Journal of Plant Biochemistry and Biotechnology (2023) 32:328–337
https://doi.org/10.1007/s13562-022-00808-y

ORIGINAL ARTICLE

Transcriptome analyses show changes in heat-stress related gene expression in tomato cultivar 'Moneymaker' under high temperature

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Received: 3 April 2022 / Accepted: 31 August 2022 / Published online: 5 October 2022

Abstract

Tomato production losses are closely associated with heat stress, mainly attributed to global warming and climate change. The present study aimed to comprehensively analyze the comparative performance of heat-sensitive tomato cultivar 'Moneymaker' under high temperature. Experimental results indicated that tomato seedlings subjected to a high temperature (42 °C) for 6 h did not show wilting phenotype and exhibited higher levels of antioxidant enzyme activities, but obvious wilting was observed in tomato seedlings exposed to the same temperature for 12 h. Correspondingly, antioxidant enzyme activities decreased significantly in the leaves of tomato plants. Using transcriptome sequencing, we found that 4696 genes (2059 up-regulated and 2637 down-regulated) were differentially expressed under high-temperature treatment for 6 h. Gene ontology (GO) annotation showed that expression of genes related to cellular process, metabolic process, and signal-organism process was significantly altered, most of which were downregulated during the high-temperature treatment. In particular, genes related to oxidoreductase activity, hydrolase activity, and protein kinase activity showed the most significant changes during the high temperature treatment, which is consistent with the findings that genes involved in response to stress and stimulus, such as *HSP17.6 A, HSP21, HSP70* were significantly enhanced in vitro. Furthermore, the appearance of many novel transcripts under high temperature indicates that these newly identified genes potentially participate in this complex process. This study provides a basis for breeding heat-resistant tomato varieties.

Keywords Transcriptome · High temperature · Gene · Tomato

Introduction

Tomato is one of the most economically important crops worldwide; however, its production is threatened by extreme environmental conditions such as high temperature (Fang et al. 2021; González-García et al. 2019). Global warming affects plant growth and yield mainly due to the damaging effect of high temperatures on plant development (Bita & Gerats 2013). Indeed, high temperature (> 40°C) can reduce tomato yields by up to 50% (Hsin et al. 2016). Continuous exposure of tomatoes to high temperature, especially in Southern parts of China, is a major constraint in tomato cultivation (Du et al. 2003) and has resulted in the cessation of tomato production in some regions. In certain cases, costly cooling equipment has been installed to decrease temperature during tomato cultivation (Yang et al. 2020). Despite significant yield losses under high-temperature conditions, tomato production is still a major economic activity in Southern China due to its high yields and abundant rainfall predominance in these areas (Cheng et al. 2009). Therefore, improving tomato varieties for heat tolerance is significant in enhancing yield and broadening genetic diversity of tomato.

The susceptibility of tomato to high temperatures varies between its growth stages. Tomato is relatively heat-sensitive at germination and seedling stages but generally tolerant to high temperatures of up to 40 °C during other growth stages (Ibrahim and El-Muqadam 2019; Mario et al. 2011). High temperature can suppress stem elongation, leaf expansion, and root growth at the 4-6-leaf stages in tomato (Hlaváčová et al. 2018; Liu et al. 2002). Extreme high temperatures can cause plant wilting or even plant death. From a physiological perspective, high temperature can significantly decrease net photosynthetic rate, accelerate

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chlorophyll degradation, and inhibit the accumulation of soluble solids at the seedling stage, further impacting the number of flowers per plant and indirectly reducing the yields in subsequent growth stages. Reduction in the ability to accumulate heat shock proteins (Hsps) was identified as a major cause of low growth vigor and yield loss in a tomato variety 'Amateur' under heat stress (Piterková et al. 2013). Increasing the levels of Hsps enhanced heat tolerance of tomato at the seeding stage (4-leaf stage). Further analysis showed that heat stress induces Hsp synthesis in tomato plants at early seedling stage (Ai-Li et al. 2013). Hsps are regulated by heat shock factors (Hsfs), which as molecular chaperone specifically recognize heat shock elements (HSE) in the promoter domains of Hsps to regulate the fold, transfer, and stability of Hsps (Cui et al. 2015; Qu et al. 2013). Thus, the levels of Hsps and Hsfs are one of the main factors affecting heat tolerance of tomato at the seedlings stage. However, some genes have also been implicated in response to stimulus, stress, and signal transduction (Xiao et al. 2017). Hydrolase was also shown to participate in heat-stress regulatory network in plants under high temperature (SøRensen et al. 2005; Yamanouchi et al. 2002). Mutual recognition and regulation of these regulatory pathways during plant response to high temperature is complex. Therefore, unraveling the role of genes related to heat-stress remains a very challenging research area.

The development of high-throughput sequencing and genome sequence data announcement in tomato has provided important methods and references for exploring complicated regulatory networks and discovering new key genes (Yuval and Speed 2012). Transcriptome sequencing (RNA-seq) has successfully revealed the mechanism of plant response to heat stress in Solanum crops such as pepper (Yang et al. 2022), eggplant (Lv et al. 2019), and tomato (Wen et al. 2019). Wen et al. (2019) discovered the key genes associated with heat tolerance in tomato (*SlCathB2*, *SlGST*, *SlUBC5*, and *SlARG1*) by RNA-sequence technology. Similar results were also reported by Jahan et al. (2021), who analyzed RNA-seq data and identified 8 genes linked to protein processing and photosynthesis under high temperature in tomato. With the advancement in molecular biology techniques, more genes involved in plant response to biotic stress will be discovered in the future.

Although Hsps and Hsfs have been identified in tomato (Bhadwal 2017), several genes related to high-temperature response have not been characterized in the crop. Considering the sensitivity of tomato growth to high temperatures at the seedling stage, it is important to explore the changes in gene expression in response to high temperatures at this stage. Thus, in the current study, we focused on analyzing the differentially expressed genes in tomato under high temperature during the seedling stage. Whole-genome transcript profiling of tomato cultivar 'Moneymaker' was performed. Tomato cultivar 'Moneymaker' was selected because of its sensitivity to high temperatures at the seedling stage. Physiological characterization of the cultivar 'Moneymaker' was also performed, and gene expression results were interpreted in the physiological context.

Materials and methods

1 Plant material and culture conditions

Tomato cultivar "Moneymaker" plants were grown in a pot with 5 L soil in a growth chamber under 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity and 16 h daily photoperiod. The day and night temperatures were 28 °C±2 °C and 20 °C±2 °C, respectively. The plants were watered once every 3 days, and the relative humidity in the chamber was kept between 65–70%. Tomato seeds were kindly provided by College of Agriculture, Ningxia University (W 106.1' N 38.5'), Yinchuan, Ningxia 750,002, P. R. China.

2 Heat treatments

High temperature experiments were conducted as described previously with slight modification (Karkute et al. 2021). Briefly, tomato seedlings at 4-6-leaf stage were exposed to 42 °C for 3 h, 6 h, 12 h, 24 h, and 48 h. Leaves were sampled from the seedlings at each time point for subsequent analysis. Each treatment contained 5 seedlings and was performed in triplicate. Control plants were subjected to 28 °C instead of 42 °C, with other conditions remaining the same.

3 Measurement of antioxidant enzyme activities and malondialdehyde (MDA) content

Antioxidant enzyme activities and MDA content were measured in the leaves of tomato seedlings. Each experiment was conducted in triplicate. MDA content was measured using thiobarbituric acid reaction as described previously (Li et al. 2013). Superoxide dismutase (SOD) activity was assayed according to the method described previously (Charles & Irwin 1971); Catalase (CAT) activity was determined using the method of Aebi (1984); Ascorbate peroxidase (APX) activity was determined in supernatant as described previously (Nakano et al. 1980), while peroxidase (POD) was measured and calculated based on the method described previously (Dionisio-Sese and Tobita 1998).

4 RNA extraction, transcriptome sequence, and data analysis

Three biological replicates were used in each experiment to ensure reproducibility and reliability of the results. Leaf samples were collected and placed in liquid nitrogen for trituration. The samples were then transferred to Trizol reagent (BBI, Shanghai, China) for RNA extraction. The concentration of RNA was determined and confirmed using Eppendorf Biophotometer and electrophoresis. Total RNA was purified further using RNeasy Mini Kit (QIAGEN).

Construction of cDNA library: Poly A + was smashed by supersonic wave to purify mRNA samples. The first cDNA strand was synthesized in reverse transcriptase system (m-Mulv, Jinrui company, China) using fragment mRNA as a template and random oligonucleotide as a primer. Then, the RNA strand was degraded by RNaseH, and the second strand of cDNA was synthesized from dNTPs using DNA Polymerase I system. The purified cDNA was repaired, and A and sequencing joints were added. For PCR amplification, 200 bp cDNA was screened using AMPure XP Beads. The PCR products are purified again using AMPure XP Beads to obtain the library.

Library quality inspection: To ensure sequencing quality, we adopted a strict quality control of accusation library construction. The testing criteria were as follows:① Analyzing the integrity of sample RNA and DNA contamination using gel electrophoresis; ② Detecting RNA purity (OD260/280 and OD260/230 ratio) using NanoPhotometer instrument; ③ Accurately quantifying RNA concentration using Agilent 2100 BioAnalyzer on Qubit2.0 Fluorometer instrument; ④Accurately detecting RNA integrity using Agilent 2100 bioanalyzer.

Analysis process: eligible RNA samples were sequenced by Illumina platform to generate raw reads. The raw reads were screened to get clean reads which mapped to reference genomes efficiently. Gene expression in the transcriptome was measured by RSEM (RNA-SEQ by Expectation Maximization). The q-value of the gene < 0.005 and |FoldChange | > 2 were considered to be significant differences for screening differential genes. Statistics of differentially expressed genes (DEGs) were performed, and GO and KEGG function analyses were conducted for genes with significant expression to discover the metabolic pathways involved in key differential genes. GSEA analysis was performed to confirm the relationship between DEGs.

5 qRT-PCR analysis

To determine the accuracy of the transcriptome data, we performed qRT-PCR to analyze the transcriptional levels of key representative genes (*SlHSFA1*, *SlHSFA2*, *SlHSP20.1*, and *SlHSP70*) in response to heat stress (Klaus-Dieter et al. 1998). Total RNA extraction and purification were performed as described above in this sub-section, and the first strand of cDNA was synthesized using the SYBRGreenPCR Master Mix (Takara, Bio, Japan). The gene-specific primers were designed using Primer software (version 5.0, Premier Company, Canada) (Table S1). qRT-PCR was performed in a qRT-PCR instrument (qTOWER3, Germany). The ubiquitin-conjugating gene *SlUbi3* was utilized as an internal control for normalizing gene expression (Hoffman et al. 1991). The $2^{-\Delta\Delta CT}$ method was used to analyze the relative transcript levels of the genes (Wang et al. 2015).

6 Statistical analysis

SPSS software (version 19.0, SPSS, Inc., USA) was used to analyze the data. Statistical significance was inferred at $p < 0.05$. The analyzed data are presented as means ± SD of three replicates for all measured parameters.

Results

1 Effect of high temperature on tomato growth

Figure 1 shows the performance of tomato seedlings under high temperature. Compared with the control, visible symptoms of plant wilting were aggravated in tomato seedlings exposed to 42 °C for 12 h (Fig. 1 A, 1D, and 1 F). However, no obvious symptoms were observed on tomato seedlings exposed to the high temperature for 3 and 6 h (Fig. 1B-Fig. 1 C). These results suggest that tomato seedlings can tolerate high-temperature stress for short periods.

2 Effect of high temperature on antioxidant enzyme activity and gene expression

High temperature stress tolerance of tomato seedlings was investigated at physiological and biochemical levels. The levels of SOD, POD, and CAT activities in the leaves of tomato seedlings were increased after 42 °C treatment for 3 and 6 h, but the enzyme activities were significantly higher at 3 h than at 6 h time (Fig. 2 A-Fig. 2 C). Notably, the trend was more obvious in CAT activity compared to POD and SOD activities (Fig. 2 C). Conversely, the MDA content decreased after 3 and 6 h of heat treatment, but the content was higher at 3 h than at 6 h time point (Fig. 2D). Meanwhile, the levels of all antioxidant enzyme activities reduced significantly in tomato seedlings exposed to 42 °C for 12 h, 24 h, and 48 h. In contrast, increased MDA contents were observed at the three time points, which was comparable to

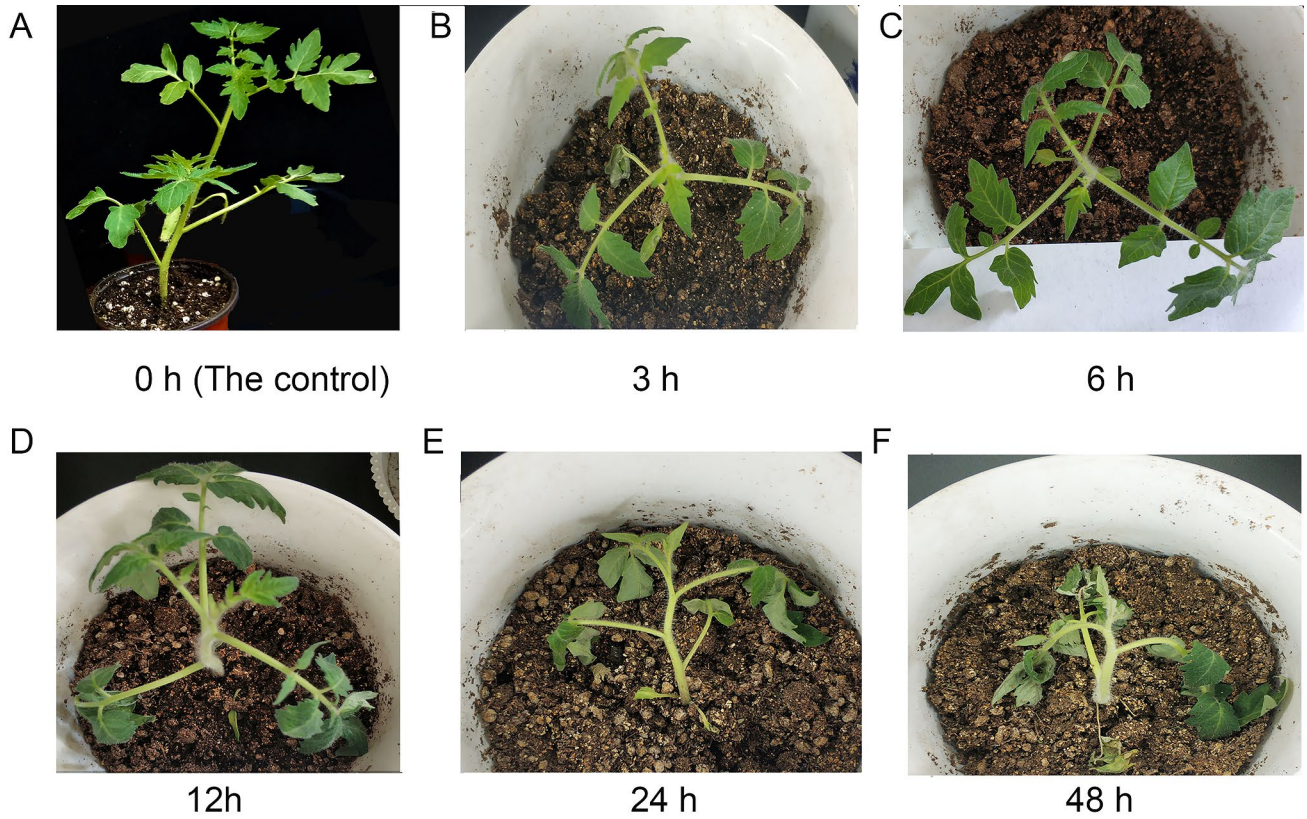

Fig. 1 Phenotype in the seedlings of tomato treated by heat stress The seedlings at 4-leafed-6-leafed was placed in the chamber with 42 °C for (A) 0 h, (B)3 h, (C) 6 h, (D) 9 h, (E) 12 h and (F) 48 h and recovery for 1 h. Each treatment includes three individuals

the content found in the tomato seedlings exposed to 42 °C treatment for 6 h.

Further, we analyzed the transcript levels of key genes related to heat stress, including *SlsHSP20.1*, *SlHSP70*, *SlHSF1*, and *SlHSFA2*, in the tomato leaves by qRT-PCR (Fig. 2E). The results showed that these genes, especially *SlHSFA2* gene, were expressed in the leaves of the tomato seedlings under high temperature (42 °C), but more strongly at 6 h followed by 3 h time points; however, the expression levels of the genes reduced significantly after 12 h. Notably, *SlsHSP20.1* showed the lowest expression level during the high temperature treatment. These results suggest that short–term exposure to heat stress can enhance the tolerance of tomato plants to high temperature by up-regulating heat-stress related genes and improving the levels of antioxidant enzyme activities.

3 Effect of high temperature on gene expression

Transcription sequencing was conducted further to test whether the expression of certain genes plays a role in tomato response to high temperature. The results showed that high temperature altered the expression of 5146 genes in the tomato plants. Among them, 2509 genes were consistently up-regulated while 2637 genes were down-regulated (Fig. 3 A). Further analysis of these genes showed that 1039 genes were localized in membrane, 504 genes in intrinsic component of membrane, 277 genes in cell periphery, and 277 genes in cell junction (Fig. 3B). All the detected transcripts were further analyzed and divided into three categories: cellular component, biological process, and molecular function, according to their expression profiles. A high percentage of the genes were implicated in biological processes, such as response to stress or stimulus, cellular response to stimulus, and cell communication (Fig. 3 C). In addition, some nosig genes were up-regulated in tomato seedlings under high temperature, and these new genes occupied the least proportion among all the differentially expressed genes; thus, their function needs to be analyzed further (Fig. 3D).

Gene Ontology (GO) analysis was conducted to evaluate the potential functions of genes with altered transcription levels under high temperature. The genes whose expression levels changed by more than 1.6-fold during high temperature treatment were analyzed and finally classified into 52 main GO categories (Fig. 4 A). Functional categories such as cellular process, metabolic process, single-organism process, catalytic activity, binding, organelle, and cell part

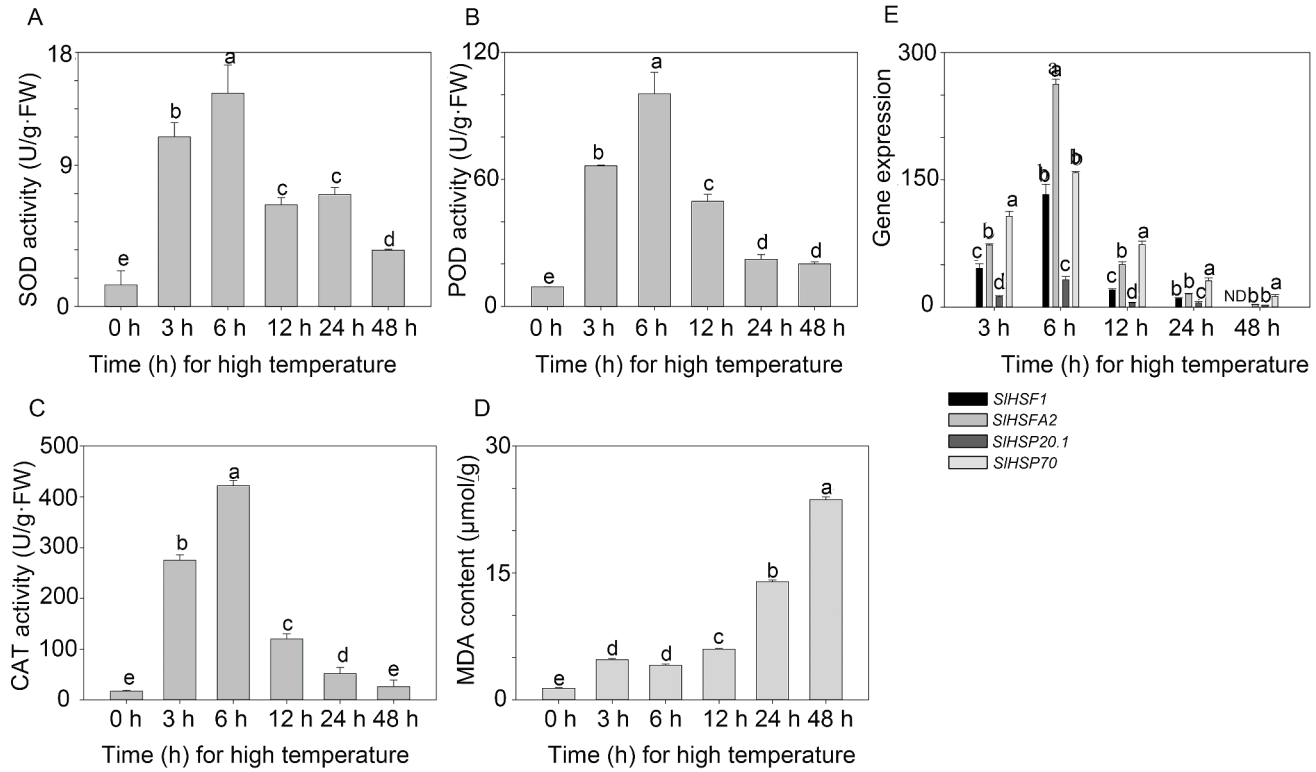

Fig. 2 The levels of antioxidase activities and MDA content in the leaves of tomato exposed to high temperature (A) SOD, (B) POD, (C) CAT and (D) gene expression The leaves were sampled at the 4-leafed-6-leafed stage, and each treatment includes three individuals. Each bar represents the means of three determinations ± SD, and values followed by different lowercase letters indicate significant difference among treatments ($P < 0.05$). FW and ND presents "Fresh Weight" and "No data"

were overrepresented under high temperature. Under high temperature, the DEGs mainly aggregate into the categories of metabolism, genetic information process, environment information process, and organism system (Fig. 4 C). To further reveal the differences between the control and the treatment groups, we displayed diagrams of various pathways related to these above-mentioned processes. The results showed that the number of genes whose expression levels changed in response to stress or stimulus under high temperature occupied 30.41 and 40.57% of the total genes, which was more than genes in other pathways (Fig. 4B and Table S2). Notably, these DEGs were directly or indirectly related to hormone signaling, suggesting that tomato seedlings may adapt to high temperature by regulating their hormone levels (Fig. 4B).

Furthermore, we screened 100 key genes expressed under heat stress from the DEGs by real-time fluorescence quantitative PCR (qPCR). A comparable number of 50 genes, especially those related to HSP and hormonal signaling, were differentially up-regulated in the leaves of the tomato seedlings under heat stress. Notably, some uncharacterized genes were also observed among the DEGs. Most down-regulated genes encode enzymes and other proteins, while some are involved in transcription factors (such as HSP family and WRIKY family) and structural genes. In addition, some hormone signaling-related genes, such as *ER24*, and *ABP19a*, were observed among the DEGs (Fig. 5).

Discussion

High temperature stress often affects physiological and biochemical development, resulting in reduced yield of tomatoes (Karkute et al. 2021). Therefore, it is essential to use high-quality tomato varieties and develop related technology to improve the growth and yield of tomatoes. The tomato cultivar 'Moneymaker', which is a high-yielding tomato cultivar was used in this study. This variety originally came from Netherlands and was bred widely for field production globally (Koornneef and Hanhart 1990). Despite its high yielding potential, the cultivar lacks essential stress resistance genes, making it easily vulnerable to various adverse and unfavourable factors (Li et al. 2014; Litvin et al. 2016). Interestingly, this study found that 'Moneymaker' could grow for more than 6 h at a high temperature of 42 °C, which was further characterized by biochemical and

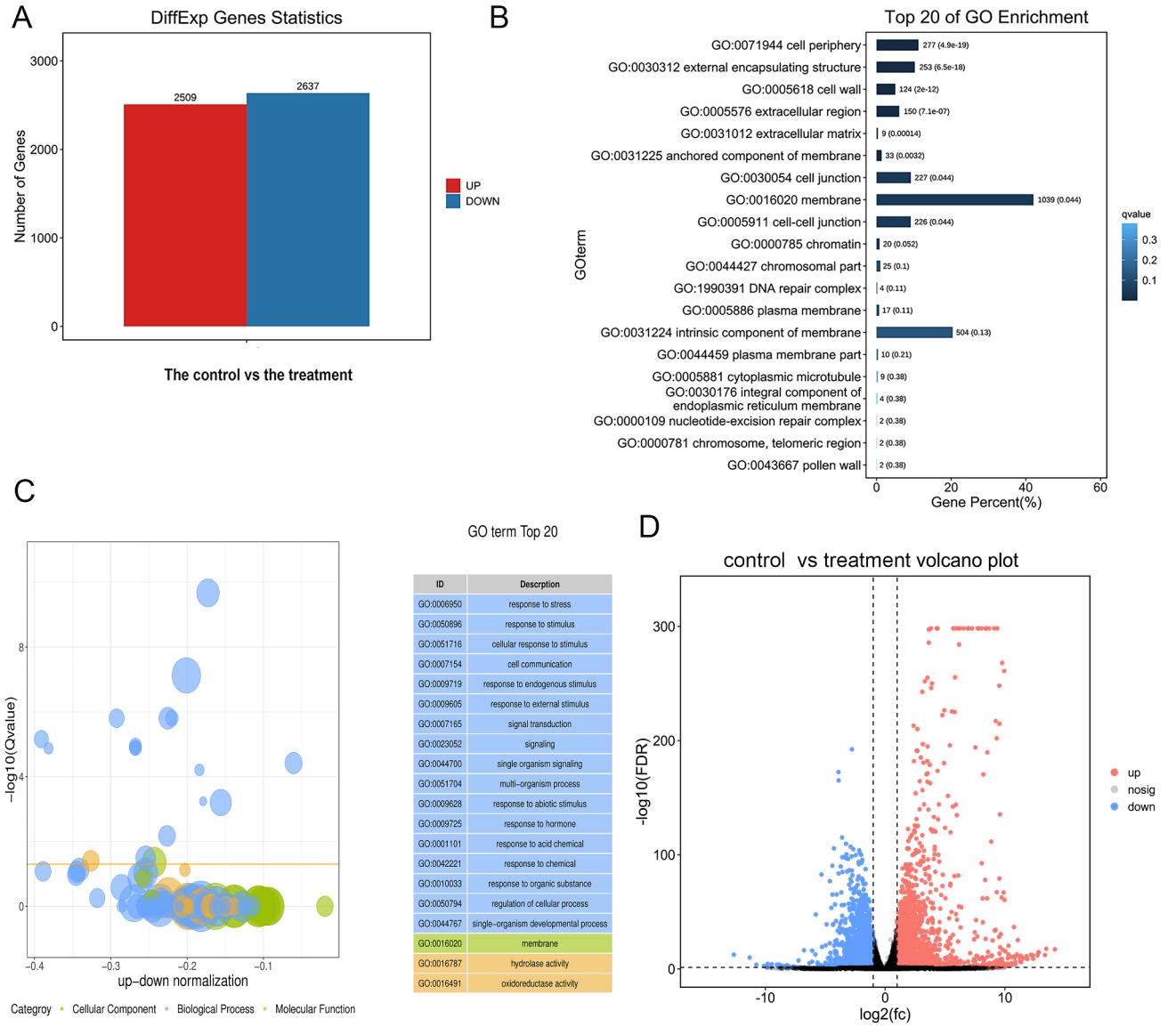

Fig. 3 GO analysis based on transcriptome data in the leaves of tomato treated by heat stress based on transcriptome sequence (A) number of differential genes, (B) top 20 of GO enrichment, (C) normalization of up or down genes and (D) volcano map of control vs. treatment

molecular parameters. This suggests that this tomato line can temporarily be resistant to high temperatures. This finding will establish the genotype as a source of genes for high-temperature stress tolerance.

The effects of stress are generally assessed by studying various parameters such as the content of antioxidants, membrane damage, and expression of stress-responsive genes (Sang et al. 2016). In this study, all these parameters were analyzed in the tomato cultivar 'Moneymaker'. The primary effect of high-temperature stress at the cellular level in plants involves damage to the cell membrane and cellular macromolecules (Liu, 2020). Specific sugars or proteins on the surface of cytomembrane perceive external signals, which are then transmitted inside the cell to induce cell metabolism when plants are subjected to environmental stress (Koiwa 2009; Luu & Maurel 2013). This could explain why most differentially expressed genes (DEGs) in this study were related to cell membrane (Fig. 3B). Additionally, this study observed that the short-term heat stress enhanced the activities of the three enzymes (SOD, POD, and CAT), but this may not be the primary reason for enhanced heat tolerance in the 'Moneymaker'. The accumulation of heat-shock proteins (HSPs) is considered an important indicator of heat-tolerance in tomato cultivars. Plants rapidly synthesize HSPs in large quantities when subjected to heat stress (Khan et al. 2020; Sadura et al. 2020; Gul et al. 2021). Heat shock factors (HSFs) regulate the synthesis of HSPs, which inhibit the uncorrected folding and transfer

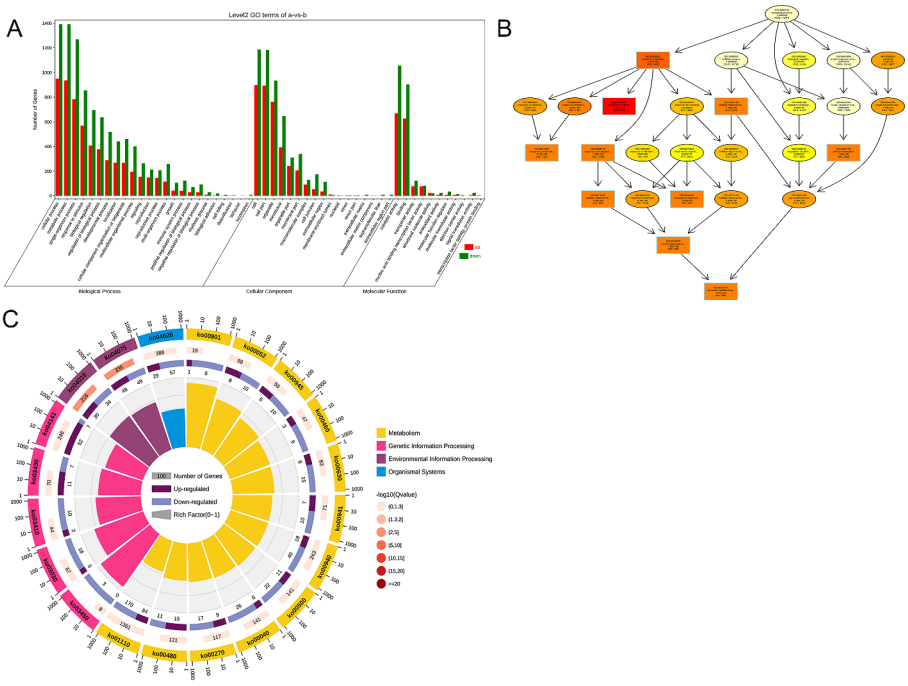

Fig. 4 KEGG analysis in the leaves of tomato treated by heat stress based on transcriptome sequence (A) genes number in different classification, (B) relationship of regulatory pathway among all differential genes, (C) Clustergram of genes involved different metabolic pathways

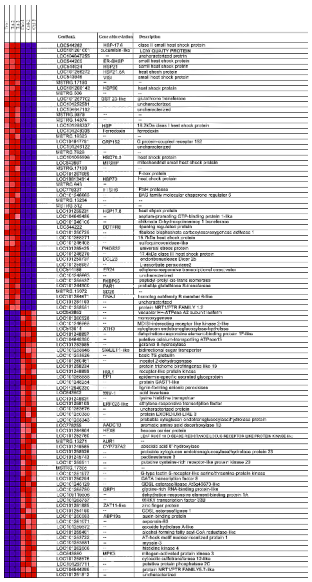

Fig. 5 Expression of key differential genes in the leaves of tomato under high temperature The leaves were sampled at the 4-leafed-6-leafed stage, and each treatment includes three individuals

of some proteins in plant cells (Lang et al. 2021; Zhai et al. 2017) also revealed that heat stress of 42 °C enhanced plant tolerance to high temperature by inducing the activities of antioxidant enzymes and *HSP* gene expression. Similar results were also found in tomato (Ali et al. 2021), pepper (Feng et al. 2019), arabidopsis (Lin et al. 2018), and rice crops (Yeh et al. 2002). Thus, we can speculate that HSP, or respond gene expression, but not the antioxidant enzymes levels, are the main and direct factors causing the improved heat tolerance in the 'Moneymaker' tomato cultivar. However, the induction mechanism at high temperatures is not widely known.

Transcriptome sequencing technology has been widely used in molecular biology studies of various plants (Crawford et al. 2010). In some Solanaceae crops, such as tomato, this technology has been widely applied (Almeida et al. 2020). RNA-seq analysis carried out in this study also identified some stimulus or stress-related genes (Fig. 3 C). The sequence data can be further analyzed by quality assessment in the current study (Table S1-S3). In this study, we found that more than 5000 genes were differentially expressed at a high temperature, and these genes were involved in HSP synthesis and hormone signaling. For example, *HSP21*, *HSP17.1 A*, and *ER24* were among the 50 up-regulated genes (Fig. 5). Interestingly, all the differentially expressed genes were directly or indirectly related to hormone signaling pathways (Fig. 4B), indicating that hormone signaling plays a crucial role in plant response to heat stress. A recent study revealed that tomato seedlings exposed to a high temperature of 41 °C exhibited higher levels of gibberellin (GA) and abscisic acid (ABA) than control plants (Zheng et al. 2020). The HSP family potentially enhances the adaptation of tomato seedlings to elevated temperatures by inducing the accumulation of hormones such as jasmonic acid (JA) (Havko et al. 2020). Therefore, hormone accumulation in plants may be considered as an indicator of plant response to high temperatures. It is noteworthy that more genes were down-regulated under high temperature treatment, which is

consistent with the results of previous studies (Cohen and Leach 2020; Almeida et al. 2020).

Large-scale analysis of the transcriptome data showed that some genes related to immunity were enriched in multiple pathways, such as HSPs accumulation and hormne metabolism (Zheng et al. 2020). The top significantly enriched KEGG pathway was the hormone signaling pathway and HSPs bio-synthetic pathway (Fig. 4). Under long-term high-temperature exposure, these metabolites are widely distributed as key trace substances in plants. Especially, HSP accumulation is crucial for tissue homeostasis and innate immune responses against high temperature (Sadura, 2020). Gene expression also showed that abundant genes related to metabolism, such as HSP70, ER24, ABP19a, were significantly enriched under high-temperature stress (Fig. 5). They involved in heat shock protein metabolism, ethylene metabolism, abscisic acid metabolism and so on. In addition, we found some new genes using transcriptome sequencing technology (Fig. 3D). This may be associated with heat stress-induced oxidation-reduction reactions, which have been shown to stimulate the expression of certain heat stress-related genes (Gaspar et al. 2002). These genes will further provide more genetic information to improve the heat tolerance of tomato plants and need to be studied in more detail in the future. We predict that tomatoes adapt to high-temperature stress through various metabolism and related pathways.

Conclusion

In the present study, transcriptome analysis was used to explore the regulatory effect of heat stress-related genes on the seedlings of tomato cultivar "Moneymaker." Based on phenotyping results, we found that tomato seedlings could resist heat stress for up to 6 h, which may be related to higher levels of antioxidant enzyme activities. Furthermore, transcriptome analysis revealed that heat-stress activated genes involved in biological processes and cell components. qRT-PCR and transcriptome data also revealed that HSP and HSF genes were up-regulated in the tomato seedlings under heat stress. Notably, all differentially expressed genes were either directly or indirectly related to hormone signaling pathways. This study provides a basis for breeding tomato varieties with heat stress resistance.

Abbreviation list

ABA	abscisic acid
APX	ascorbate peroxidase
CAT	catalase
DEGs	differentially expressed genes
GA	gibberellin
HSE	heat shock element
HSF	heat shock factor
HSP	heat shock protein
JA	jasmonic acid
MDA	malondialdehyde
qPCR	real-time fluorescence quantitative; PCR
POD	peroxidase
SOD	superoxide dismutase

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13562-022-00808-y

Acknowledgement Authors wishes to express thanks to Professor Xiao-Min Wang and Meng Guo for suggestion and for providing the test platform and sample.

Author contributions Hai-Zhe Su designed experiments and carried out experiments; J.S. analyzed experimental results. Guo-Xin Cheng analyzed data and wrote the manuscript.

Funding This work was supported through funding from the Natural Science Foundation of Ningxia Province (CN) (No. 2020AAC03092), the National Key Research and Development Program of China (2021YFD1600300), and Key R & D Program of Ningxia Province (2021BBF02024).

Statement and declarations

Ethics approval and consent to participate Not applicable.

Consent for publication All authors approved for publication.

Availability of data and materials All data generated or analyzed in this study are included in the published article and supplementary files.

Competing Interests The authors declare that they have no conflict of interest.

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