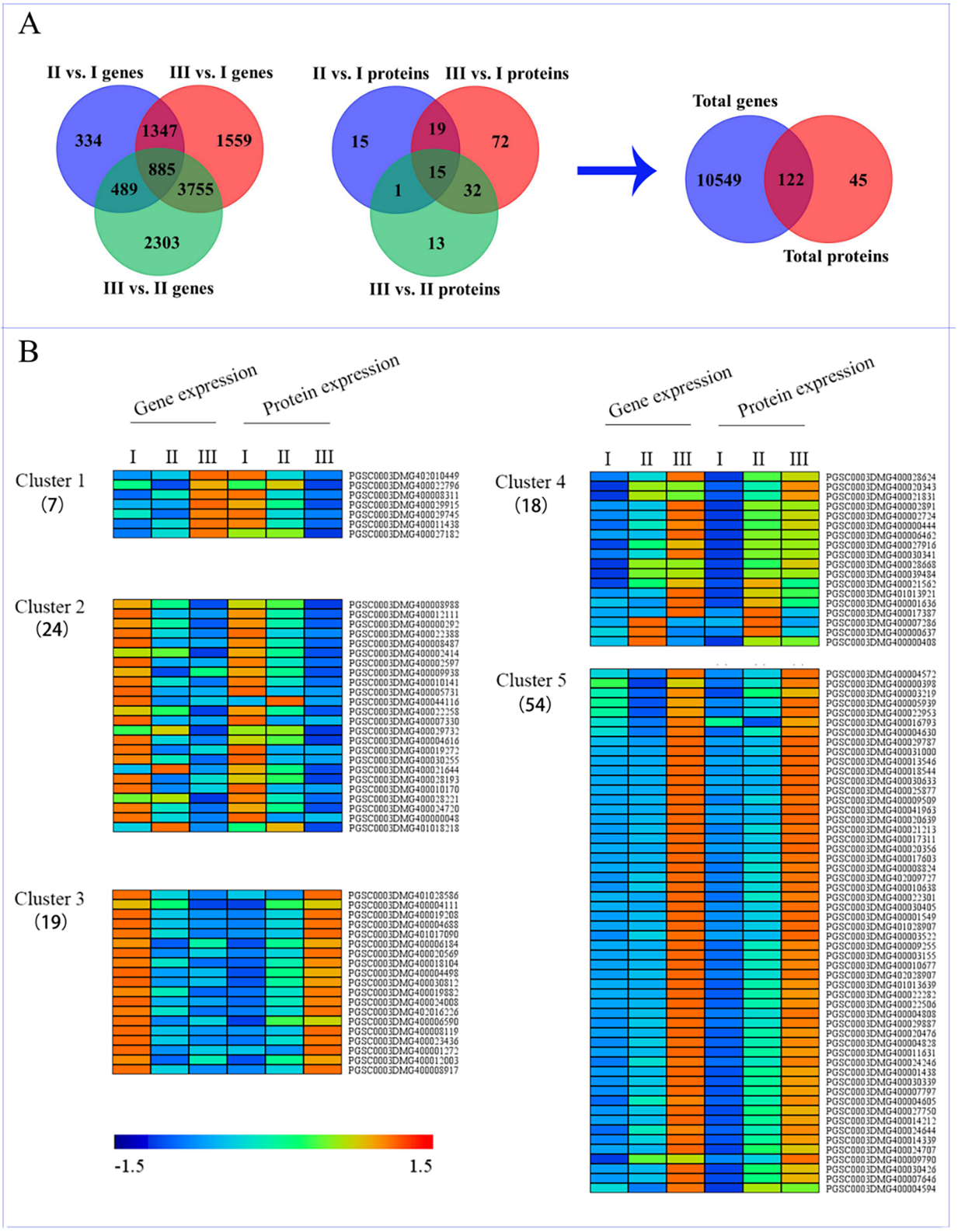

Fig. 3. K-means clustering of differently expressed genes and proteins from potato tubers under different temperature storage. Group I: Potatoes stored at 15 °C for 20 days; Group II: Potatoes stored at 4 °C for 20 days; Group III: Potatoes stored at 0 °C for 20 days.

temperature to inhibit sprouting and decay, which caused a series of physiological and biochemical responses. The soluble sugars such as sucrose, glucose and fructose were significantly increased under low temperature storage through the function of granule-bound starch synthase 1, beta-amylase, invertase inhibitor and fructokinase. Fifteen members of heat shock proteins were induced by low temperatures, which may act individually or synergistically to prevent cellular damage and to re-build cellular homeostasis in potato tubers under cold stress.

Table 2
Selected genes and proteins involved in sugar metabolism in potatoes stored for 20 days under 15 °C, 4 °C, and 0 °C.

Annotation	Gene ID	Gene expression (FPKM value)			Protein expression		
		15 °C	4 °C	0 °C	15 °C	4 °C	0 °C
Granule-bound starch synthase 1, chloroplastic/amyloplastic	PGSC0003DMG400012111	2107.63	587.92	368.67	2.03	1.02	0.01
Beta-amylase	PGSC0003DMG400001549	12.07	32.62	238.40	0.72	0.89	1.62
Invertase inhibitor	PGSC0003DMG400004616	274.19	132.68	85.12	0.91	0.85	0.53
Sucrose synthase 2	PGSC0003DMG400013546	48.3	53.64	1006.84	0.64	0.68	1.47
Fructokinase	PGSC0003DMG400024246	37.82	68.82	138.22	0.83	0.90	1.00
UDP-glucose: glucosyltransferase	PGSC0003DMG400019882	7.26	3.15	0.22	0.83	0.9	1.02
Pyruvate kinase	PGSC0003DMG400006590	202.87	79.83	108.55	0.73	0.88	0.92
Pyruvate decarboxylase	PGSC0003DMG400022953	24.19	13.77	33.40	0.94	1.00	1.14
Beta-glucan-binding protein 4	PGSC0003DMG400044116	57.35	9.07	17.88	0.88	1.09	0.86

Table 3
Heat shock proteins in response to low temperatures in potatoes during postharvest storage.

Classification	Annotation	Gene ID	Gene expression (FPKM value)			Protein expression		
			15 °C	4 °C	0 °C	15 °C	4 °C	0 °C
	17.6 kD class I small heat shock protein	PGSC0003DMG400030339	3.96	60.37	338.74	0.74	1.10	1.42
	17.6 kD class I small heat shock protein	PGSC0003DMG400030426	115.06	913.96	10245.01	0.65	0.96	1.09
	17.6 kDa class I heat shock protein	PGSC0003DMG400030341	36.11	744.48	2516.14	0.8	1.16	1.18
Small Hsp	Small heat-shock protein	PGSC0003DMG400028624	4.13	87.22	310.88	0.87	1.10	1.15
	Small heat-shock protein homolog protein	PGSC0003DMG400009255	2.93	41.71	1914.64	0.78	0.97	1.25
	Mitochondrial small heat shock protein	PGSC0003DMG400004808	43.80	204.03	1314.81	0.59	0.81	1.31
	Chloroplast small heat shock protein class I	PGSC0003DMG400011631	0.53	5.53	51.78	0.73	0.99	1.24
	Low molecular weight heat-shock protein	PGSC0003DMG400039484	1136.29	10516.67	11353.57	0.96	1.22	1.23
	Small heat shock protein, chloroplastic	PGSC0003DMG400003219	0.24	0.06	0.44	0.80	1.19	1.39
Hsp70	Heat shock cognate 70 kDa protein	PGSC0003DMG400000444	25.67	60.19	113.06	0.86	1.07	1.13
	Heat shock 70 kDa protein, mitochondrial	PGSC0003DMG400010677	84.19	133.82	840.31	0.84	0.93	1.06
	Hsc70	PGSC0003DMG400027750	125.46	271.35	1017.29	0.87	1.08	1.21
	Heat shock cognate 70 kDa protein 2	PGSC0003DMG400019208	4846.00	1423.2	794.44	0.82	0.98	1.44
	Heat shock protein 70	PGSC0003DMG400008917	13432.77	5877.31	3821.86	0.80	0.98	1.56
	Heat shock protein 70–3	PGSC0003DMG400000398	472.74	168.73	560.71	0.83	0.99	1.29
	Heat shock cognate 70 kDa protein 1	PGSC0003DMG400030405	82.16	70.79	636.87	0.78	0.96	1.64
Hsp80	Heat shock protein 83	PGSC0003DMG400009509	49.58	128.72	1545.46	0.68	0.91	1.62
	Heat shock protein 83	PGSC0003DMG401028907	2.10	32.57	423.33	0.55	0.76	1.66
Hsp90	Heat shock protein 90	PGSC0003DMG402028907	3.05	31.50	270.32	0.54	0.78	1.32
	Molecular chaperone Hsp90-1	PGSC0003DMG400029787	180.88	132.29	591.29	0.95	0.96	1.19
Hsp100	101 kDa heat shock protein	PGSC0003DMG400024644	56.05	402.78	915.90	0.80	1.01	1.26

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Declaration of Competing Interest

The authors have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.124991.

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