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Early transcriptional responses to chilling stress in tomato fruit with hot water pre-treatment

Abraham Cruz-Mendívil[a], José A. López-Valenzuela[a,b], Carlos L. Calderón-Vázquez[c], Misael O. Vega-García[a,b], Cuauhtémoc Reyes-Moreno[a,b], Angel Valdez-Ortiz[a,b,*]

[a] Programa Regional de Posgrado en Biotecnología, Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa (FCQB-UAS), Culiacán 80030, Mexico
[b] Posgrado en Ciencia y Tecnología de Alimentos, FCQB-UAS, Culiacán 80030, Mexico
[c] Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Sinaloa, Guasave 81101, Mexico

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ABSTRACT

We previously demonstrated the effectiveness of a hot water (HW) treatment (40 °C for 7 min) to reduce chilling injury (CI) in tomato fruit and described the global transcriptional changes associated with CI tolerance and susceptibility after 2 weeks of cold storage. In this study we describe the early transcriptional responses of HW treated and non-treated tomato fruit after a short-term cold storage (2 and 24 h at 5 °C). RNA-Seq analysis detected a large number of differentially expressed genes that varied from 575 (control fruit after 2 h at 5 °C) to 5100 (HW treated fruit after 24 h at 5 °C). The protective effect of HW treatment against chilling stress was related first with the up-regulation of AP2/EREBP and C_2H_2-type zinc finger transcription factors, which are known to induce the expression of cold-regulated genes, and second with the up-regulation of chaperonins and peptidyl-prolyl *cis–trans* isomerases, which prevent the denaturation and aggregation of proteins. Also, some genes related to pathogen resistance (TIR, NBS and LRR families) were up-regulated in HW treated fruit after chilling, suggesting a crosstalk between biotic and abiotic stress responses. Transcriptional changes that were induced in HW treated fruit at early stages of chilling and maintained after long-term cold storage included the up-regulation of genes related to heat stress and the down-regulation of genes related to cell wall degradation.

1. Introduction

Harvested fruit and vegetables are usually stored at low temperatures in order to extend their shelf life by reducing the respiratory rate and thus delaying the ripening and senescence processes (Lurie and Pedreschi, 2014). However, exposing tropical and subtropical commodities to temperatures below 12 °C causes the development of chilling injury (CI), a physiological disorder that greatly reduces fruit quality, causing undesirable changes such as abnormal ripening, pitting, browning and wilting, frequently rendering the product unmarketable (Aghdam et al., 2013).

Tomato (*Solanum lycopersicum*) is the second most important horticultural crop worldwide, with an annual production over 161 million tonnes in 2012 (FAOSTAT, 2014). Nevertheless, its susceptibility to develop CI restricts the use of refrigerated transportation to reach distant markets, or the use of long-term cold storage to extend its seasonal availability. Storage at low temperature (2–6 °C) for 2 weeks is sufficient to induce internal damage in tomato fruit, leading to development of CI visual symptoms when they are transferred to ripening conditions (20–22 °C) (Vega-García et al., 2010; Cruz-Mendívil et al., 2015).

Postharvest heat treatments have been used to modify fruit responses to cold stress, maintaining fruit quality during storage and modulating the rate of fruit ripening; the treatment conditions vary widely from a few minutes at 40–60 °C in hot water (HW) to hours or days at 30–40 °C in hot air (HA); these treatments have shown to delay or prevent the development of CI in many commodities, which has been associated with the prolonged presence of heat shock proteins (HSPs) in the tissue and the protective effect they exert (Lurie and Pedreschi, 2014).

A comparative metabolomic analysis between control and HW (7 min at 40 °C) treated tomato fruit (cv. Micro-Tom) after cold storage (14 days at 2.5 °C), showed that the metabolites arachidic acid and 2-ketoisocaproic acid were increased only in HW treated fruit after chilling, and they may be associated with CI tolerance

* Corresponding author at: Programa Regional de Posgrado en Biotecnología, Facultad de Ciencias Químico-Biológicas, Universidad Autónoma de Sinaloa (FCQB-UAS), Culiacán 80030, Mexico. Tel.: +52 667 715 7641; fax: +52 667 713 6615.
E-mail address: angelvaldezortiz@yahoo.com.mx (A. Valdez-Ortiz).

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mechanisms; meanwhile, arabinose and fructose-6-phosphate were increased only in control chilled fruit and may serve as metabolite markers for incipient CI in mature-green tomato fruit (Luengwilai et al., 2012). On the other hand, Zhang et al. (2013) reported that a HA treatment (12 h at 38 °C) enhanced the expression of two genes encoding arginase (*LeARG1* and *LeARG2*) and effectively alleviated CI in tomato fruit after 28 days at 2 °C; in addition, the HA treatment increased the activities of antioxidant enzymes (SOD, CAT and APX) and promoted the accumulation of arginine, proline and putrescine. Recently, an RNA-Seq analysis in tomato fruit (cv. Micro-Tom) after a HW treatment (7 min at 40 °C), cold storage (14 days at 5 °C) and subsequent ripening (14 days at 20 °C), showed that the induced tolerance to CI after a heat shock seems to be associated with the up-regulation of genes related to heat stress, detoxification, sugars metabolism, and biosynthesis of ethylene and carotenoids, as well as the down-regulation of genes associated with degradation of cell wall and lipids (Cruz-Mendívil et al., 2015).

In the present study we performed an RNA-Seq analysis in tomato fruit with HW pre-treatment during a short-term cold storage (2 and 24 h at 5 °C) to identify genes and TFs involved in the first molecular responses to heat shock and chilling, rather than those involved in the development of CI and appearance of visual symptoms. A functional categorization of differentially expressed (DE) genes was conducted to provide an overview of the main cellular responses, regulatory pathways and metabolic pathways associated with chilling tolerance and susceptibility at early stages.

2. Material and methods

2.1. Plant material and postharvest treatments

Tomato fruit (*S. lycopersicum* cv. Micro-Tom) were harvested and treated as previously reported (Cruz-Mendívil et al., 2015). Briefly, mature green fruit were selected based on epidermis color ($a^* = -13$ to -10) and weight (2–3 g). Tomato fruit were immersed for 7 min in water at 40 °C (HW) or at 20 °C (control), air-dried for 30 min at room temperature, and stored at 5 °C for 2 and 24 h.

2.2. RNA isolation and high-throughput sequencing

Tomato fruit from both control and HW treatments were sampled after 2 and 24 h at 5 °C, and named C-2 h, C-24 h, HW-2 h and HW-24 h, respectively. Five fruits were pooled for each condition; pericarp tissues were ground in liquid nitrogen and stored at −70 °C until use. Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA) and purified using RNeasy MinElute Cleanup Kit (Qiagen, Hilden, Germany), following the manufacturer's protocols. RNA quality was monitored by gel electrophoresis, A_{260}/A_{280} ratio, and RNA integrity number (RIN). High quality RNA samples (25 μg each) were used for cDNA libraries preparation and massive sequencing on a 5500 SOLiD System (with Exact Call Chemistry module), following the protocols from Applied Biosystems (Foster City, CA). Two technical replicates of sequencing (independent runs) were performed per pooled sample. In total, 3.6 Gb of single-end reads (75 bp length) were obtained per pooled sample. Raw sequence data is available for download at NCBI Sequence Read Archive under the BioProject Accession: PRJNA229516.

2.3. Bioinformatic analysis

The trimming and mapping of SOLiD reads were conducted on CLC Genomics Workbench 6.0 software (CLC bio, Aarhus, Denmark) using the same parameters as Cruz-Mendívil et al. (2015). The number of uniquely mapped reads per gene were used as expression values and represented as unique gene reads (UGR). The resulting UGR values were subjected to quantile normalization to remove the bias of sequencing depth across all samples. Genes with normalized UGR means <1 were discarded before statistical analysis with Baggerly's *t*-test, to have more certainty that the statistical differences between samples had a biological significance. Multiple testing corrections were performed by controlling the false discovery rate of *P*-values and expressed as *Q*-values. Genes with $Q < 0.01$ and fold change (FC) of at least ±2 were considered as DE. Venn diagrams of DE genes across samples were obtained with the online tool VENNY (Oliveros, 2007).

2.4. Functional categorization

Lists of gene identifiers and log 2 FC values from each comparison were imported into the MapMan 3.6 software (Thimm et al., 2004), and assigned to functional categories (BINS) using the mapping tomato file 'Slyc_ITAG2.3'. The Wilcoxon rank sum test was used to calculate the probability that the response of the genes assigned to a functional category, was statistically different from that of all remaining genes. Significant ($P < 0.01$) categories in at least one condition were graphically displayed, showing their average expression values (log 2 FC) in a color scale generated with the Heat Map tool of FiRe 2.2 software (Garcion et al., 2006).

3. Results and discussion

3.1. RNA-Seq and differential expression analysis

Transcriptome sequencing of tomato fruit yielded 48,646,261 raw reads (75 bp length) from the four sequenced samples (C-2 h, C-24 h, HW-2 h and HW-24 h), and after trimming by quality and length, 32,531,854 reads (66.9%) were retained. From these reads, 22,964,924 (70.6%) were uniquely mapped in color-space format to the tomato reference transcriptome 'ITAG2.3_cdna' (http://solgenomics.net/), and conserved for further analyses. A summary of trimming and mapping statistics is provided in Supplementary Table S1.

Gene expression values from the four samples were compared to those from control fruit at time zero (C-0 h) (Cruz-Mendívil et al., 2015). Transcripts that met the cut-off criteria ($Q < 0.01$, FC ± 2) were considered as DE. These ranged from 575 after 2 h of cold storage in control fruit to 5100 after 24 h of cold storage in HW treated fruit (Table 1). Complete details of DE genes from all comparisons are provided in Supplementary Table S2.

DE genes were visually represented in a Venn diagram (Fig. 1). A total of 7743 distinct genes were DE as a consequence of HW or cold treatments, which correspond to 22.3% of the total genes in the tomato genome. After 2 h of cold storage, 126 and 705 genes were uniquely expressed in control and HW treated fruit, respectively, while 51 genes were expressed in common. After 24 h of cold storage the number of commonly expressed genes increased to

Table 1
Effects of hot water treatment and short-term cold storage on the transcriptome of tomato pericarp.

Pairwise comparison	Differentially expressed genes		
	Up-regulated	Down-regulated	Total
C-2 h/C-0 h	230	345	575
C-24 h/C-0 h	2003	2253	4256
HW-2 h/C-0 h	1131	1172	2303
HW-24 h/C-0 h	2431	2669	5100

Gene expression values from control (C) and hot water (HW) treated tomato fruit after 2 and 24 h at 5 °C were compared to those from control time zero (C-0 h). Genes were considered as differentially expressed at $Q < 0.01$ (Baggerly's *t*-test) and fold change of at least ±2.

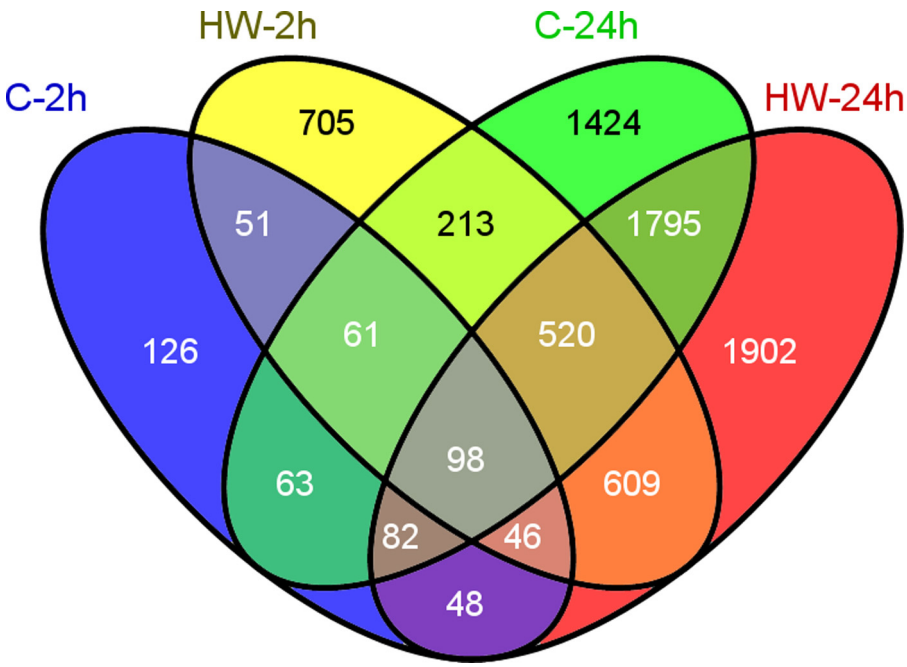

Fig. 1. Venn diagram showing common and unique expression profiles among samples. Numbers represent differentially expressed genes in control (C) and hot water (HW) treated tomato fruit after 2 and 24 h of cold storage (5 °C).

1795, while the number of uniquely expressed genes increased to 1424 and 1902 in control and HW treated fruit, respectively. As reported previously by Cruz-Mendívil et al. (2015), genes uniquely expressed in control fruit could be associated with chilling-susceptibility, genes uniquely expressed in HW treated fruit could be associated with chilling-tolerance, and genes expressed in common between control and HW treated fruit could be associated with chilling-response.

3.2. Functional categorization of DE genes

DE genes during short-term cold storage from control and HW treated fruit were assigned to MapMan BINS. In Fig. 2, the BINS that were significant ($P < 0.01$) in at least one condition are shown and their average expression changes (log 2 FC) are represented on a color scale (red = repression, blue = induction). Detailed information of these functional categories in all conditions is provided in Supplementary Table S3. In addition, the transcriptional changes of DE genes after 2 and 24 h of cold storage were graphically represented into the MapMan pathways of cellular response overview (Fig. 3), regulation overview (Fig. 4) and metabolism overview (Fig. 5).

After 2 h of cold storage, control fruit showed down-regulation of transport proteins and ATPases involved in protein degradation, in addition to the up-regulation of phototrophic response proteins; on the other hand, HW treated fruit showed down-regulation of cell wall degradation, simple phenols, peroxidases, homeobox TF family, subtilases, receptor kinases and storage proteins; as well as the up-regulation of heat stress, heat shock TF (HSTF) family, metalloproteases, protein folding and plasma membrane intrinsic proteins (Fig. 2).

After 24 h of cold storage, the common transcriptional changes in control and HW treated fruit included down-regulation of photosynthetic processes, protein synthesis, lipid transfer proteins and receptor kinases, as well as the up-regulation of DOF (DNA-binding with one finger) TF family and ATPase's involved in protein degradation. Some of these common responses to chilling at early stage were also observed during long-term cold storage (Cruz-Mendívil et al., 2015); for instance, the chilling-response regulon (after 2 weeks at 5 °C) included the down-regulation of photosynthesis and lipid transfer proteins, as well as the up-regulation of DOF TFs and protein degradation.

The unique responses of control fruit after 24 h of cold-storage included the down-regulation of histones, and the up-regulation of cellulose synthases, phenylpropanoids, UDP glucosyl and glucoronyl transferases, MYB-related TF family, posttranslational modification proteins and ATP-binding cassette transporters. On the other hand, the unique responses of HW treated fruit after 24 h of cold-storage included the down-regulation of cell wall degradation, isoprenoids, ethylene signaling, glycosidases and peptide transporters; as well as the up-regulation of phenylalanine ammonia-lyases, pathogenesis resistance proteins, heat stress and some TF families (AP2/EREBP, GATA, C_2H_2 zinc finger, C_3H zinc finger and PHOR1) (Fig. 2). Henceforth, we will focus on describing the most prominent pathways and categories affected by HW treatment and short-term cold storage in tomato fruit, in order to obtain new insights about the chilling tolerance and susceptibility mechanisms at early stages.

3.3. Abiotic stress

HSPs are stress-responsive proteins classified into five main families based on their molecular weight: small HSPs, HSP60, HSP70, HSP90 and HSP100. The expression of heat-shock genes encoding different HSPs is regulated by HSTFs that have the ability to sense heat, cold and oxidative stress. Moreover, HSPs not only confer protection against the original stress, but also against any subsequent stress situation, mainly through their chaperone activity, which consists of the recognition and binding to unfolded proteins, the prevention of protein aggregation, and the contribution to renaturation of aggregated proteins (Sevillano et al., 2009; Aghdam et al., 2013).

The heat stress BIN (20.2.1) was significantly up-regulated in HW treated fruit during short-term cold storage (Fig. 2). Specifically, we observed that 71 and 59 transcripts related to heat stress were up-regulated in HW treated fruit after 2 and 24 h at 5 °C, respectively; meanwhile, 4 and 32 transcripts of the same BIN were down-regulated in control fruit (Fig. 3). A total of 38 transcripts related to heat stress were up-regulated in common between HW treated samples (HW-2 h

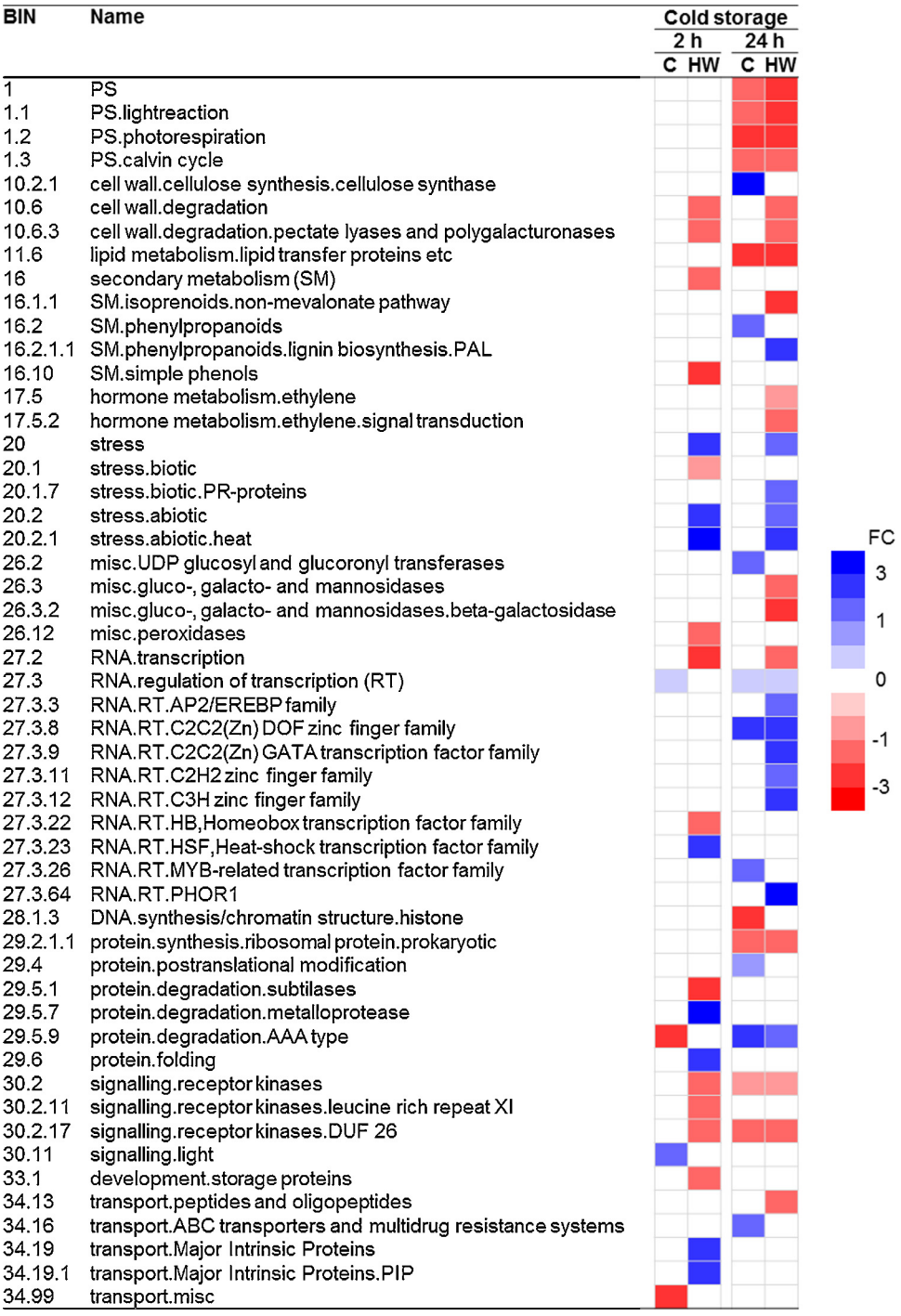

Fig. 2. Functional categorization and global gene expression changes in tomato fruit after short-term cold storage. Control (C) and hot water (HW) treated samples were grouped per storage time (2 and 24 h at 5 °C). MapMan BINS that were significant ($P < 0.01$) in at least one condition were displayed, and the average expression changes (log 2 FC) were represented on a color scale (red = repression, blue = induction) only in those conditions where the BIN was significant (for complete details, see Supplementary Table S3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and HW-24 h) and were not present in control samples; therefore, these genes could be associated with chilling tolerance mechanisms. This cluster included 10 chaperone proteins, 13 small HSPs, nine HSP70, two HSP90, three HSTFs and one phosphosulfolactate synthase. In agreement with these results, a transcriptome profiling of HW treated tomato fruit after 2 weeks of cold storage showed the up-regulation of 12 *HSPs* (six small HSPs, four HSP70 and two HSP90) and one *HSTF* (Cruz-Mendívil et al., 2015). Also, proteomic studies have shown the accumulation of small HSPs as part of CI tolerance mechanisms in tomato fruit (Page et al., 2010; Sanchez-Bel et al., 2012). According to Aghdam et al. (2013), CI mitigation due to increasing HSPs gene expression and protein accumulation could be attributed to three main processes: (1) the characteristic chaperone activity of this protein family; (2) the action of small HSPs as stabilizers of cell membranes; and (3) the synergistic action of HSPs with antioxidant systems.

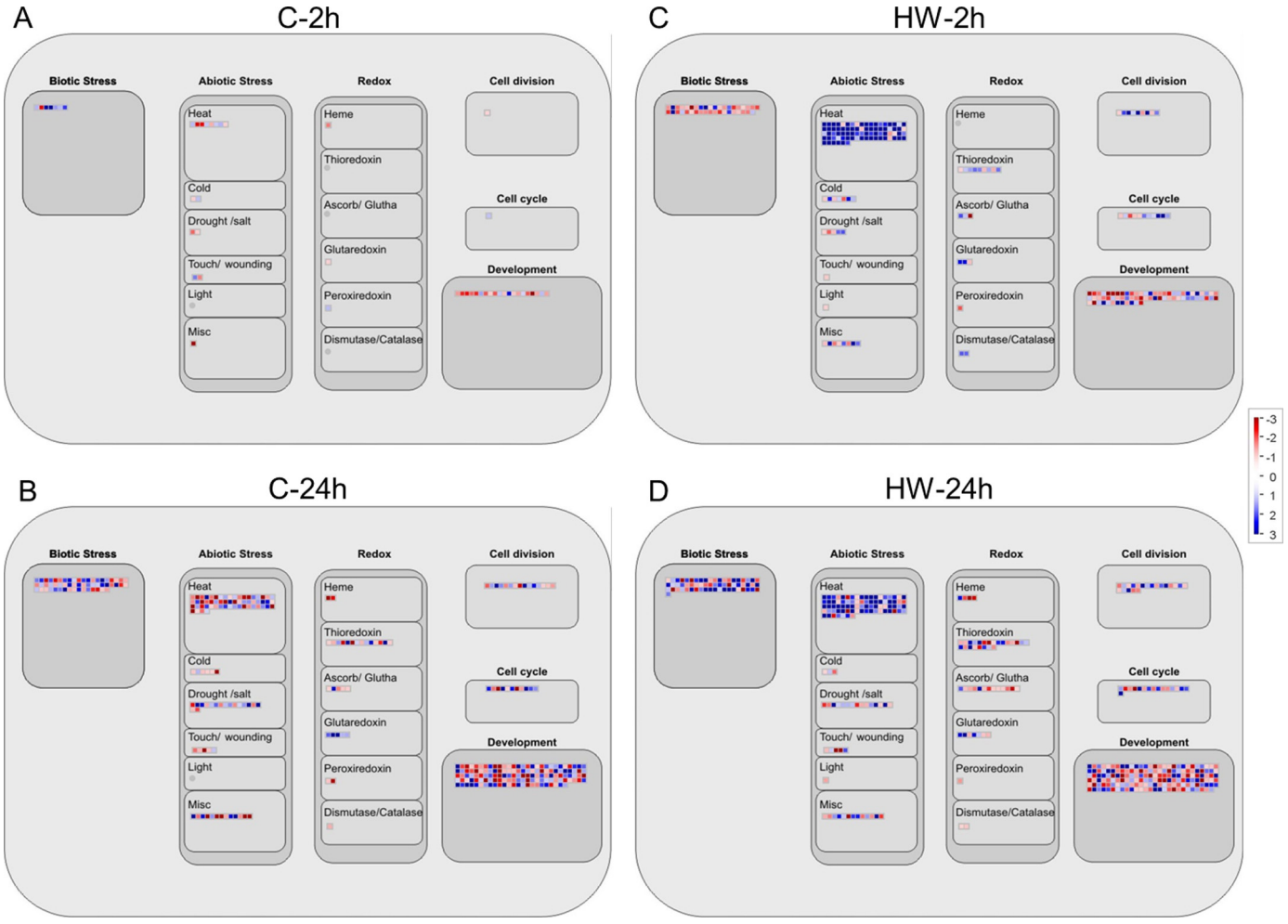

Fig. 3. Overview of differentially expressed transcripts involved in cellular responses after heat and cold stress. Control fruit after 2 h (A) and 24 h (B) at 5 °C. Hot water treated fruit after 2 h (C) and 24 h (D) at 5 °C. Transcript expression changes (log 2 FC) were visualized on a color scale (red = repression, blue = induction). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Biotic stress

We found that the response to biotic stress was also affected by the heat and cold treatments in tomato fruit. The pathogen resistance (PR) BIN (20.1.7) was significantly up-regulated in HW treated fruit after 24 h of cold storage (Fig. 2), showing 11 up-regulated transcripts that belong to PR gene families (Fig. 3) such as toll/interleukin-1 receptor (TIR), nucleotide-binding site (NBS), leucine-rich repeat (LRR) and Kunitz trypsin inhibitor (KTI). These results suggest the existence of crosstalk between biotic and abiotic stress responses in postharvest tomato fruit.

TIR-encoding genes represent one of the most important families of disease resistance genes in plants and act as signal mediators that respond to pathogens, but some studies suggest additional roles of TIR-encoding genes (Sun et al., 2014). For instance, the overexpression of *CHS1* (TIR–NBS protein) alleviates chilling damage and enhances plant growth in *Arabidopsis* by limiting chloroplast damage and cell death at low temperature (Zbierzak et al., 2013). Also, a mutation in *CHS3* (TIR–NB–LRR protein) showed arrested growth and chlorosis in *Arabidopsis* plants when grown at low temperature, suggesting a mutual interaction between cold signaling and defense responses (Yang et al., 2010).

3.5. Regulation of transcription

The APETALA 2/ethylene-responsive element binding protein (AP2/EREBP) is a large family of plant-specific TFs that includes DREBs/CBFs, which activate the expression of abiotic stress-responsive genes via specific binding to the DRE/CRT *cis*-acting element in their promoters (Mizoi et al., 2012). In the present work, the AP2/EREBP family (BIN 27.3.3) was significantly up-regulated in HW treated fruit after 24 h of cold storage (Fig. 2), showing 20 up-regulated AP2/EREBP TFs in the HW-24 h sample. In this sense, Kang et al. (2011) reported that the overexpression of *FTL1/DDF1*, which encodes an AP2 TF, enhanced the tolerance to cold, drought and heat stresses in *Arabidopsis* plants.

On the other hand, C_2H_2-type zinc finger proteins are induced by different abiotic factors such as cold, salinity, drought and oxidative stresses, and they have been shown to play diverse roles in the plant stress response and the hormone signal transduction (Kielbowicz-Matuk, 2012). Here, the C_2H_2 zinc finger family (BIN 27.3.11) was significantly up-regulated in HW treated fruit after 24 h of cold storage (Fig. 2), showing 17 up-regulated C_2H_2 zinc finger TFs in the HW-24 h sample. In this sense, Rugkong et al. (2011) reported that four TFs coding for C_2H_2-type zinc finger proteins were up-regulated in tomato fruit after 4 weeks of cold storage. Also, the overexpression of *SlCZFP1*, a C_2H_2-type zinc finger

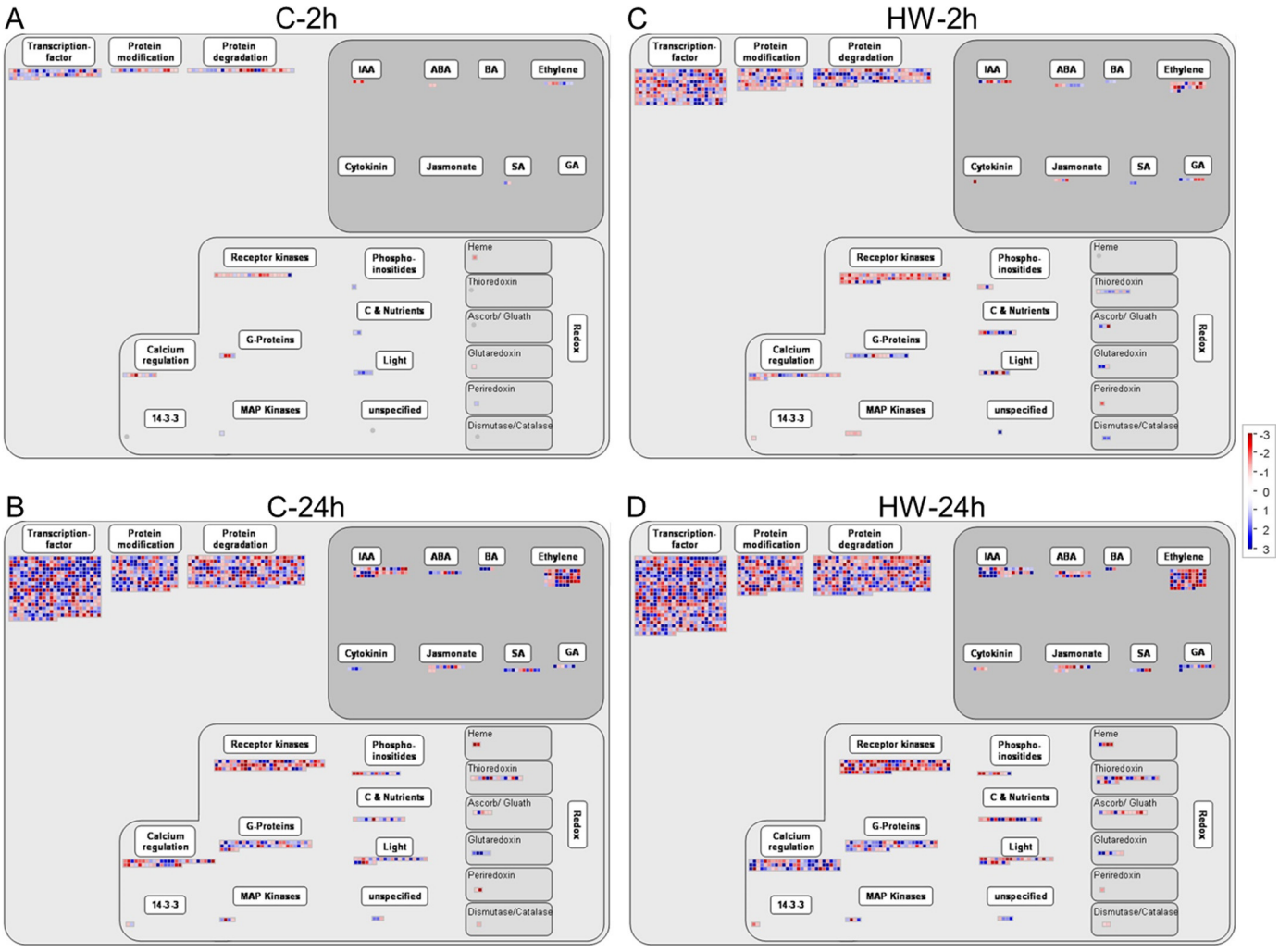

Fig. 4. Overview of differentially expressed transcripts involved in regulatory pathways after heat and cold stress. Control fruit after 2 h (A) and 24 h (B) at 5 °C. Hot water treated fruit after 2 h (C) and 24 h (D) at 5 °C. Transcript expression changes (log 2 FC) were visualized on a color scale (red = repression, blue = induction). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

protein from tomato, induced the constitutive expression of cold-regulated (COR) genes and enhanced the cold tolerance in transgenic *Arabidopsis* and rice plants (Zhang et al., 2011).

These evidences suggest that the up-regulation of AP2/EREBP and C_2H_2 zinc finger TFs, together with HSTFs (Section 3.3), may be one of the first transcriptional responses involved in the acquisition of CI tolerance after a HW treatment in tomato fruit, followed by the down-stream induction of COR genes.

Finally, the DOF TF BIN (27.3.8) was significantly up-regulated in both control and HW treated fruit after 24 h of cold storage (Fig. 2), with four DOF TFs that were up-regulated in common. In agreement with these results, Cruz-Mendívil et al. (2015) reported the up-regulation of 14 DOF TFs in both control and HW treated fruit after 2 weeks of cold storage. This evidence supported the role of DOF TFs in the common response to chilling in tomato fruit, which we suggest is related to the down-stream induction of glutathione S-transferases and enhanced protection against oxidative stress.

3.6. Protein metabolism

It has been reported that postharvest chilling of tomato fruit increases the autoproteolytic activity and reduces the content of insoluble proteins, which are predominantly localized in cell membranes, thus suggesting that CI causes the degradation of membrane proteins (Re et al., 2011). In this report, the category of AAA-ATPases involved in protein degradation (BIN 29.5.9) was significantly up-regulated after 24 h of cold storage in both control and HW treated fruit (Fig. 2), showing 15 up-regulated transcripts in common between control and HW samples: seven 26S protease regulatory subunits, four AAA-ATPases and two ftsH cell division proteases (Fig. 4). Cruz-Mendívil et al. (2015) also reported that the category of protein degradation (BIN 29.5) was significantly up-regulated in both control and HW treated tomato fruit after 2 weeks of cold storage.

Proteomic approaches also support the role of proteases in CI development. Page et al. (2010) isolated six spots related to protein/peptide degradation in cold-stored tomatoes, two of them corresponded to key proteins involved in the proteasome, and the others were proteinases, including aspartic, cysteine and Arg/Lys protease. Also, Vega-García et al. (2010) detected two spots, mitochondrial processing peptidase and 26S proteasome ATPase subunit, that were more abundant in damaged pericarp tissues of chilled tomato fruit. In contrast, Sanchez-Bel et al. (2012) found two spots, 26S proteasome subunit RPN11 and aspartic proteinase, which were down-regulated in response to cold stress in tomato fruit. According to Vega-García et al. (2010), the removal of non-functional but potentially harmful polypeptides arising from

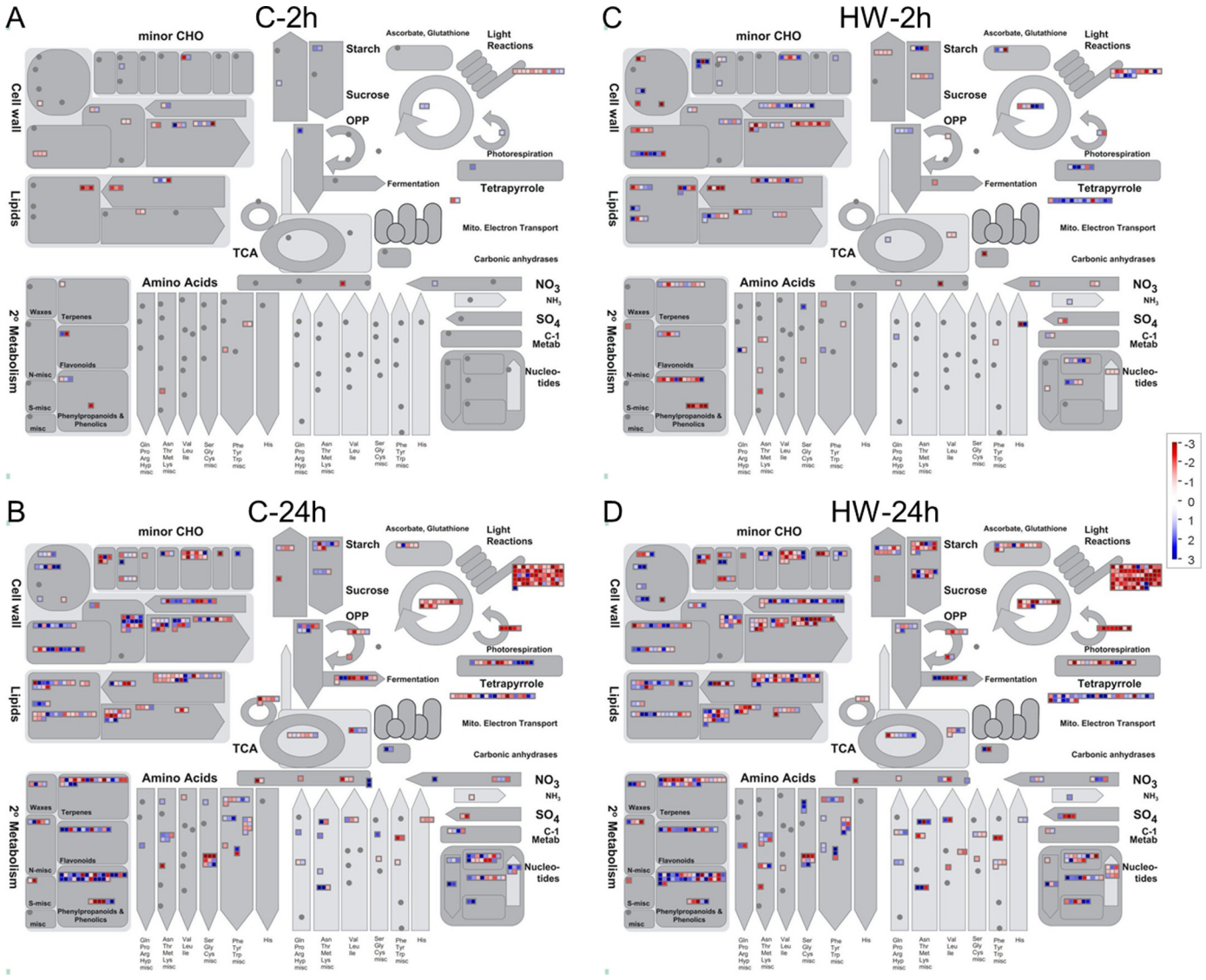

Fig. 5. Overview of differentially expressed transcripts involved in metabolic pathways after heat and cold stress. Control fruit after 2 h (A) and 24 h (B) at 5 °C. Hot water treated fruit after 2 h (C) and 24 h (D) at 5 °C. Transcript expression changes (log 2 FC) were visualized on a color scale (red = repression, blue = induction). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

misfolding, denaturation, or aggregation is important for the maintenance of cellular homeostasis during chilling stress in tomato fruit.

On the other hand, the category of protein folding (BIN 29.6) was significantly up-regulated in HW treated fruit after 2 h of cold storage, showing 13 up-regulated transcripts: six chaperonins, three peptidyl-prolyl *cis–trans* isomerases, two grpE proteins and two unknown proteins (Figs. 2 and 4). In addition, 14 transcripts of the same BIN were up-regulated in HW treated fruit after 24 h of cold storage, showing six chaperonins expressed in common. Protein folding in vivo is mediated by two systems: foldases and molecular chaperones; foldases include protein disulfide isomerase and peptidyl prolyl isomerase, which rearrange disulfide and peptide bonds; whereas molecular chaperones include a broad range of protein families whose common property is that they recognize non-native proteins and prevent their aggregation (Boston et al., 1996; Saibil and Ranson, 2002). Since chilling stress can cause protein denaturation, the up-regulation of transcripts related to protein folding in HW treated fruit (Fig. 2) seems to play an important role in the induced tolerance to CI.

3.7. Ethylene metabolism

The category of ethylene signal transduction (BIN 17.5.2) was significantly down-regulated in HW treated fruit after 24 h of cold storage, showing the down-regulation of 11 transcripts: six ethylene receptors, four ethylene responsive TFs and one PPPDE peptidase domain-containing protein (Figs. 2 and 4). Also, nine transcripts of the same category were down-regulated in control fruit after 24 h of cold storage, showing eight genes expressed in common. In agreement, Rugkong et al. (2011) reported the down-regulation of ethylene receptor genes in tomato fruit during cold storage. Since exposure to low temperature induce alterations in cell membrane conformation and structure (Sevillano et al., 2009), ethylene receptors localized in the endoplasmic reticulum membrane could also be affected by cold storage, leading to delayed or uneven ripening in tomato fruit (Rugkong et al., 2011).

3.8. Cell wall metabolism

The category of cellulose synthesis (BIN 10.2.1) was significantly up-regulated in control fruit after 24 h of cold storage, showing the

up-regulation of nine transcripts coding for cellulose synthases, four of which were uniquely expressed in control fruit and five were expressed in common with HW treated fruit after 24 h of cold storage (Figs. 2 and 5). Although the role of cellulose synthase in CI development is not clear, other studies have reported the up-regulation of cellulose synthase genes as a response to cold stress in sugarcane (Nogueira et al., 2003) and *Thlaspi arvense* (Sharma et al., 2007) plants.

On the other hand, the category of cell wall degradation (BIN 10.6) was significantly down-regulated in HW treated fruit during cold storage; specifically, 15 and 26 transcripts related to cell wall degradation were down-regulated in HW treated fruit after 2 and 24 h of cold storage, respectively, and from these, 12 transcripts were down-regulated in common between both storage times, including seven polygalacturonase family proteins, three endo-glucanases, one pectate lyase and one rhamnogalacturonate lyase (Figs. 2 and 5). In agreement with these results, Cruz-Mendívil et al. (2015) reported the down-regulation of cell wall degradation genes in HW treated tomato fruit after 2 weeks of cold storage, and lower levels of water loss and wilting (CI symptoms) after additional 2 weeks at ripening temperature, suggesting that HW treatment contributed to maintain the cell wall integrity and turgor during cold storage. The results of the present work support this hypothesis, and also show that the protection to cell wall degradation in HW treated tomato fruit is exerted since the initial stages of cold storage.

4. Conclusions

Unique transcriptional responses to short-term cold storage in HW treated fruit could be associated with chilling tolerance; they included the up-regulation of genes related to regulation of transcription (AP2/EREBP and C_2H_2-type zinger protein), pathogen resistance (TIR, NBS and LRR families) and protein folding (chaperonins and peptidyl-prolyl *cis–trans* isomerases). On the other hand, the transcriptional changes induced in HW treated fruit during short-term cold storage and maintained after 2 weeks at 5 °C (Cruz-Mendívil et al., 2015) included the up-regulation of heat stress genes (HSTFs and HSPs) and the down-regulation of cell wall degradation genes. The results of this study contribute to a better understanding of the molecular mechanisms associated with induced tolerance to CI, and may be helpful for breeding cold-tolerant tomato genotypes.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2015.06.015.

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