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ORIGINAL ARTICLE

Transcriptome analysis of heat stress response genes in potato leaves

Ruimin Tang[1,2] · Sanjay K. Gupta[3] · Suyan Niu[2,4] · Xiu-Qing Li[2] · Qing Yang[1] · Guanshui Chen[2,5] · Wenjiao Zhu[1] · Muhammad Haroon[2]

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Abstract

Heat stress has a severe impact on potato growth and tuberization process, always resulting in the decrease of tuber yield and quality. Therefore, it is of great significance for potato breeding to illuminate the mechanism of heat stress on potato and explore heat resistant genes. In this study, two cDNA libraries from normal potato leaves (20 °C day/18 °C night) and potato leaves with 3 days of heat treatment (35 °C day/28 °C night) were constructed respectively. Totally, 1420 differentially expressed genes (DEGs) were identified. The expression patterns of 12 randomly selected genes detected using droplet digital PCR agreed with the sequencing data. Gene ontology analysis showed that these DEGs were clustered into 49 different GO types, reflecting the functional diversity of the heat stress response genes. The results of KEGG pathway enrichment showed the potential biological pathways in which the DEGs were involved, indicating that these pathways may be involved in heat tolerance regulation. Most potato heat transcription factors (*StHsfs*) and heat shock proteins (*StHsps*) were not expressed efficiently based on expression profile of these DEGs. *StHsp26-CP* and *StHsp70* were markedly increased after 3 days of heat treatment. These data will be useful for further understanding the molecular mechanisms of potato plant tolerance to heat stress and provide a basis for breeding heat-tolerance varieties.

Keywords Potato · Heat stress · Transcriptome sequencing · Differentially expressed genes · Droplet digital PCR

Introduction

Potato (*Solanum tuberosum* L.) is a thermophilic crop of perennial nightshade and is considered as a vegetable and staple crop in many countries because of its high nutrition contents [1]. During potato cultivation in the field, various abiotic stresses would exert detrimental impact on its growth

Qing Yang is the first corresponding author and Xiu-Qing Li is the second corresponding author.

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✉ Xiu-Qing Li
Xiu-Qing.Li@canada.ca; lixiuqing2008@gmail.com

✉ Qing Yang
qyang19@njau.edu.cn

Ruimin Tang
ruimin_tang829@hotmail.com

Sanjay K. Gupta
gupta020@umn.edu

Suyan Niu
Suyanniu_happy@126.com

Guanshui Chen
gshchenfafu@163.com

Wenjiao Zhu
wenjiaozhu@njau.edu.cn

Muhammad Haroon
Muhamamd.Haroon@canada.ca

[1] College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, China

[2] Fredericton Research and Development Centre, Agriculture and Agri-Food Canada, Fredericton, NB E3B 4Z7, Canada

[3] Department of Soil, Water and Climate, University of Minnesota, 1991 Upper Bufford Circle, St. Paul, MN 55108, USA

[4] Institute of Bioengineering, Zhengzhou Normal University, Zhengzhou 450044, China

[5] College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China

status, such as drought stress, salt stress and extreme temperatures [2]. Among these abiotic factors, temperature is the most indisciplinable factor affecting potato growth and development [3]. For potato, the optimal temperature for the growth of aerial portion is approximately 20–25 °C and the best temperature for tuber formation is around 15–20 °C [4]. Due to global warming prediction, heat stress has become a severe agricultural problem in many areas, with significantly influencing the potato growth and tuberization process and subsequently impacting on the tuber yield and quality [5–7]. Therefore, it is of great significance for potato breeding to illuminate the mechanism of heat stress on potato, reveal the response mechanism of potato to heat stress and explore heat resistant genes.

To delve into the associated response mechanisms to heat stress and characterize the significantly differentially expressed genes in various biological pathways, the next generation sequencing (NGS) technology has been widely used in many plant species [8]. NGS technology, also called massive parallel deep-sequencing technology, can be performed in three platforms: Roche (454) GS FLX sequencer, Solexa/Illumina genome analyzer and Applied Biosystems SOLiD sequencer [9]. These platforms have their own advantages and disadvantages, and have been extensively applied in the biotic and abiotic stress response mechanisms due to their rapid sequencing and lower cost characteristics [6]. For instance, Qi et al. [10] investigated the molecular mechanisms response to water logging stress, and also profiled the expression levels of differentially expressed genes in cucumber plants via this method. In *Ammopiptanthus mongolicus*, the candidate genes and developed SSR markers involved in drought tolerance were identified by this method [11]. And a wide range of heat-associated biological pathways have been mapped in KEGG database and numerous genes have been identified to be related to heat stress in a lot of plant species, such as *Arabidopsis*, switchgrass, spinach and so on [6, 12, 13].

At present, many researches have demonstrated that the expression of many heat response genes could change significantly in a very short time under heat stress, especially *Hsfs* and *Hsps* which are sensitive to high temperature [14]. For example, most *OsHsfs* members were observed to be up-regulated expressed after 10 min of heat stress in rice [15]. In our previous study, we found that the transcript levels of most *StHsf* members began to show dramatic changes at 2 or 6 h, indicating that *Hsfs* are sensitive to heat stress in potato [2]. Similarly, in potato, most *Hsp20* members were extremely increased after being treated for 3 h and 24 h underheat stress, and few members were up-regulated after a 24 h heat stress [14]. However, gene expression is a dynamic process with the extension of heat treatment time. The expression levels of plentiful heat response genes were dramatically increased in the first several hours after heat stress, but decreased when heat lasted for more than several hours [2], suggesting that different kinds of heat response genes perform functions during different periods of heat stress. In many countries, the effects of high temperature on potato cultivation tend to last for 3 days or longer, not only several minutes or several hours.

Therefore, in order to excavate the heat response genes under continuous heat stress, the transcriptome sequencing between non-stressed and 3 days of heat-stressed potato leaves (cv. Russet Burbank) was performed. The differentially expressed genes (DEGs) were identified and aligned to GO database and KEGG database, which provided important information to gain insight into the potential regulatory biological pathways in respond to heat stress. Some candidate genes that likely played vital roles in the relative long-term heat stress were identified based on the differential gene expression profiles. These results will be useful for further understanding the molecular mechanisms of potato plant tolerance to heat stress and provide the theoretical and practical basis for breeding heat-tolerance varieties.

Methods and materials

Plant materials and growth conditions

This research used Russet Burbank (*S. tuberosum*) as the potato material and was conducted in the Fredericton Research and Development Centre, Agriculture and Agri-Food Canada. The 1-month-old tissue culture seedlings were moved in clay pots and grown in an illumination and aeration chamber with 70% of humidity under 14 h light/10 h dark regime at 20 ℃ day/18 ℃ night. Two weeks later, half of the plantlets were moved to another chamber with the same condition except the temperature (35 ℃ day/28 ℃ night) for 3 days; while the rest plants were remained in the original chamber as control. The second fully expanded leaves of the plantlets from two different temperature treatments were harvested and blended separately. All collected samples were immediately immersed in liquid nitrogen and stored at − 80 ℃ prior to RNA extraction.

RNA extraction and library preparation

Total RNA was extracted from 0.5 g potato leaf samples using the RNeasy Maxi kit (Qiagen, Germany). The quality and concentration of the total RNA samples were examined using the 2100 Bioanalyzer (Agilent Technologies) and NanoDrop 1000 Spectrophotometer (Thermo Scientific). After that, the total RNA samples were divided into two parts. One part of the total RNA samples was used for cDNA library construction; the other part was retained for the validation of transcriptome sequencing results.

The messenger RNA (mRNA) was isolated from total RNA sample of each treatment using oligo (dT) attached beads. The purified mRNA was subsequently trimmed into shorter fragments (~20 nt) by specific buffer and reverse-transcribed to synthesize cDNA. After end-repair, 3′ end adenylation and ligation of the Illumina sequencing adapters, the cDNA fragments were purified and amplified by PCR to build the final libraries.

Transcriptome sequencing and analysis of differential expression genes (DEGs)

The tested cDNA libraries were ready for RNA sequencing on a HiSeq-2000 platform at the Canadian Centre for Computational Genomics. Millions of short sequencing fragments (reads) were obtained. The raw reads were pre-processed by trimming from the sequencing adapters using Trimmomatic [16] and removing the low quality reads whose length less than 32 bp. The filtered reads were aligned to a potato reference genome (*S. tuberosum* assembly 4.03) using STAR [17].

Different exploratory data analyses were conducted in gene expression levels to detect the potential outliers by exploring the homogeneity of biological replicates and appreciating the global effects of the different experimental variables. The differential gene expression analysis was performed using DESeq [18] and edgeR [19] of R Bioconductor packages.

Validation of the RNA-seq results using droplet digital PCR

RNAs were extracted from non-stressed and 3 days of heat-stressed leaves as described above. The specific primers were designed by Primer-Blast in NCBI website (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Droplet digital PCR (ddPCR) was performed on QX200 Droplet Digital PCR System (Bio-Rad) which consists of the QX200 Droplet Generator and the QX200 Droplet Reader. The PCR samples were mixed by 2 µL DNA sample (2–4 ng), 0.5 µL of 10 µM primer mixture, 11.5 µL EvaGreensupermix and 9 µL ddH_2O. Then 20 µL PCR samples were transferred into the individual sample walls of the 8-channel droplet generator cartridge (DG8, Bio-Rad) and 70 µL of droplet generation oil was added to the oil walls of DG8 cartridge. After the creation of monodispersed droplets in the QX200 Droplet Generator, 40 µL droplets were used for PCR using the following cycling program: 95 °C for 5 min, then 40 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, 4 °C for 5 min, 90 °C for 5 min and 10 °C for keeping the PCR products. The numbers of PCR-positive and PCR-negative droplets were counted by the associated QuantaSoft™ software of the QX200 Droplet Reader. The expression of each gene was normalized and calibrated against the reference gene *EF1α*, which has a relative stable expression level under different conditions.

Functional annotation and analyses of gene ontology (GO) and KEGG pathway enrichment

GO was used to describe the function attributes of genes or gene products in organisms. GO enrichment analysis illustrates the significantly enriched GO items in DEGs compared with the genome background, and provides a method to elucidate which biological functions are significantly associated with the DEGs [6]. The sequences of all the detected genes were blasted to the Gene Ontology database using Blast2GO to extract their GO annotation information [20]. Then topGO function in bioconductor software was applied to perform GO enrichment analysis of the differentially expression genes with the p value ≤ 0.05 [21].

To further understand the biological function of DEGs and to detect the main pathways in which the DEGs involved, the gene sequences were mapped to the KEGG database by BLASTX with an E-value cutoff of $< 10E{-}5$ (https://www.genome.jp/kegg/). According to the KEGG annotation information of the differentially expressed genes, the pathway enrichment analysis of these genes was conducted using hypergeometric inspection [22]. After multiple testing corrections, the pathways with p value ≤ 0.05 were selected as the significant enriched pathways.

Results

Construction of expression profile of DEGs in potato leaves under heat stress

Potato plantlets with similar growth status were treated with different temperatures (control check: 20 °C day/18 °C night; heat treatment: 35 °C day/28 °C night). As shown in Fig. 1, compared with the control group, part of the leaves of the potato plants in the treatment group were slightly darker in color and covered with a thin layer of wax. This structure helps to reflect sunlight and reduce radiation absorption.

The second fully expanded leaf (usually the fourth or the fifth leaf) from each plant after 3 days of different treatments was collected and used for RNA sequencing by Illumina Hiseq-2000 system. After trimming the Illumina adapters and removing the low quality reads, a total of 48,646,835 and 46,181,488 clean paired reads were obtained from 49,200,771 and 46,654,357 raw paired reads which produced from non-stressed (CK) and heat stressed (HS) cDNA library, respectively. Finally, 21,681 genes were identified by alignment of the filtered reads and a potato reference genome (*S. tuberosum* assembly 4.03). Among

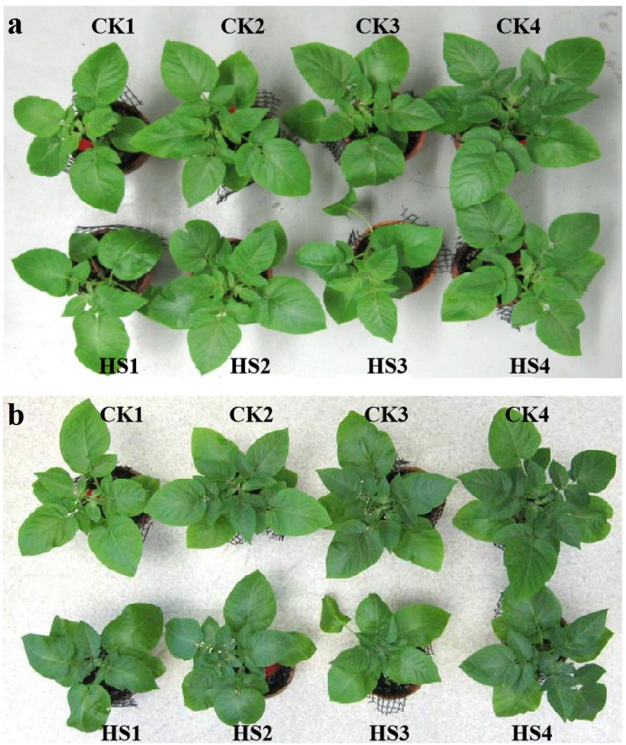

Fig. 1 Potato plants before and after different temperature treatments. **a** Potato plants before treatments and **b** potato plants after 3 days of different temperature treatments. *CK* contrast check (20 °C day/18 °C night), *HS* Heat stressed potato plants (35 °C day/28 °C night)

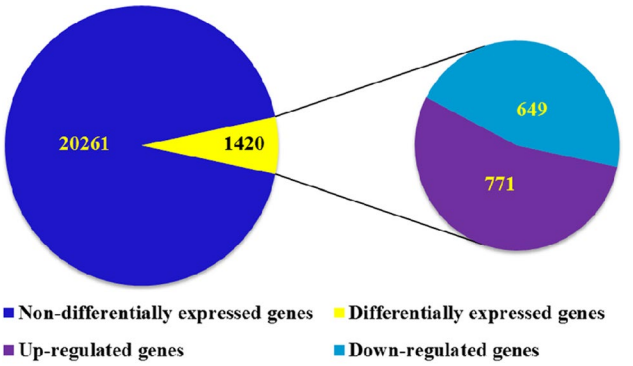

Fig. 2 Numbers of DEGs between potato leaves under different temperature treatments. Totally, 21,681 genes were identified by alignment of the filtered reads and a potato reference genome (*Solanum tuberosum* assembly 4.03). Among these detected genes, 20,261 genes showed no significantly differential expression; 1420 genes showed significantly differential expression levels between HS and CK cDNA samples: 771 and 649 genes were found to be over-expressed and under-expressed respectively. *CK* 20 °C day/18 °C night, *HS* 35 °C day/28 °C night

these detected genes, 1420 (7%) genes showed significantly differential expression levels between HS and CK cDNA samples (deseq p value ≤ 0.05 or edger p value ≤ 0.05, and $|\log_2$ (fold change)| > 1): 771 (4%) and 649 (3%) genes were found to be over-expressed and under-expressed respectively (Fig. 2). The transcriptome sequencing information of these 1420 DEGs were presented in ESM 1. The identification of these DEGs paves the way for further investigation on molecular mechanisms of heat tolerance in potato.

3.2. Validation of Illumina-generated expression profile by droplet digital PCR analysis

To verify the reliability of RNA sequencing results, the expression patterns of 12 randomly selected genes were detected using droplet digital PCR (ddPCR) analysis. The primer pairs of these genes were presented in Table 1. The ddPCR data showed that the expression trends of these 12 genes were basically consistent with the result of RNA sequencing detection (ESM 2), and the two results had a very high correlation under two different detection methods (Fig. 3). Therefore, the results obtained from transcriptome sequencing were credible and could be used for the further analyses.

Functional category of DEGs by GO-term analysis

In this study, these 1420 identified DEGs were aligned to GO database and classified into different categories according to their functions. Finally, the DEGs could be grouped into 49 GO categories after cutting off the GO terms including less than 9 genes (Fig. 4, ESM 3). Of these GO annotations, 27 GO terms were associated with biological process (BP), 16 GO terms were assigned to molecular function (MF), and 6 were assigned to cellular component (CC). In the category of biological process, "response to stimulus", "response to stress", "response to chemical" and "response to abiotic stress" including 127 (8.94%), 70 (4.93%), 57 (4.01%) and 36 (2.54%) DEGs respectively, were the predominant GO terms. GO terms like "catalytic activity" and "oxidoreductase activity" were the leading terms in the category of molecular function, especially "catalytic activity", which contained the most DEGs (21.83%) among these 49 GO terms. In cellular component category, "plastid" and "plastid part" were the most representative terms and contained 55 (3.87%) and 31 (2.18%) DEGs, respectively. These results suggested that high temperature treatment may lead to various stresses on plants except heat stress, such as chemical stress, light stress and oxidative stress. Synergistic effects of intricate stresses might activate the gene expression of numerous enzymes like oxidoreductase, hydrolase, transferase and peptidase, to resist the adverse environment. Furthermore, under heat stress, the genes involved in the synthesis of plastid components were probably activated and helped to alleviate the stress damage on cellular components in plant organisms.

Table 1 List of primer sequences of 12 selected genes and reference gene used for the ddPCR analysis

Primer name	Forward primer	Reverse primer
α-xyl	CCTGTGGATGCCTGCTTGTATTC	GTATCCCTGTAACCAGCCCTCAT
Rubisco	GACAACACGCTGGATGGATTCT	TGACTTCCCTTGACCTTTCCCT
BAG6	CCACAGAGCAGCCTACAGATACT	GGATAGTTGAGGTTCCACCGATT
C2H2	CGCAAGAGACAGGTCATCATCAC	TCCAACTCATCAACATCGCTACC
Hsf30	CCAGTGCTGAGAACCTTCAAGATG	CAACAGAAGCCGACCTGACATTG
R2R3-myb	AGGAACTTCTACGATACGGGTGG	ACATCCATAGAGTGTTTGGGCAAT
Ubi	CGTCAGCGGGAATCAATAAAGGAT	CCTGAATGGCAGCCTTAACATCT
Hsp70	GTGAAGGCTACTGCTGGAGACAC	CTGAGCAGTGGATGAAAGGGTCC
As-pero	CAAGACAGAACCACCTCCAGAAG	CCAGAGTGTGACCACCAGATAAAG
WRKY	TTCATTTGATGCCACACCAACAC	CATCCAAGGTCTCAAGTTGTGTCT
CoA-redu	TCCGACCTACGACGAATTGATGG	TCATGTTGGTCCCTCTTCCTTGT
HsfA3	CGGAAGTTCTTCTGGATCATCTG	CCATCTGCTTCTGTCTTTGTTCT
EF1α	CTGTTAAGGATCTGAAGCGTGGT	AATGTGGGAAGTGTGGCAGTCG

Primer name	RNAseq (log_2 fold change)	ddPCR (log_2 fold change)
α-xyl	-4.824702155	-3.301301444
Rubisco	4.899948541	4.87082335
BAG6	3.666442323	3.746022478
C2H2	-4.857280151	-5.900556662
Hsf30	-3.612183968	-4.026557707
R2R3-MYB	-5.452200057	-4.372729363
Ubi	-3.372741886	-3.693761096
Hsp70	0.684039286	0.548387301
As-pero	3.414071085	3.214208753
WRKY	4.187996575	2.714599415
CoA-redu	4.075763669	3.494108349
HsfA3	-1.748445089	-1.915990178

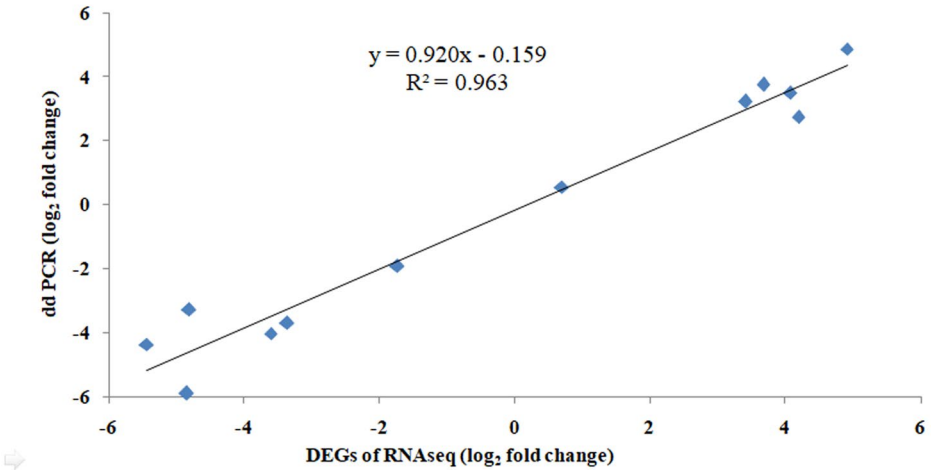

Fig. 3 Correlation analysis of the results obtained from RNA-seq and ddPCR. Twelve DEGs, randomly selected from the RNA-seq data, were used to detect their expression changes between CK and HS by the method of ddPCR. The value in the table above showed the expression changes of DEGs under two different methods, that is log_2 (DEG expression amount under heat stress/its expression amount under normal condition)

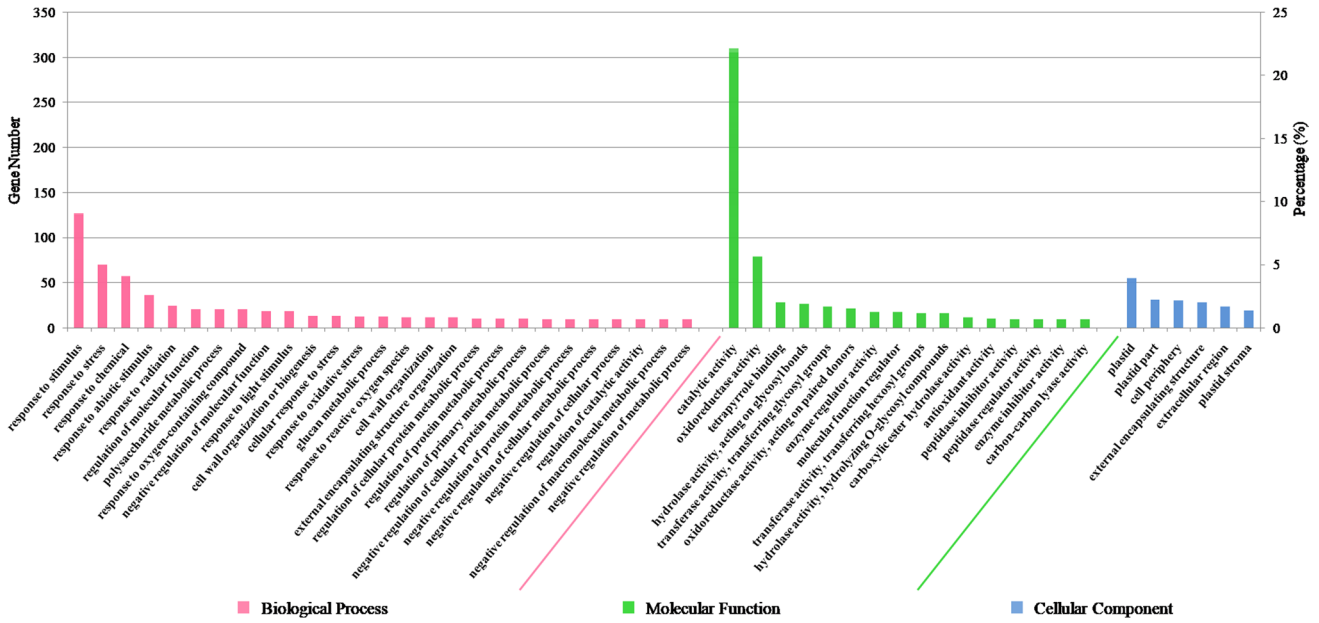

Fig. 4 Gene ontology classification analysis of DEGs between non-stressed potato leaves and heat-stressed potato leaves. GO functions were represented in X-axis; the number of DEGs annotated in each GO term was presented in left Y-axis; and the right Y-axis showed the percentage of DEGs which were annotated in each GO term

Analysis of KEGG pathways enrichment of DEGs

In order to further investigate the biological functions of the differentially expressed genes, these DEGs were mapped to the KEGG database and were found to be involved in 110 metabolic pathways (ESM 4). The top 20 enriched biological pathways were shown in Fig. 5 according to enrichment significance. The pathways with p value ≤ 0.05 were considered as significant enriched pathways. They were mainly related to "Limonene and pinene degradation" (ko00903), "starch and sucrose metabolism" (ko00500), "plant hormone signal transduction" (ko04075), "Carotenoid biosynthesis" (ko00906), "Stilbenoid, diarylheptanoid and gingerol biosynthesis" (ko00945), "plant pathogen interaction" (ko04626) and so on.

Moreover, among these pathways, "Metabolic pathways", "Biosynthesis of secondary metabolites", "Plant-pathogen interaction", "Plant hormone signal transduction" and "Starch and sucrose metabolism" contained more DEGs (203, 130, 93, 79, 32 DEGs respectively) than any other pathways (Fig. 5, ESM 4). These results indicated that the expression levels of genes involved in these biological pathways changed significantly to respond to the heat stress. Also, the transcriptomic data provided basis for the deep research aimed at investigating the regulation mechanism of specific pathways in potato leaves under heat stress.

Expression analysis of *Hsfs* and *Hsps* in potato leaves under heat stress

Previous studies have shown that *Hsfs* and *Hsps* are sensitive to heat stress, which means they could be expressed in large quantities within a few minutes. Based on the result of transcriptome sequencing, only two *Hsf* members were identified to be differentially expressed. They were *HsfA2* (PGSC0003DMG400008223) and *HsfA3* (PGSC0003DMG401002683), whose expression levels were dramatically down-regulated in potato leaves after 3 days of heat treatment (Table 2). Unlike *Hsfs*, the expression of many *Hsps* had significant changes, such as some *Hsp90s*, *Hsp70s* and *sHsps* members. These *StHsps* could be enriched in the metabolic pathway of "protein processing in the endoplasmic reticulum" (Fig. 6). As shown in Fig. 6, the expression amounts of most *Hsps* in the cytoplasm was markedly decreased after 3 days of heat treatment (marked with green box). However, the expression levels of some *Hsp70s* and *sHsps* members were up regulated significantly (marked with red box). Totally, 9 *Hsps* with up-regulated expression were screened out (Table 2). Among them, the expression of *Hsp26-CP* (PGSC0003DMG400003219) and *Hsp70* (PGSC0003DMG400027611) were extremely up-regulated (more than 10-fold) under heat stress, especially *sHsp-CP* whose expression increased to about 433-fold compared with the control. These results indicated that sHsp-CP and Hsp70 might play an important role in protecting cells

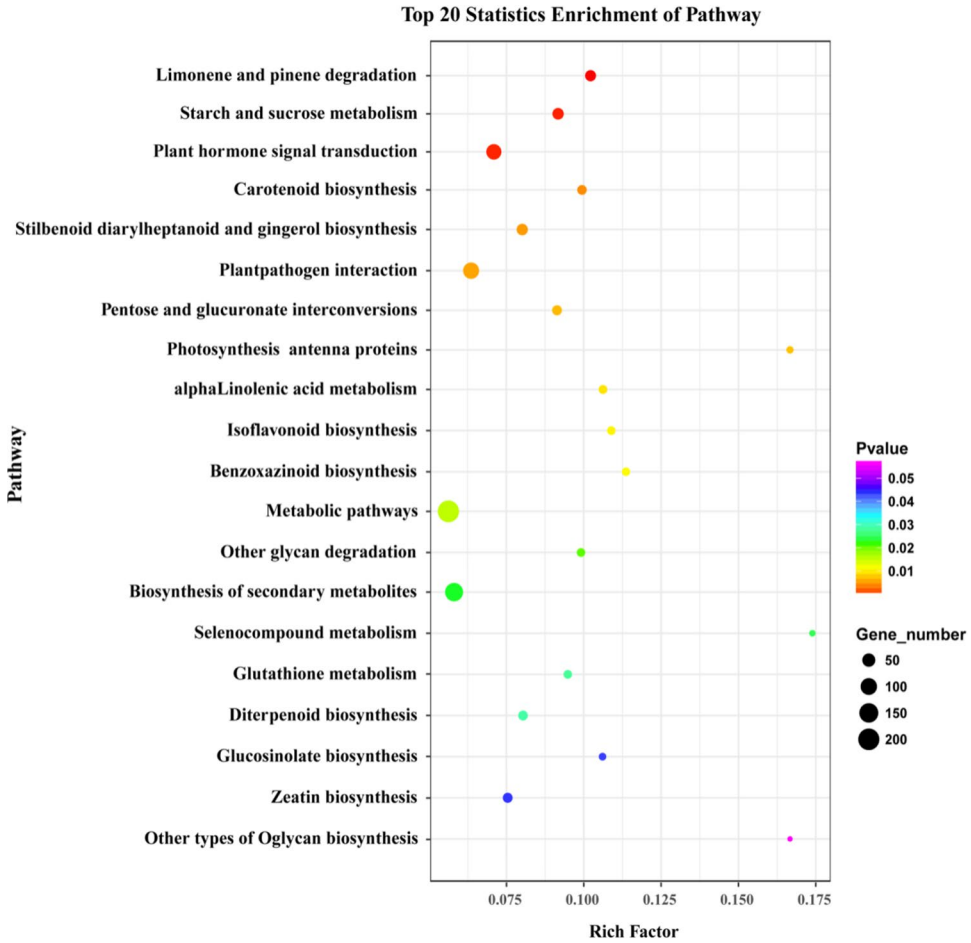

Fig. 5 Top 20 biological pathways enrichment of the DEGs in potato leaves after 3 days of heat treatment. The dot size represented the number of the differentially expressed genes involved in each pathway. The different dot color represented the p value of each pathway; the pathways with p value ≤ 0.05 were significant enriched pathways. Rich Factor represented the ratio between the number of DEGs and all genes which were annotated in each pathway. (Color figure online)

and tissues during the long-term thermal stress response of potato.

Discussion

The differentially expressed genes in response to heat stress in potato leaves have diverse functions

Heat stress becomes a major concern for crop growth, development and productivity due to global warming effect [23]. During the summer, potato plants often suffer from heat damage lasting for 3 days or longer. In order to reflect the actual situation during the potato planting period, the gene expression profiles were constructed in potato leaves under 3 days of heat treatment. Through transcriptome sequencing, the expression patterns of potato genes in response to heat stress at high temperature were analyzed, and heat-resistance related genes were identified. This study provides a basis for the study of the molecular mechanism of potato heat resistance.

Russet Burbank, which was used as the plant material in this research, is a very important and prevalent potato cultivar in the world, and occupied 70% of the processed potato market in North America [24]. After 3 days of high temperature treatment, several leaves of heat-stressed plants covered with a thin layer of wax, which helps to reflect sunlight and reduce radiation absorption (Fig. 1). According to RNAseq data, a total of 1420 DEGs were identified, including 771 up-regulated genes and 649 down-regulated genes (Fig. 2, ESM 1). The method of droplet digital PCR (ddPCR) was used to validate the credibility of the results obtained from Illumina platform. The detection results under two different methods had the similar trend, and the correlation coefficient between the RNA-seq data and ddPCR test results was 0.9352 (Fig. 3), suggesting that the RNA-seq results were reliable for further research.

These identified DEGs were subjected to GO enrichment analysis. Totally, 21% DEGs were found to be involved in "catalytic activity" (GO: 0003824), suggesting that heat stress triggered the expression changes of many genes encoded the enzymes which participate in different regulatory pathways in response to adverse environment (Fig. 4). For example, under heat stress, the

Table 2 Expression changes of Hsfs and Hsps among the DEGs

ID	Gene_symbol	log_FC	HS	CK
PGSC0003DMG400008223	Heat_shock_factor_protein_Hsf30 (HsfA2)	− 3.666	35	428
PGSC0003DMG401002683	Heat_stress_transcription_factor_A3	− 1.807	1064	3575
PGSC0003DMG400003219	Small_heat_shock_protein,_chloroplastic	8.696	12,572	29
PGSC0003DMG400027611	Heat_shock_protein_70	3.339	707	67
PGSC0003DMG400024707	Luminal_binding_protein (Bip)	3.461	93	8
PGSC0003DMG400009255	Small_heat-shock_protein_homolog_protein	3.189	58	6
PGSC0003DMG400012480	Heat_shock_protein_70_(HSP70)-interacting_protein	2.767	22	3
PGSC0003DMG400030340	17.6_kD_class_I_small_heat_shock_protein	2.153	56	12
PGSC0003DMG400030340	17.6_kD_class_I_small_heat_shock_protein	2.153	56	12
PGSC0003DMG400011631	Chloroplast_small_heat_shock_protein_class_I	2.146	323	70
PGSC0003DMG400039484	Low_molecular_weight_heat-shock_protein	1.96	620	153
PGSC0003DMG400004784	Heat_shock_protein_70_(HSP70)-interacting_protein	− 1.932	3559	13,043
PGSC0003DMG400030339	17.6_kD_class_I_small_heat_shock_protein	− 1.971	146	550
PGSC0003DMG400014405	Heat_shock_cognate_protein_80	− 1.98	850	3221
PGSC0003DMG400021737	Class_II_small_heat_shock_protein_Le-HSP17.6	− 2.019	161	627
PGSC0003DMG400031821	Heat_shock_protein_binding_protein	− 2.562	17	97
PGSC0003DMG400004808	Mitochondrial_small_heat_shock_protein	− 2.577	291	1668
PGSC0003DMG400019137	18.1_kDa_class_I_heat_shock_protein	− 2.781	130	859
PGSC0003DMG400008554	Heat_shock_protein_binding_protein	− 2.945	3	23
PGSC0003DMG400030405	Heat_shock_cognate_70_kDa_protein_1	− 3.211	730	6492
PGSC0003DMG400009173	Small_heat_stress_protein_class_CIII	− 3.471	15	161
PGSC0003DMG400030542	Heat_shock_protein_binding_protein	− 3.485	1	12
PGSC0003DMG400019136	18.1_kDa_class_I_heat_shock_protein	− 3.588	3	36

synthesis of starch might be hindered because of down-regulated *starch synthase* gene (*SS*). In contrast, the gene encoded β-amylase was expressed significantly up-regulated, which promotes the hydrolysis of starch. This result was in accordance with the trends of starch content and reducing sugar content in tubers (ESM 5). In addition, many DEGs were activated to respond to different stimulus (GO: 0050896), stresses (GO: 0006950), chemical (GO: 0042221) and oxidative stress (GO: 0006979) to help the plants survive in the adverse environment caused by heat. Heat stress always causes other stresses, such as drought stress and oxidative stress [25, 26]. The interaction of these multifarious stresses has a worse effect on plants growth and development [23]. Similar results have been found in other plant species, such as switchgrass and spinach [6, 13]. Besides, many genes were clustered into the terms of "plastid" (GO: 0009536) and "plastid part" (GO: 0044435), indicating that these genes, such as a potato chloroplast sHsp (PGSC0003DMG400003219), would participate in repairing the cellular components, especially plastid components, to alleviate the destruction of membranaceous structures of cell and organelle caused by high temperature. In *Arabidopsis*, it has been demonstrated that AtHsp21 (a kind of chloroplast sHsp) could interact with the plastid nucleoid protein pTAC5 to maintain the function of plastid-encoded RNA polymerase, PEP [27]. However, the exact role of this sHsp in potato remains unknown.

The analysis of enriched pathways for differentially expressed genes in this study specifically showed the most potential biochemical pathways in which these DEGs involved under heat stress (Fig. 5). The major pathways included limonene and pinene degradation (map00903); starch and sucrose metabolism (map00500); plant hormone signal transduction (map04075); plant–pathogen interaction (map04626); metabolic pathway (map01100); biosynthesis of secondary metabolites (map01110) and so on. Under heat stress, most of the DEGs participated in the metabolic pathway which consists of a connected series of biochemical reactions catalyzed by abundant enzymes in a cell. Also, various signal transduction molecules were produced to generate a series of innate defensive reactions in response to heat stress [23, 28]. For example, as a stress hormone, ABA has been demonstrated to play an important role in the regulation of heat stress. In *Arabidopsis*, ABA signaling pathway and ABA-mediated heat responses were connected by HsfA6b [29]. In addition, many DEGs were observed to take part in the plant–pathogen interaction pathway and biosynthesis of secondary metabolites. Sustained high levels of heat stress may lead to plant susceptibility to pathogens

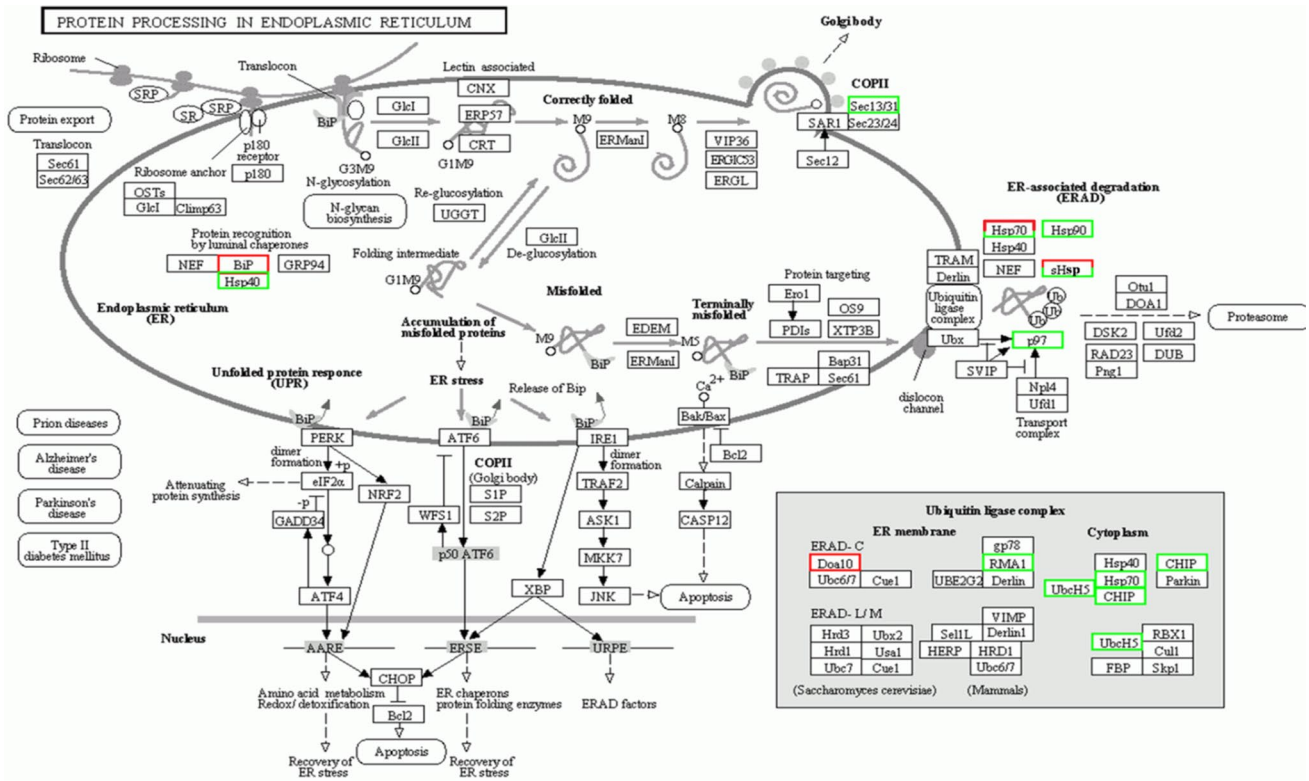

Fig. 6 Expression changes of Hsps in potato leaves with 3 days of heat treatment. The gene in the red box had significantly up-regulated expression; gene in the green box had significantly down-regulated expression; gene in the gray box was unsignificantly expressed. Heat stress condition: 35 °C day/28 °C night; normal condition: 20 °C day/18 °C night. (Color figure online)

[30, 31]. To defend against plant pathogens, many secondary metabolites, such as flavonoid, were induced to inhibit fungal growth [32, 33]. Therefore, many DEGs were also observed to be clustered in the plant–pathogen interaction pathway and biosynthesis of secondary metabolites under heat stress.

Most potato *Hsfs* and *Hsps* cannot be expressed efficiently under long-term heat stress

In our previous research, 27 *Hsfs* were identified in potato and most of them were highly expressed under 2 h of heat stress [2]. However, only *StHsfA2* and *StHsfA3* were observed to be significantly down-regulated in potato leaves under 3 days of heat treatment; while the expression of other *Hsfs* members had no significant difference. As the downstream genes of *Hsfs*, only few *Hsps* were expressed significantly increased after heat treatment. These results indicated that the continuous high temperature has a very serious effect on the expression of *Hsfs*, thus affecting the expression of their downstream genes. Numerous studies have shown that the expression of most *Hsfs* and *Hsps* could be activated in a short time after heat shock. For example, in rice, 16 of the 23 *OsHsfs* genes were significantly up-regulated expressed after 10 min to 30 min of heat stress [15]. In pepper, most *CaHsfs* and *CaHsp20s* were found to be strongly expressed after short-term thermal stress (40 ℃ 2 h) [34, 35]. Potatoes and peppers, both of which belong to the nightshade family, are closely related. Therefore, the expression of most *StHsfs* and *StHsps* in potato might be activated under short-term heat stress, but gradually decreased under continuous heat treatment.

Although most Hsps are activated under short-term heat stress, some *Hsps* genes with significantly up-regulated expression, such as *Hsp26-CP* and *Hsp70*, could still be found in potato leaves after 3 days of heat treatment. Hsp70 is one of the most conserved and widely distributed heat shock proteins. Under non-stress condition, Hsfs remain inactive state by binding with Hsp70s. While under heat stress, Hsp70s preferentially bind to the denatured proteins, and the released Hsfs would form into an active trimer that is transferred to the nucleus to activate the expression of *Hsps* [36]. Hsp70s can help the denatured proteins fold correctly, degrade denatured proteins and move them out of the cell to prevent protein aggregation. Therefore, they play an important role in plant response to heat stress [37].

StHsp26-CP, which belongs to sHsp family, was significantly expressed after heat stress. Although the

transcription level of the gene cannot directly reflect its protein level, it can still provide a basis for in-depth study of the function of Hsps [38]. Transcriptome sequencing results showed that the expression levels of most cytoplasm *sHsps* and mitochondria *sHsps* were significantly down-regulated; while some chloroplast *sHsps* were up-regulated expressed significantly, especially *Hsp26-CP*, whose expression was 433 times as that of the control group. Therefore, the high level expression of *Hsp26-CP* under continuous heat stress indicated that Hsp26-CP is likely to play an important role in the response of potato to long-term heat stress. It has been found that the chloroplast sHsp (AtHsp21) could maintain the function of plastid-encoded RNA polymerase (PEP) and protect chloroplast by binding to plastid transcriptionally active 5 (pTAC5) in *Arabidopsis* [27]. This result is consistent with the GO clustering analysis (Fig. 4), indicating that numerous heat stress response genes might be involved in the repair of plastid components. However, further studies need to be conducted to find out whether potato chloroplast sHsp performs a similar function as AtHsp21.

Conclusion

In conclusion, a total of 1420 DEGs induced by 3 days of high temperature (35 °C day/28 °C night) were identified in potato leaves. Among them, 771 genes were significantly up-regulated and 649 genes were significantly down-regulated. These DEGs could be clustered into 49 different GO types and enriched into multiple metabolic pathways, reflecting the diversity of their functions. The transcriptome profiling of potato leaves between two different treatments facilitated the identification of major DEGs and regulatory mechanisms for the heat tolerance of potato plant. After 3 days of heat stress, most *StHsfs* members and *StHsps* members were not efficiently expressed, and only a few *Hsps* members (such as *Hsp26-CP* and *Hsp70*) could be expressed at high level. These data provide a basis for the study of gene function and the mechanism of heat tolerance regulation.

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Author contributions RT, QY, and XL conceived and designed the research. MH, RT and XL performed ddPCR analysis. RT, SN, GC and MH collected the samples. RT, WZ and SG analyzed the data and performed the bioinformatics analysis. RT and SN determined the starch content of potato tubers. SG detected the reducing sugar content in potato tubers. RT and QY wrote the manuscript. XL and SG edited the English language in this manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This research is about transcriptomic analysis in plant (potato). Human participants/animals were not involved in this study.

Informed consent Not applicable in this study.

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