ORIGINAL ARTICLE

Plant, Cell & Environment | WILEY

A transcriptomic, metabolomic and cellular approach to the physiological adaptation of tomato fruit to high temperature

Juliana Almeida | Laura Perez-Fons | Paul D. Fraser [ID]

Department of Biological Sciences, Royal Holloway University of London, Egham, UK

Correspondence
Paul D. Fraser, Department of Biological Sciences, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK.
Email: p.fraser@rhul.ac.uk

Abstract

High temperatures can negatively influence plant growth and development. Besides yield, the effects of heat stress on fruit quality traits remain poorly characterised. In tomato, insights into how fruits regulate cellular metabolism in response to heat stress could contribute to the development of heat-tolerant varieties, without detrimental effects on quality. In the present study, the changes occurring in wild type tomato fruits after exposure to transient heat stress have been elucidated at the transcriptome, cellular and metabolite level. An impact on fruit quality was evident as nutritional attributes changed in response to heat stress. Fruit carotenogenesis was affected, predominantly at the stage of phytoene formation, although altered desaturation/isomerisation arose during the transient exposure to high temperatures. Plastidial isoprenoid compounds showed subtle alterations in their distribution within chromoplast sub-compartments. Metabolite profiling suggests limited effects on primary/intermediary metabolism but lipid remodelling was evident. The heat-induced molecular signatures included the accumulation of sucrose and triacylglycerols, and a decrease in the degree of membrane lipid unsaturation, which influenced the volatile profile. Collectively, these data provide valuable insights into the underlying biochemical and molecular adaptation of fruit to heat stress and will impact on our ability to develop future climate resilient tomato varieties.

KEYWORDS
carotenoids, fruit quality, fruit ripening, heat stress, isoprenoids, metabolomics, plastoglobuli, tomato, transcriptomics

1 | INTRODUCTION

Plant growth and development are vulnerable to abiotic stresses such as higher temperatures, leading to detrimental effects on agricultural yield (Battisti & Naylor, 2009; Bita & Gerats, 2013). Increased global mean temperatures and extreme climate-related events are now occurring with increased frequency and intensity (Horton et al., 2015; IPCC, 2013). The consequences arising from the warmer temperatures have already been observed and hold potential to destabilise food systems and to threaten local to global food security (Lesk et al., 2016; Zhao et al., 2017). Higher temperatures can trigger developmental, physiological, cellular stress responses in plants which is highly dependent on duration and severity of stress as well as sensitivity of plant cell type and developmental stage (Larkindale et al., 2007; Ohama et al., 2017). When heat stress is moderate, the changes occurring to crops may be rapidly reversible, but severe episodes of elevated temperatures are irreversible and can lead to crop failure (Zhang et al., 2010).

Tomato (*Solanum lycopersicum*) is among the crops for which yield losses have been well documented when different high-temperature regimes occur during reproductive phase. Yield (e.g., fruit number and weight) is adversely affected by daily mean temperatures above 29°C, ranging from a few days—when pollen development or fruit set is disturbed—to a whole developmental period (Peet et al., 1998; Pressman et al., 2002; Sato et al., 2000, 2006). Notably, the tomato vegetative development is less sensitive to episodic temperature increases as structural damages of photosystem II were not detected for temperatures reaching 38°C (Lu et al., 2017; Spicher et al., 2017). Nevertheless, the influence of heat stress on tomato nutritional composition and quality has received less attention. This is surprising considering tomato is one of the most widely consumed fruits globally (Bergougnoux, 2014), it is grown worldwide and is an important source of vitamins and bioactives in the human diet (Viuda-Martos et al., 2014).

Metabolite composition of tomato fruit is affected by adverse environmental conditions (Quinet et al., 2019). While some abiotic stresses as water deficit lead to an increase in sugars, organic acids, vitamin C and carotenoids (Albert et al., 2016), high-temperature conditions seem to have a diverse impact on fruit quality. Most information relies on studies performed with differing post-harvest conditions. Early studies have demonstrated that the long-known failure to achieve normal pigmentation of excised tomatoes ripening at high temperatures (Tomes, 1963) is associated with changes in ethylene production, fruit softening and colour development, a phenotype that could be reversed when fruits were transferred to optimal temperature (Lurie et al., 1996; Picton & Grierson, 1988). More recent evaluations corroborated these findings highlighting the heat-sensitivity of antioxidant accumulation as carotenoids and vitamin C (Gautier et al., 2008; Massot et al., 2013). For vine-attached fruits, the detrimental effects of high ambient temperature on bioactive compounds have also been observed (Hernández et al., 2015; Mulholland et al., 2003). Vitamin C (ascorbate) and carotenoids (provitamin A) are significantly lower when a heat-stress treatment is imposed during the advanced stages of fruit development. However, when the temperature is raised at earlier stages, the lack of effects indicates differential thermo-sensitivity of fruit developmental stages (Hernández et al., 2015). While these changes in tomato metabolism have been reported, a comprehensive evaluation exploring the cellular and molecular modifications associated with heat response awaits elucidation.

Alteration in metabolite composition can be direct consequence of several molecular mechanisms underlying the heat stress responses. A typical signature response is a wide-scale transient reprogramming of gene expression, including the expression of heat shock proteins (HSPs, Kotak et al., 2007; Ohama et al., 2017). The majority of HSPs function as molecular chaperones which act not only in protection against stress damage but also in folding, intracellular distribution and degradation of proteins (Mishra et al., 2002). Interestingly, HSPs seem to be important for tomato fruit ripening (Fragkostefanakis et al., 2015; Neta-Sharir et al., 2005). To maintain membrane stability and deal with oxidative stress generated in

response to heat, plants induce synthesis of hormones, and other protective molecules including osmoprotectants and antioxidants (Gray & Brady, 2016; Wahid et al., 2007). The antioxidant network, in part, is based on the action of several molecules including carotenoids, tocopherols (vitamin E), ascorbate and phenolic compounds, all with potential to contribute to fruit nutritional proprieties (Li et al., 2018b). Furthermore, as heat can lead to membrane damage caused by lipid hyper-fluidity and lipid peroxidation, modifying membrane lipid composition, particularly the acyl moieties of glycerolipids, is another critical aspect of plant thermotolerance (Falcone et al., 2004; Murakami et al., 2000; Higashi & Saito, 2019) that is linked to fruit quality. Changes in 18-carbon (C18) polyunsaturated fatty acids can affect the enzymatic/nonenzymatic formation of oxylipins derived thereof (Feussner & Wasternack, 2002). Lipoxygenase (LOX) pathway produces hydroperoxide intermediates for the synthesis of different compounds, including jasmonic acid and volatiles, the latter an essential aspect of fruit quality (Tieman et al., 2017). Lipid-derived signaling molecules as oxidised derivatives constitute important components of heat stress response, acting, for example, in the control of gene expression related to protective responses (Balogh et al., 2013; Farmer & Mueller, 2013; Hou et al., 2016).

In this present study, transcriptomic, metabolomic and cellular analysis has been applied to tomato fruits at different ripening transitions, following exposure to transient high-temperature treatment (40°C day/ 30°C night). These conditions may replicate events of heat stress experienced during commercial production. Collectively, the data provide new insights into the metabolic plasticity of tomato fruit to heat stress episodes and may contribute generically to the development of climatic resilient crops.

2 | MATERIAL AND METHODS

2.1 | Plant material, growth conditions and transient high-temperature treatment

Tomato plants (*Solanum lycopersicum*, cv. Ailsa Craig) were grown under greenhouse conditions with a 16/8-hr day night photoperiod at 25°C /19°C, respectively.

For transient heat stress treatment (H), the greenhouse chamber temperatures were set at 40°C/30°C (day/night) over a 48 hr duration. Photoperiod and lighting were the same as the control plant chamber, and watering adjusted to keep soil water content near field capacity. Non-stressed plants were kept at control conditions (C) in a parallel chamber. Flowers were tagged at anthesis. Treatment H was imposed on three different groups formed by at least five tomato plants (between 12- and 13-week-old), each one used to harvest a specific fruit ripening stage, that was heat-stressed only once. After H, five biological replicates of fruits, that is, fruits from five different plants, were harvested as follows: mature green (H_{MG}, 37–39 days after anthesis, DAF), breaker (H_B, 39–41 DAF) and 3 days post breaker (H_{B3}, 42–44 DAF). Leaves from heat-stressed plants (H_L) were also collected. Plants dedicated to H_B and H_{B3} treatment could

recover (R) under control conditions, and fruits were harvested at red ripe stage (7 days post breaker, B7); for H_B, after 7-days recovery period (H_BR7); for H_{B3}, after 4-days recovery period ($H_{B3}R4$). Overall fruit from five plants, representing five biological replicates, were collected for each treatment described above and each biological replicate was analysed independently. Samples were frozen immediately in liquid N_2 upon collection and stored at −80°C until metabolic and molecular analysis.

2.2 | Isoprenoid determination and quantification

Isoprenoids (carotenoids, tocochromanols and chlorophylls) were extracted from lyophilised tissue powder (15 mg) as described by Enfissi et al. (2010). Compounds were analysed by reverse-phase chromatography using an ultra-performance liquid chromatography (UPLC) system (Acquity, Waters) equipped with a Photo Diode Array (PDA) detector (Acquity, Waters). A UPLC BEH-C18 column (100 mm × 2.1 mm; 1.7 μm, Acquity, Waters) was used for separation as described by Nogueira et al. (2013). Peak identification was achieved by comparison of characteristic UV/Vis spectrum with authentic standards, reference spectra and retention times (Fraser et al., 2007). Quantification was performed using dose–response curves obtained from authentic standards.

2.3 | Metabolite profiling by gas chromatography (GC)-MS

Polar extracts were prepared from freeze-dried fruit powder (10 mg), extracted with 1 ml of solution containing methanol and water acidified with 0.1% formic acid [80:29.9:0.1, (v/v/v)] and agitated for 1 hr. After centrifugation, the polar extract was spiked with ribitol (1 mg/ml in MeOH; 10 μg final concentration) as the internal standard. For nonpolar extracts, alkaline hydrolysis with KOH was performed with a fruit powder aliquot (10 mg) during 1 hr at 40°C followed by extraction as described for isoprenoids. Nonpolar extracts were spiked with deuterated myristic acid-$_{d27}$ as the internal standard. The dried residues were derivatised in methoxyamine hydrochloride (in pyridine) followed by silylation with N-methyl trimethylsilyl trifluoroacetamide. The GC–MS analysis was achieved on Agilent 7890A GC system interfaced with a 5975C mass-selective detector as described in Uluisik et al. (2016).

For lipid and fatty acid compositional analysis, extraction was performed as described for isoprenoids and resolved on high-performance thin-layer chromatography (HTLC) silica gel 60 F254 plates (Merck) developed in a solvent mixture of acetone, toluene, and water [91:30:7, (v/v/v)]. Regions containing the lipid classes were identified based on the comparison with authentic standards visualised with iodine vapour and scraped from the HTLC plate. Elution and conversion to fatty acid methyl esters (FAMEs) by acid-catalysed transmethylation, followed by quantification using GC–MS were performed as previously described by Nogueira et al. (2013). FAMEs were quantified using myristic-$_{d27}$ acid as an internal standard.

Components were identified using a mass spectral library built from in-house standards and NIST11 database. Each analytical batch was validated with quality control samples.

2.4 | Profiling of volatile compounds by GC–MS

Frozen fruit samples were ground in liquid N_2 and aliquots (0.5 g) used for the analysis of volatile compounds. Homogenates were weighed out into screw-top headspace amber glass vials (20 ml) and spiked in with deuterated acetophenone-$_{d3}$ as internal standard (20 ppb). Capped vials were incubated at 40°C and shaken for 30 min. Volatile compounds were then adsorbed onto a SMPE fibre (Car/DVB/PDSM) for 20 min, followed by desorption into the injection port for 5 min. Chromatographic separation was conducted in a DB-5MS 30 m × 250 μm × 0.25 μm column (J&W Scientific, Folsom, CA), equipped with a 10 m guard column and using a step-temperature gradient from 40 to 300°C at 5°C/min. The linear temperature gradient included a 2 min hold-temperature and then steps at 40, 120, 250°C and 5 min at 300°C. Helium was employed as the carrier gas and the flow rate was 1 ml/min. The inlet and the mass spectrometer transfer line were heated to 250°C. A 7890B-5977B GC-MS system (Agilent Technologies, Palo Alto, CA) was used in splitless mode, and data processing and analysis proceeded using AMDIS (version 2.73) software.

2.5 | Subchromoplast fractionation

Chromoplasts were isolated from fruits (90 g) at B3 to B4 stage, and sub-compartments were fractionated using a discontinuous gradient of sucrose, according to Nogueira et al. (2013).

2.6 | Transmission electron microscopy

Pericarp fruit segments were fixed at room temperature in solution [3% (v/v) glutaraldehyde, 4% (v/v) formaldehyde buffered with 0.1 M PIPES buffer pH 7.2] and then stored at 4°C for at least 24 hr until processing. Samples were post-fixed in buffered 1% (w/v) osmium tetroxide and uranyl acetate, washed, dehydrated in a graded series of acetone, and embedded in resin. Ultrathin sections were stained with Reynolds lead citrate and imaged on a Tecnai T12 Transmission Electron Microscope (Field Electron and Ion Company).

2.7 | qPCR expression analyses

Total RNA was extracted from frozen leaves and fruit pericarps using the RNeasy kit (Qiagen) according to manufacturer's instructions. RNA from at least four biological replicates was prepared from each tissue and ripening stage. RNA quality was assessed by agarose gel electrophoresis. Total RNA (1 μg) was treated with DNase and converted into cDNA using the QuantiTect Reverse Transcription kit (Qiagen),

according to the manufacturer's protocols. Real-time quantitative PCR (qPCR) assays were performed in technical duplicates using RotorGene SYBR green PCR kit (Qiagen) on Rotor-Gene Q, with approximately 10 ng of reverse-transcribed RNA. Primer sequences are listed in Table S1. Relative expression was calculated as described by Quadrana et al. (2013). For reference gene selection, expression stability of five known reference genes (CAC, EXP, GAGA, ACT1 and ACT2) (Cheng et al., 2017; Exposito-Rodriguez et al., 2008) was evaluated on control and heat-stressed samples using GeNorm (Vandesompele et al., 2002). ACT2 and CAC were selected based on lowest expression stability values (M) of 0.362 and 0.438, respectively.

2.8 | RNA sequencing

Total RNA from three biological replicates samples was isolated using Trizol RNA Purification kit (Thermo Fisher Scientific). cDNA libraries were prepared and sequenced by IGA Technology Services facility (Udine, Italy). Single-end sequence reads (75 nt) at a read depth of 31.3 million reads on the average per sample (24.1 to 39.9 M reads) were obtained from the NextSeq500 platform (Illumina). Raw reads were processed using ERNE (Del Fabbro et al., 2013) and Cutadapt (Martin, 2011) software. The reads were mapped onto tomato genome (S. lycopersicum, cv. Heinz) reference SL3.0, with gene models ITAG3.10, using STAR (Dobin et al., 2013) applying default parameters. Assembling and quantification of full-length transcripts were accomplished by Stringtie (Pertea et al., 2015). The counting was achieved by HTseq-count (Anders et al., 2015). Gene ontology (GO) term annotation was performed using Blast2GO Pro (version 5.2.5) (Conesa et al., 2005).

All raw RNA sequencing data are available on NCBI, under the Bioproject accession number PRJNA603594.

2.9 | Data analyses

Significant differences between the control and heat-stressed conditions were determined by Student's t test or ANOVA followed by a Dunnett's multiple comparison with the level of significance set to 0.05, using GraphPad Prism software. For pair-wise differential expression analysis of transcriptome data, statistical analyses were performed by DeSeq2 (Love et al., 2014). Differentially expressed genes were determined using false discovery rate (FDR) ≤ 0.01 (adjusted p-value) and |fold-change| ≥ 1.5 (or $|\log_2 FC| \geq 0.58$). GO enrichment analysis with Fisher's Exact Test was conducted using Blast2GO.

3 | RESULTS

3.1 | Heat stress at advanced ripening stages negatively affects carotenoid accumulation

Plants grown under control conditions (C) were exposed to 48 hr high-temperature treatment (H). The H was imposed when plants possessed fruits undergoing specific ripening transitions, from mature green to breaker (H_B) and yellow to light-red transition (H_{B3}) (Figure 1). For comparison, an additional H treatment at early to late mature green (H_{MG}) was included. After H, plants were returned to control conditions (recovery, R) until fruits ripened (B7 stage).

The levels of plastidial isoprenoids were determined by UPLC-PDA. H negatively influenced the carotenoid levels in tomato fruit, and changes from the heat stress were highly dependent on the fruit stage used (Table 1). Remarkably, phytoene, the first carotene product of the pathway, was reduced both in H_B (10-fold) and H_{B3} (4.6-fold) compared to their corresponding non-stressed fruits. The other carotenes in the pathway, phytofluene and ζ-carotene, when detected, responded similarly to phytoene when H was applied. Lycopene, the predominant carotenoid found in red ripe tomato, was significantly lower, below detection in H_B fruits and reduced by 50% in H_{B3} fruits, compared to corresponding non-stressed controls. Interestingly, lycopene levels only partially recovered in $H_{B3}R4$ fruits, to about 60% of the content found in ripe C_{B7} fruit, despite the levels of phytoene and phytofluene precursors being fully restored at this stage (Table 1).

The total fruit carotenoid content was consistent with lycopene levels at later stages of ripening. While lycopene decreased in H_{B3} and $H_{B3}R4$ fruits, total carotenoids remained unchanged both in H_B fruits and in those fruits allowed to recover from heat stress (H_BR7). By contrast, H_{MG} carotenoid levels were mostly similar to the control, except for a modest increase in β-carotene levels. The same response was also observed in heat-stressed leaves (H_L). Additionally, lutein levels as well as total carotenoid showed an increase in H_L compared to control conditions.
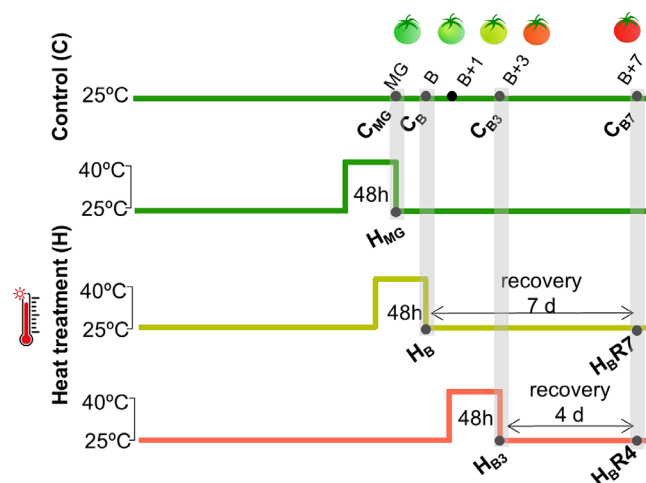

FIGURE 1 Outline of heat stress experiment. Tomato plants were kept at 25°C day/20°C night (control, C) or exposed to transient high-temperature treatment (H) at 40°C/30°C (day/night) for 48 hr. After H, fruits at the following stages were harvested: mature green (H_{MG}), breaker (H_B) and 3 days post-breaker (H_{B3}). Fruits allowed to recover (R) under normal conditions were harvested at ripe stage (H_BR7 and $H_{B3}R4$)

TABLE 1 Transient changes in isoprenoid profile of tomato fruits and leaves exposed to high-temperature treatment

μg/g DW	Fruit									Leaf	
	C_{MG}[a]	H_{MG}[a]	C_B[a]	H_B[a]	C_{B3}[a]	H_{B3}[a]	C_{B7}[a]	H_BR7[a]	$H_{B3}R4$[a]	C_L[a]	H_L[a]
Phytoene	nd	nd	2.7 ± 1.5	**0.3 ± 0.2***	103.5 ± 15.7	**21.9 ± 17****	207.8 ± 39.1	201.8 ± 44.9	147.9 ± 32.3	14 ± 3.3	13.1 ± 5.2
Phytofluene	nd	nd	1.8 ± 1.5	nd	**78.2 ± 13.3**	**15.7 ± 13.4****	172.9 ± 28.7	165.7 ± 32.8	125.8 ± 25.5	nd	nd
ζ-Carotene	nd	nd	nd	nd	3.1 ± 1.3	**0.7 ± 0.4***	6.7 ± 1.1	7.7 ± 1.6	8.2 ± 1	nd	nd
Lycopene	nd	nd	4.4 ± 5.4	nd	616.3 ± 93.6	**227.9 ± 188.3****	1724.7 ± 258.6	1,670.3 ± 279.5	**1,066.2 ± 117.3****	nd	nd
γ-Carotene	nd	nd	nd	nd	30.6 ± 6.6	19.9 ± 8.5	46.4 ± 6	38.6 ± 4.3	29.9 ± 10.2	nd	nd
β-Carotene	48.9 ± 1.1	**54.1 ± 2.5****	64.8 ± 2.4	**54.7 ± 6.1***	136.9 ± 7.8	145.5 ± 8.5	242.6 ± 8.4	217.3 ± 12.6	231.7 ± 38.4	499.3 ± 58.6	**614.9 ± 61.2***
δ-Carotene	nd	nd	nd	nd	3.8 ± 1.5	2.5 ± 1.3	3.4 ± 0.7	**7.8 ± 2.6****	2.8 ± 0.6	nd	nd
Lutein	57.8 ± 4.5	64.4 ± 6.6	63.2 ± 2.5	61.6 ± 4.6	71.8 ± 11.3	70 ± 3.9	146.3 ± 1.5	**158.3 ± 3.1****	145.8 ± 2.2	697.9 ± 84.4	**943.2 ± 87.3****
Total carotenoid	106.7 ± 5.5	118.5 ± 8.8	136.9 ± 11.6	116.7 ± 10.5	1,044.2 ± 130.8	**504 ± 229.2****	2,550.7 ± 329.5	2,467.5 ± 308.4	**1758.1 ± 170.6****	1,211.2 ± 142.5	**1,571.2 ± 150.4****
α-Tocopherol	210.7 ± 9.2	**284.3 ± 7.8****	291.9 ± 46.6	271.3 ± 28.9	341.8 ± 45.7	**421.7 ± 13.5***	425.2 ± 36.2	**507.8 ± 33.3****	**523.8 ± 35.8****	1,583.3 ± 213.6	1913.4 ± 431.3
β/γ-tocopherol	nd	nd	nd	nd	15.2 ± 3.1	10.1 ± 5.0	25.1 ± 3.9	**17.1 ± 5.6***	**7.7 ± 3.7****	nd	nd
PC-8	36.7 ± 5.1	**48.6 ± 8.5***	38.1 ± 2.9	43.3 ± 4.7	38.9 ± 7.5	44.2 ± 14.2	38.8 ± 5.2	43.3 ± 3.6	44.8 ± 11.6	67.1 ± 13.1	102.1 ± 36.3
Total Chl	311.5 ± 66.2	281.7 ± 39.3	376.5 ± 48.3	430.1 ± 101.9	18.4 ± 7.8	16.1 ± 11.7	nd	nd	nd	6,168.4 ± 824.3	**7,970.6 ± 868.7***

Levels of other lipid-soluble antioxidants were also influenced by H. α-tocopherol increased 1.2-fold in H_{MG} and H_{B3} fruits. Interestingly, its levels were still higher even after a recovery period in H_BR7 and $H_{B3}R4$ fruits compared to control conditions (Table 1).

3.2 | Metabolite profiling of tomato fruit exposed to transient heat stress

Metabolite profiling using GC–MS was carried out on fruits exposed to heat stress and concurrently samples were collected for transcriptome analysis. Principal component analysis (PCA) was used to compare primary metabolism of fruits under C, H and R conditions.

The score plot obtained from polar and nonpolar extracts could not discriminate non-stressed and heat-stressed conditions among the fruit stages evaluated, though a clear separation between early- and late-ripening stages was achieved (Figure. 2a, b). Overall primary metabolism remained unchanged after H and followed by R (Table S2).

Our high-temperature treatment did not appreciably affect the levels of known osmoregulators such as proline and GABA, and only a few metabolites responded significantly to H mostly in a fruit stage-dependent manner (Figure 2c, d). Sucrose was very responsive to heat, consistently accumulating in fruits (H_{MG} and H_B) at early ripe stages (Figure 2c). Some amino acids changed in content, particularly threonine was increased in H_B and H_{B3}. The tricarboxylic acid (TCA)
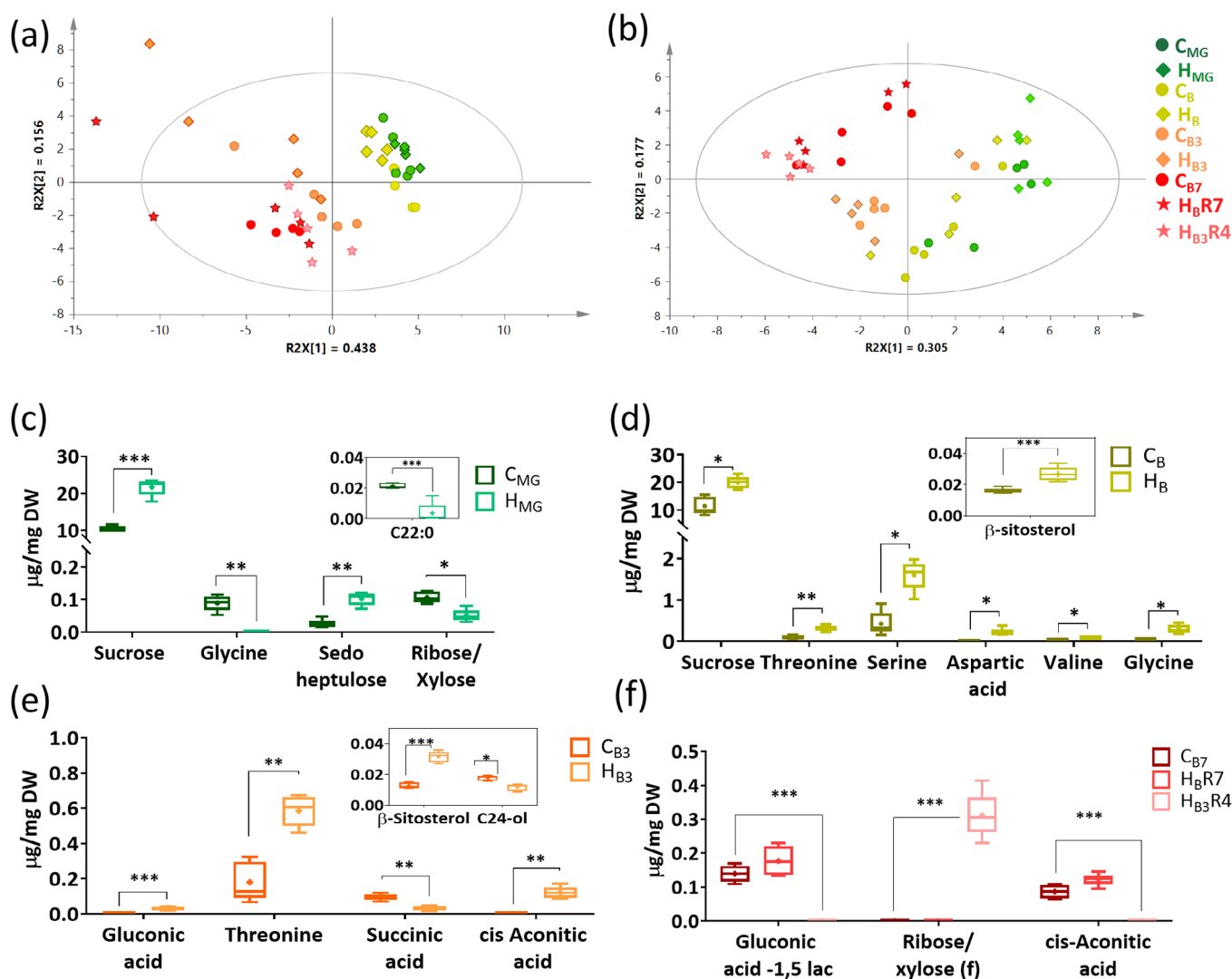

FIGURE 2 Effect of heat stress on tomato fruit metabolite profiling. Scores plot obtained by Principal Component Analysis (PCA) for metabolite levels measured in polar (a) and nonpolar (b) extracts. Metabolites at mature green, MG (c), breaker, B (d), breaker+3, B3 (e), red ripe, B7 (f) stage of fruits kept at C, exposed to H or followed R. Quantification was determined relative to the internal standard and values are presented as mean ± *SD* from five biological replicates. Nonpolar compounds are shown as left insets in the graphs. Only significant changes compared to respective control are shown (pair-wise *t* test corrected for multiple comparison using Holm-Sidak's post-test; * Adjusted $p < .05$, ** $p < .01$, *** $p < .001$). Full data set available in Table S2. C22:0, Docosanoic acid; C24-ol, tetracosanol; Ribose/xylose (f), Ribose/xylose in furanose ring form [Colour figure can be viewed at wileyonlinelibrary.com]

intermediates showed a variable response, with aconitic acid accumulating upon H. From non-polar fraction, β-sitosterol levels significantly accumulated in H_B and H_{B3}.

After R, primary metabolism of ripe fruits previously heat-stressed at B transition (H_BR7) was largely the same than their non-stressed counterparts, except for high levels of α-tocopherol. This quantitative tocopherol response to heat was observed both within the GC–MS and UPLC-PDA derived datasets confirming the utility of the approaches (Table S2).

3.3 | Fruit lipid metabolism is highly responsive to heat

Lipid remodelling while-decreasing the level of lipid unsaturation is a crucial aspect of plant thermotolerance under suboptimal temperature conditions (Falcone et al., 2004). Nevertheless, the analysis of the total lipid fraction by GC–MS showed no significant differences between C and H in fatty acid composition. To increase the sensitivity and address the potential changes in complex lipid moieties, lipid species were first separated by TLC and then analysed by GC–MS.

Analysis of lipid classes revealed a relative increase in the storage lipid triacylglycerol (TAG) under H at both stages B and B3 compared to control (Figure 3a, c). Extraplastidic classes of phospholipids, phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylethanolamine (PE) showed variable response to heat. For the fatty acid composition of the lipid classes (Figure 3), the general trend was the lower levels of the trienoic fatty acid C18:3 under H, particularly in the plastidial membrane lipids mono-galactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) in comparison to control conditions. This decline was predominantly mirrored by an increase in the corresponding dienoic fatty acid precursor, linoleic acid (C18:2), and other less unsaturated and saturated acyl moieties (Figure 3b, d). Consistently, a greater reduction of the C18:3/C18:2 ratio was observed in MGDG and DGDG (Table S3). In contrast, H_B fatty acid composition of the TAG fraction showed an opposite response with higher levels of the trienoic acid C18:3 proportion compared to control C_B (Figure 3). Overall, tomato fruit response to high-temperature conditions includes lipid remodelling, which leads to TAG accumulation, and decreasing the level of membrane lipid unsaturation, particularly trienoic acid composition.
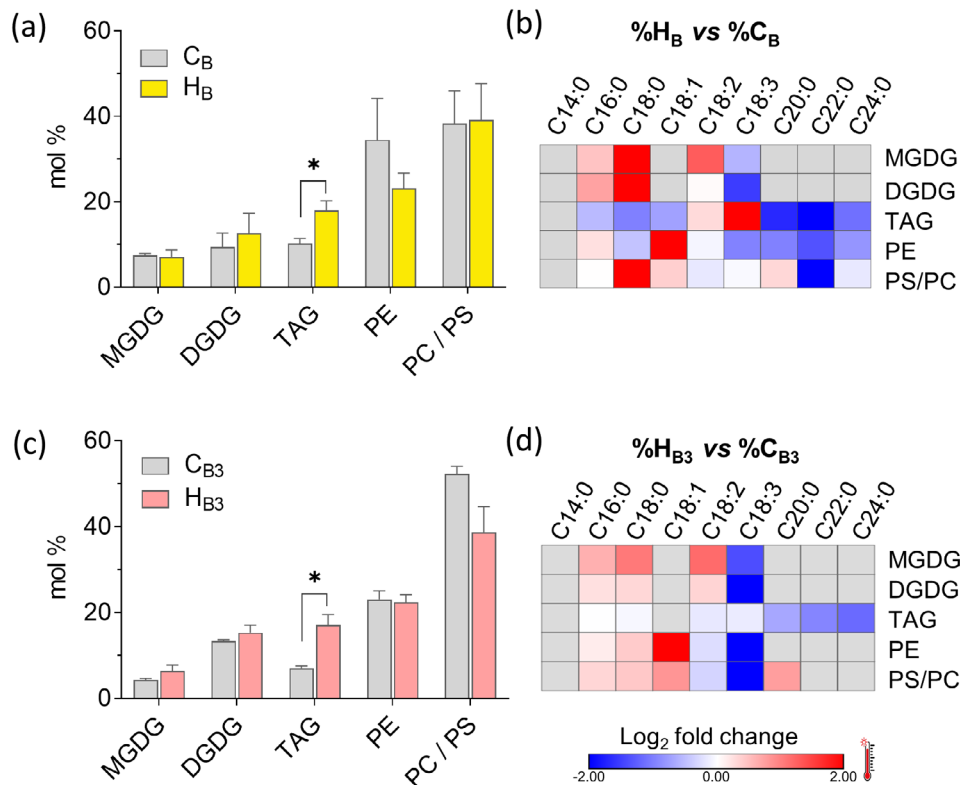

FIGURE 3 Lipid profile of tomato fruits after heat stress. Lipids were separated by TLC and quantified by GC–MS. (a, c) Relative abundance of lipid content (mol % of total) based on detector response calculated from internal standard. The values are shown as mean ± *SD* of three measurements from a pool of four biological replicates. (b, d) Heat map representation of changes in fatty acid composition (mol% of total) of each lipid class under heat. The ratio % in H versus % in C were obtained and showed as log$_2$ fold change. Dark red or dark blue indicates that the acyl moiety is relatively increased or decreased, respectively, under high-temperature conditions. Grey colour indicates acyl moieties not detected. MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PC/PS, phosphatidylcholine/phosphatidylserine; PE, phosphatidylethanolamine; TAG, triacylglycerol [Colour figure can be viewed at wileyonlinelibrary.com]

The volatile organic compounds (VOC) analysis revealed C18 fatty acid-derived volatiles were altered in heat-stressed fruits (Table S4). For the C18:3-derived flavour molecules produced via LOX action, a notable decrease in C6 volatiles was observed. Interestingly, levels of the C18:2-decomposition product hexanal were significantly lower only at H_B fruits, whilst H_{B3} exhibited hexanal at the same levels of non-stressed fruits. Contrastingly, C5 volatiles as pentanal and 2-pentanone accumulated in H_{B3} fruits. Carotenoid-derived volatiles detected were unaffected except for the lycopene derived 6-methyl-5-hepten-2-one; which was found to be lower in H_{B3} compared to C_{B3}. These data complement the lower levels of lycopene found in these samples following H. Finally, the accumulation of different VOCs belonging to terpenoid parent molecules, such as α-pinene, δ-4-carene, cymene, β-phellandrene was detected in at least one ripening stage either immediately after stress and/or after recovering. A similar trend was found for the phenolic derived molecule o-guaiacol (Table S4).

3.4 | Chromoplast structure of fruits exposed to high temperatures

Heat stress-induced changes in carotenoids, tocopherols and neutral lipid levels may be associated with perturbations not only in the metabolic pathways but also in compound sequestration (Spicher et al., 2017; Zhang et al., 2010). To ascertain whether high temperature alters the distribution of liposoluble antioxidants into plastidial sub-compartments, analysis of fractionated chromoplast from heat-stressed and non-stressed fruits was carried out (Figure 4).

First, the total amounts obtained from the sum of all fractions (i.e., plastoglobuli, envelope membranes, stroma, thylakoids) corroborated the lower levels of phytoene, phytofluene and lycopene in H_{B3} fruits (Table S5). Overall, the proportions of all carotenoids, tocopherols and plastochromanol (PC-8), this latter also an antioxidant typically accumulating at *plastoglobuli* (Kruk et al., 2014) followed the same distribution pattern immediately after H, suggesting that sub-compartmentation was not largely altered by heat stress. Yet, the lower phytofluene levels found in H_{B3} fruits preferentially accumulated into plastoglobular fraction (Figure 4b). No changes in the proportion of carotenoids and tocopherols arose in the plastoglobular fraction in the fruits that undergone heat stress at B stage following a short recovery (H_BR4). However, the presence of the fractions termed membrane II was more pronounced in H_BR4, which indicates the persistence of thylakoids remnants (Figure 4d).

Analysis of chromoplast ultrastructure by transmission electron microscopy suggested that non-stressed fruit chromoplasts displayed more mature lycopene crystals (Figure S1) while fruits experiencing
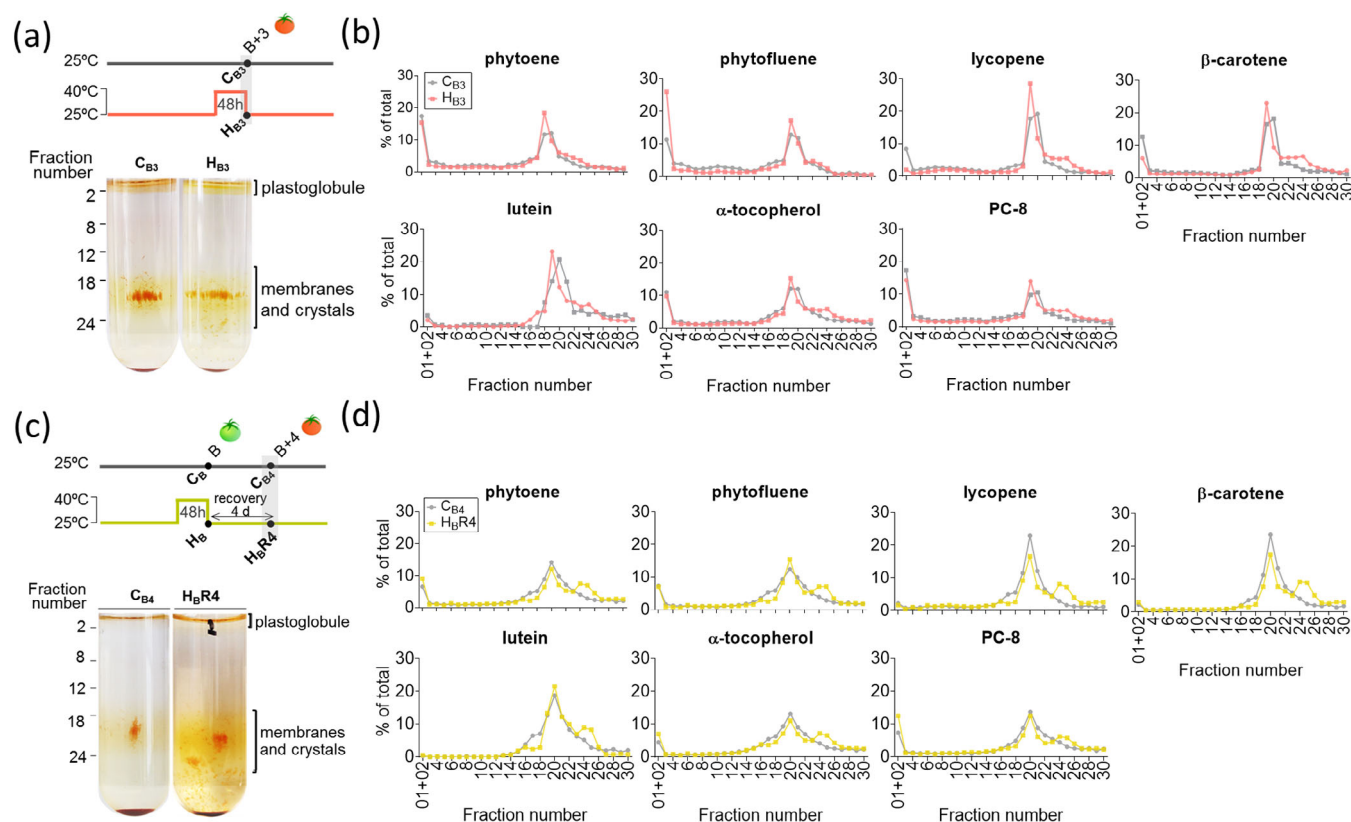

FIGURE 4 Profile of carotenoid and tocopherol across the chromoplast subcompartments after heat stress. (a, c) Fruit harvest time point and separation of membranes from isolated tomato chromoplasts (H_{B3} and H_BR4) by flotation on a discontinuous sucrose gradient. (b, d) Plastidial isoprenoid profile of fractions. Values represent % of the total amount found in each fraction [Colour figure can be viewed at wileyonlinelibrary.com]

stress at B stage (H$_B$R4) had smaller lycopene crystals and a notable persistence of thylakoid membrane which could reflect delays in fruit ripening.

3.5 | Transcriptome analysis

To address the molecular mechanisms underlying metabolic responses to heat stress, transcriptome analysis of B and B3 fruits under stressed and non-stressed conditions was carried out. Numerous

differentially expressed (DE) transcripts were detected as a result of H imposed (Table S6). In total, 8,141 and 7,006 genes were found DE in the comparison C$_B$ versus H$_B$ and C$_{B3}$ versus H$_{B3}$, respectively. Among the DE, 2067 genes were up-regulated, and 2,368 were down-regulated under heat, irrespectively of the fruit stage when the stress was imposed. These subsets of commonly heat-stressed regulated genes were used in the gene ontology (GO) enrichment analysis (Figure 5a).

Considering the most specific GO terms, up-regulated genes were significantly enriched for only a few biological process GOs
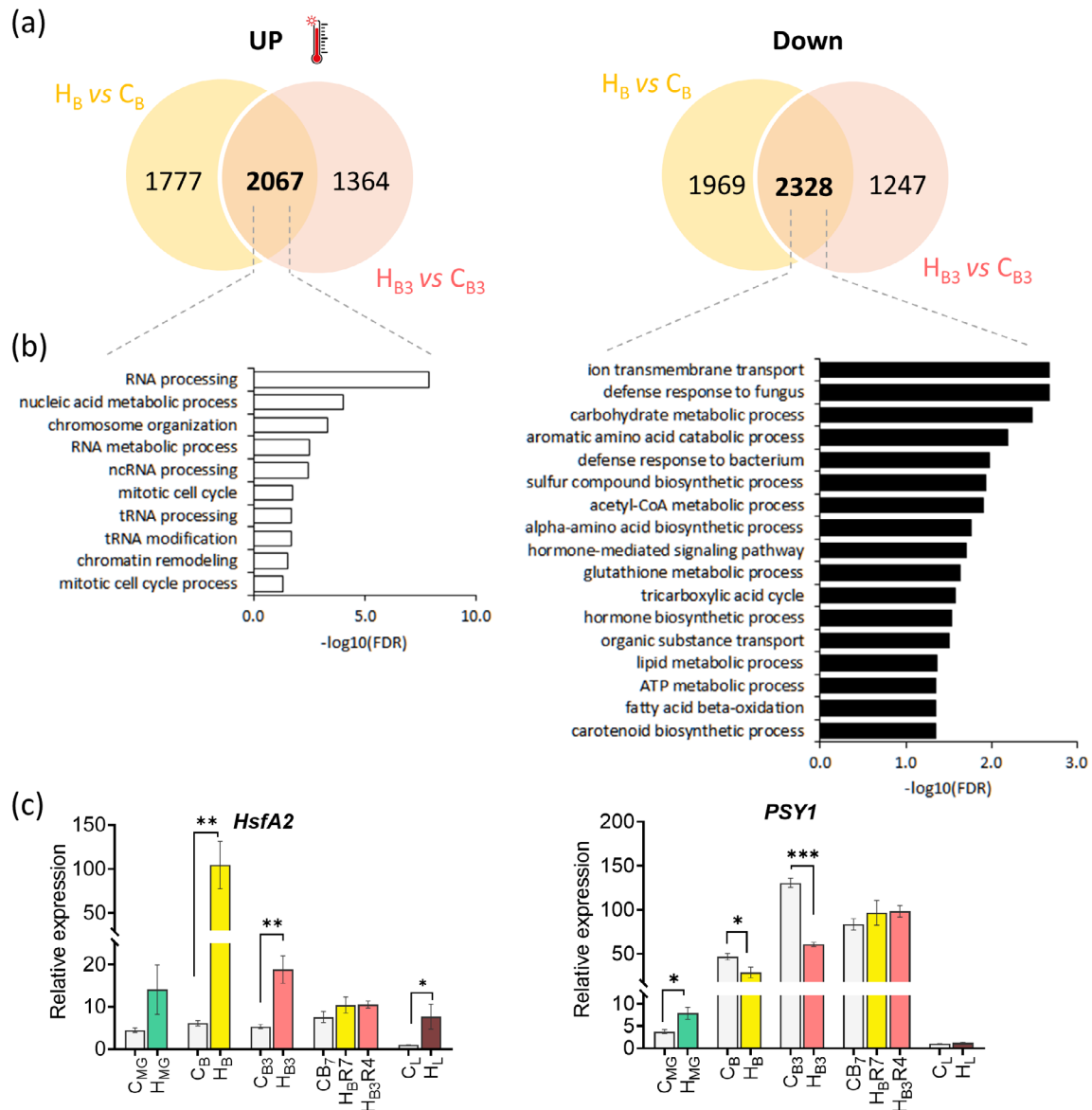

FIGURE 5 Gene expression changes associated with heat stress in tomato fruit. (a) Venn-diagrams of the up-regulated and down-regulated differentially expressed (DE) genes following H at B and B3 fruit stages. (b) GO terms enriched in the common set of DE genes observed at B and B3 stages according to Fisher's exact test (FDR < 0.05). Only the most specific GO terms for biological process category were shown. (c) Relative expression of *HsfA2* and *PSY1* by qPCR. Abbreviations and colour codes for fruit treatments are the same as in Figure 1. Nonstressed leaves (C$_L$) and heat-stressed (H$_L$, brown bars) leaf samples were included for comparison. Values are expression levels normalized to *CAC* and *ACT2* reference genes (mean ± *SEM* of at least four biological replicates) from samples kept at C, exposed to H or followed R. Significant differences (Student's t test, * p < .05, ** p < .01, *** p < .001) between heat and control conditions at corresponding organ/developmental stage are shown [Colour figure can be viewed at wileyonlinelibrary.com]

(10) associated with general terms as RNA processing, mitotic cell cycle and chromatin remodelling (Figure 5b). When a more relaxed significance threshold (p-value <.01) was applied, GO terms as "mRNA splicing, via spliceosome" (GO:0000398; p-value 2.15 E^{-03}), and molecular function "SWI/SNF superfamily-type complex" (GO:0070603, p-value 4.68 E^{-04}), the latter acting in chromatin remodelling processes (Table S7) were found. DNA de novo methylation results in part from the activity of DOMAIN REARRANGED METHYLTRANSFERASE (DRM) and chromatin remodelers as DEFECTIVE IN RNA-DIRECTED DNA METHYLATION1 (DRD1) (Zhang et al., 2018). As a response to heat, for example, gene encoding tomato homologs of DRM (Solyc10g078190, Solyc05g053260) and DRD1 (Solyc01g109970) were found up-regulated upon H (Table S6). The epigenetic mechanisms also featured when the heat-induced genes were queried for each comparison separately; "histone modification" and "RNA processing" processes were overrepresented among up-regulated H_{B3} genes (Table S7).

By contrast, genes down-regulated by H were enriched for GOs mainly associated with defence response to biotic stress, hormone synthesis and signaling pathway, metabolic processes related to lipids, carbohydrate, amino acids, and redox-related compound glutathione (Figure 5b). GO terms found overrepresented such as "carotenoid biosynthetic process," "carbohydrate metabolic process," "alpha-amino acid biosynthetic process" are closely related to the metabolic reprogramming triggered by heat in fruits.

Extreme temperature is known to induce the expression of *HEAT SHOCK TRANSCRIPTION FACTORS* (*Hsfs*). In tomato, *HsfA1*, which is constitutively expressed and post-translationally regulated, is responsible for the initial heat stress response controlling the HS-induced expression of *HsfA2* and *HsfA3* (Fragkostefanakis et al., 2015; von Koskull-Döring et al., 2007). Both *HsfA2* and *HsfA3* were found upregulated under H in RNA-seq dataset compared to control conditions (Table S6). qPCR assays confirmed higher *HsfA2* transcript levels not only for H_B and H_{B3} but also in heat-stressed leaves (H_L), though significant differences for H_{MG} were not detected. Importantly, higher *HsfA2* transcripts were not sustained after R (Figure 5c).

3.6 | Ripening regulators and targeted pathways

Given that processes related to fruit ripening were significantly enriched among the genes repressed by heat (Figure 5b), we explored the heat-induced transcriptional regulation associated with ripening as well as downstream targeted pathways such as isoprenoid metabolism (Karlova et al., 2014; Li et al., 2019; Quadrana et al., 2013) (Figure 6a, Table S8).
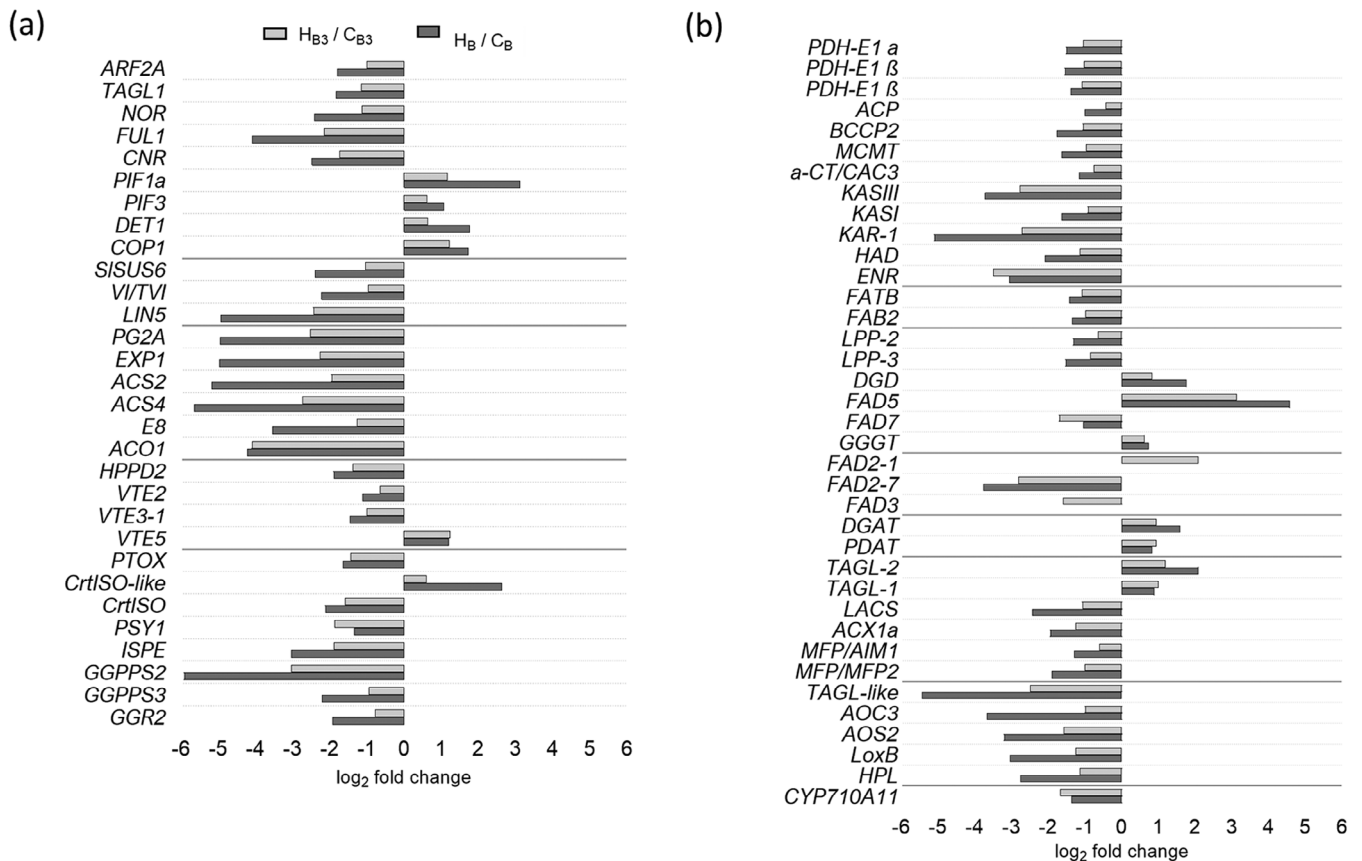

FIGURE 6 Transcriptional regulation of genes involved in fruit ripening and lipid metabolism under heat stress. Bars represent the log$_2$-fold change based on transcriptome comparison of H versus C at B (dark grey) and at B3 (light grey) for DEG. (a) Ripening-related genes. (b) Lipid-related genes. Full data set available in Tables S8 and S9. Gene abbreviatures according to Tables S8 and S9

In tomato, climacteric ripening is controlled by several transcription factors in conjunction with different phytohormones, such as ethylene (Karlova et al., 2014). Genes encoding key ripening-associated transcription factors such as COLORLESS NON-RIPENING (CNR), NON-RIPENING (NOR), FRUITFULL1 (FUL1), TOMATO AGAMOUS-LIKE1 (TAGL1) and AUXIN RESPONSE FACTOR2A (ARF2A) were strongly suppressed by heat. Interestingly, the expression of RIPENING-INHIBITOR (RIN) was not found to be heat-sensitive (Figure 6a). Transcripts encoding key repressors of photomorphogenesis as PHYTOCHROME INTERACTING FACTORS (PIFs), which are degraded in the light upon interaction with photoactivated phytochromes (Leivar & Monte, 2014), specifically PIF1a and PIF3, as well as DEETIOLATED1 (DET1/HP2), CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1) responded positively to heat at both ripening stages analysed.

Ripening-inducible genes related to ethylene biosynthesis and signaling, namely 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase and ACC synthase (ACO1, ACS2, ACS4), cell wall metabolism (e.g., polygalacturonase, PG2A), were significantly down-regulated as a result of heat stress exposure (Figure 6a). Moreover, expression of the gene encoding the fruit specific phytoene synthase (PSY1), the first enzyme of carotenoid pathway, was significantly repressed under H. This result was also confirmed by qPCR analysis in H_B and H_{B3} samples (Figure 5c). Indeed, the expression profile obtained from all samples suggests that PSY1 suppression under heat seemed to be linked to later ripening stages when chromoplast differentiates, because in H_{MG} fruits the PSY1 expression was yet slightly up-regulated.

Besides PSY1, other carotenogenic-related genes were DE upon heat exposure. Genes encoding 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (ISPE), geranylgeranyl pyrophosphate synthase (GGPPS2, GGPPS3), plastidial terminal oxidase (PTOX), carotenoid isomerase (CrtISO) were significantly down-regulated. Genes associated with vitamin E metabolism showed also lower expression (VTE2, VTE3.1, HPPD2) under H. Regarding the plastoglobuli-related proteins involved in the isoprenoid sequestration, tomato genes encoding FIBRILLIN (FBN) homologous to those described in Arabidopsis thaliana (van Wijk & Kessler, 2017) responded differently to heat stress. While some highly expressed members were down-regulated as FBN4, other members with less abundant transcripts in fruits were consistently up-regulated under H (Table S8).

The sucrose accumulation in heat-stressed fruit may be related to differences in sucrose turnover enzyme abundance (Qin et al., 2016). Although sucrose accumulated only in H_B fruits (Figure 2c), perturbations in the expression of genes related to sugar metabolism were detected at both B and B3 stages, with notable repression of the transcripts of vascular invertase (VI), cell wall invertase (LIN5) and sucrose synthase (Susy6) (Figure 6a). Sucrose turnover enzymes coding genes were higher repressed in H_B than H_{B3} (e.g., for VI, fold change ratio was about four-fold and two-fold in C_B vs H_B and C_{B3} vs H_{B3}, respectively), though the transcripts encoding a vacuolar invertase inhibitor (VIF) were found slightly up-regulated in H_{B3}, which suggests further capping to invertase activity.

3.7 | Lipid metabolism

Upon heat stress, lipid metabolism-related transcripts were found to be overrepresented among downregulated genes (Figure 5(b)). From a manually curated list derived from tomato loci showing homology to Arabidopsis genes associated with acyl-lipid metabolism (Higashi et al., 2015), a subset of genes putatively involved in the plastidial de novo fatty acid biosynthesis was suppressed under H in both B and B3 stages (Figure 6b, Table S9). First, the tomato homologs encoding the plastidial pyruvate dehydrogenase complex (PDH-E1 α, E1 β) producing acetyl-CoA precursors that feed fatty acid synthesis; then the subunits of heteromeric acetyl-CoA carboxylase (α-CT/CAC3, BCCP2), the acyl carrier protein (ACP), the malonyl-CoA:ACP malonyltransferase (MCMT), the 3-ketoacyl-ACP synthase 3 and 1 (KASIII, KASI) and the reductase (KAR), the hydroxyacyl ACP dehydratase (HAD), the enoyl-ACP reductase (ENR) were all strongly down-regulated under heat. The same trend was observed for the genes related to the acyl-ACP hydrolysis (FATB) involved in the acyl moieties releasing for the trafficking between plastid and endoplasmic reticulum (ER, Hölzl & Dörmann, 2019).

For the plastidial galactolipid synthesis, heat-inducible transcripts included those involved in the remodelling of galactolipids; for example, the galactolipid galactosyltransferase (SFR2/GGGT), which converts MGDG to oligogalactolipids and diacylglycerol (DAG, Higashi & Saito, 2019), and digalactosyl-DAG synthase (DGD). In contrast, genes encoding members of the plastid-localized lipid phosphatidate phosphatases (LPP), promoting dephosphorylation of phosphatidic acid (PA) that yields DAG, were suppressed by heat (Figure 6b).

In Arabidopsis, fatty acid desaturation, a key aspect of thermotolerance (Falcone et al., 2004), is regulated by genes encoding for the plastid localised fatty acid desaturase (FAB2/SAD, FAD4, FAD5, FAD6, FAD7, FAD8) and the ER-localized fatty acid desaturases (FAD2 and FAD3). For the plastid-residing FADs, genes encoding homologs for the 18:0-ACP desaturase (FAB2) and the 16:2/18:2 galactolipid ω3 desaturase (FAD7/FAD8/LeFAD7, Nakamura et al., 2016) were repressed. The latter is in line with the decrease in the plastid membrane trienoic fatty acids (Figure 3). In the present study, a specific 16:0-MGDG Δ7-desaturase (FAD5) coding gene was found to be highly up-regulated after H. In addition, major differences were detected in ER lipid biosynthetic gene transcripts in the H_{B3} samples, where the highly expressed gene coding for 18:1-phosphatidylcholine ω6 desaturase (FAD2-1, Lee et al., 2020) isoform and 16:2/18:2 galactolipid ω3 desaturase (FAD3, Yu et al., 2009) were repressed.

For TAG biosynthesis, higher levels of expression were associated with biosynthetic pathway genes. Different types of enzymes can synthesise TAG from DAG, including acyl-CoA dependent enzymes, acyl-CoA:DAG acyltransferases (DGATs), and diacylglycerol acyltransferase (PDAT) which uses PL as acyl donor (Fan et al., 2017). Genes encoding DGAT and PDAT were upregulated under heat, following the higher levels of TAG observed in H_B and H_{B3} (Figure 3). A similar trend was

found for the transcripts encoding proteins associated with TAG hydrolysis, which were predominantly upregulated at both stages analysed (Figure 6b).

Fatty acid β-oxidation pathway was overrepresented among the genes down-regulated by heat (Figure 5b). In accordance, the genes encoding enzymes were found actively repressed under heat, such as acyl-CoA oxidase (*ACX1a*, Li et al., 2005) and members of the multifunctional protein (*MFP*), as well as the peroxisomal isoform of long-chain acyl-CoA synthetase (LACS), which activates free fatty acids to acyl-CoA thioesters to generate acyl-CoA derivatives. Indeed, genes associated with oxylipin biosynthesis were repressed after H, including allene oxide cyclase (*AOC3*), allene oxide synthase (*AOS2*) and acyl-hydrolase patatin-like, involved in the production of jasmonate from polyunsaturated fatty acids.

For the volatiles production, 13-LOXs and hydroperoxide lyase (HPL) are the main enzymes catalysing the conversion of C18 polyunsaturated fatty acids to C5 and C6 volatiles in tomato fruit (Shen et al., 2014). Among the tomato 13-LOXs, LoxC is essential for the generation of fruit C5- (1-penten-3-ol, 1-penten-3-one, pentanal, (Z)-2-penten-1-ol, and 1-pentanol) and C6-flavour volatiles (Shen et al., 2014), while LoxB and LoxA possibly support C5 synthesis (Griffiths et al., 1999). The heat-induced response of genes encoding tomato *13-LOX* varied between H_B and H_{B3}, except for *LoxB*, which was downregulated at both ripening stages addressed. Importantly, ripening-inducible *LoxC* was found only repressed in H_B. *HPL* transcripts were concordantly repressed under H (Figure 6b, Table S9).

As the levels of β-sitosterol increased after heat stress (Figure 2e), the transcripts encoding sterol 22-desaturase (CYP710A11), involved in the conversion of β-sitosterol to stigmasterol, were checked, revealing a heat-sensitive expression pattern.

4 | DISCUSSION

Our study has undertaken an integrative approach to address the metabolic, cellular and molecular changes associated with transient heat stress imposed on tomato fruits, elucidating several key features that impact on fruit quality traits.

4.1 | Nutritional attributes were altered by heat stress

Our findings revealed that transient heat stress can alter carotenoid accumulation in tomato fruits, with sensitivity to temperature increasing as ripening advanced. The negative impact of heat on carotenoid levels was associated with changes in the initial steps in carotene formation.

Our study showed that ripe fruits have tremendous plasticity to restore carotenoid levels following a heat wave, suggesting no permanent damage was achieved. The temperature of 40°C caused moderate stress in tomato as reported earlier (Spicher et al., 2017). Reversible effects of heat treatment have been observed on vine

detached fruits previously (Lurie et al., 1996). Nevertheless, the lower carotenoids levels in ripe fruits experiencing heat at B3 transition implies that the length of the recovery period may be critical. Boosting in carotenoid synthesis during fruit ripening is achieved predominantly by the up-regulation of genes encoding key biosynthetic enzymes (Hirschberg, 2001; Enfissi et al., 2017). Thus, the heat-induced transcriptional misregulation at advanced ripening stages (Figure 6a) may explain the decrease in fruit carotenoid levels. Firstly, both phytoene formation and subsequent isomerisation are potentially compromised as the expression of fruit-specific *PSY1* (Figure 5), encoding the major flux-controlling enzyme of carotenogenic pathway (Fraser et al., 2002, 2007), and *CrtISO* (Isaacson et al., 2002) were repressed by heat. Secondly, efficient carotenoid desaturation conducted by phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS) depends on the redox status of plastoquinone/plastoquinol pool dependent on the activity of PTOX (Shahbazi et al., 2007) whose transcripts were found heat-sensitive. Finally, methyl-erythritol phosphate (MEP)-derived precursors for carotenogenesis (Almeida et al., 2015; Nogueira et al., 2018) might be altered as the expression of *ISPE*, *GGPPS3* and the ripening-induced *GGPPS2* were suppressed by heat. Together, these perturbations emphasise the transcriptional heat-sensitivity of the early carotenogenesis in fruits.

Carotenogenic enzymes are also post-translationally regulated by protease complexes which are highly active under heat (D'Andrea et al., 2018). The MEP enzyme DXS and carotenogenic PSY are direct substrates of Clp protease complex whose activity, in turn, is counterbalanced by other chaperones, for example, orange (OR; Welsch et al., 2018). Their interaction adjusts DXS and PSY functional forms controlling enzyme level and activity. Interestingly, Clp-defective tomato fruits have improved carotenoid accumulation under higher temperature post-harvest treatment compared to control (D'Andrea et al., 2018). Therefore, it is expected some contribution of post-translational mechanisms curbing the activity of carotenoid-related enzymes under heat stress. It is worth noting that this response is likely fruit-specific as heat-stressed leaves showed an opposite effect on carotenoids; indeed, the increased levels of lutein observed in H_L were consistent with a previous report using tomato (Spicher et al., 2017).

Higher vitamin E levels found in heat-stressed fruits and their recovered counterparts may serve as a molecular signature of fruits which have experienced stress previously irrespective of their developmental stage. The production of tocopherols has been linked to high-temperature response in tomato leaves supported by transcriptional regulation (Spicher et al. 2016, 2017). In our study, the lack of correlation between fruit tocopherol content (Table 1) and the expression of genes involved in tocopherol biosynthesis (Figure 6a), could be associated with the redirection of isoprenoid precursors from carotenoid formation into tocopherol formation instead (Almeida et al., 2015; Fraser et al., 2007). Importantly, carotenoid and tocopherol heat-responsive genes typically exhibit ripening-associated expression pattern (Quadrana et al., 2013; Sato et al., 2012;) and the changes observed may be due to inhibition of fruit ripening.

4.2 | Ripening related processes are misregulated in heat-stressed fruits

In tomato, fruit ripening encompasses highly coordinated processes orchestrated by a network of interacting genes and signaling pathways, which involves differentiation of chloroplasts into chromoplasts (Liu et al., 2015; Seymour et al., 2013). A peak in ethylene production and burst in cellular respiration are associated with profound metabolic transitions, leading to alterations not only in pigmentation but also in sugar accumulation, tissue softening and volatile production (Klee & Giovannoni, 2011; Rambla et al., 2015).

Several transcription factors acting as regulators of tomato fruit ripening including *CNR*, *NOR*, *FUL1*, *TAGL1* and *ARF2a* (Bemer et al., 2012; Giovannoni, 2007; Hao et al., 2015; Manning et al., 2006; Vrebalov et al., 2009) were transcriptionally suppressed by heat. Besides, repressors of carotenogenesis *PIF1a*, *DET1* and *COP1*, which transduce phytochrome-sensed changes in the environmental light, hence affecting carotenoid biosynthesis and plastid development (Enfissi et al., 2010; Liu et al., 2004; Llorente et al., 2015), were found to be up-regulated under heat conditions. As PIF1a binds to *PSY1* promoter region and represses *PSY1* transcription in tomato fruits (Llorente et al., 2015), the higher *PIF1a* expression may cause temperature-induced *PSY1* repression.

Expression profile of the ripening regulators mined from public database (TomExpress; Zouine et al., 2017) at non-stressed conditions supports the view that heat-induced repression of *CNR* and *NOR* are not likely due to ripening delay (as it cannot be ruled out for *FUL1* and *TAGL1*) but rather a bona fide feedback transcriptional regulation of fruits in response to higher temperatures. Similarly, for the known carotenogenic repressors PIF1a, COP1 and DET1 and possibly PIF3, all plausibly act as core components of heat-triggered transcriptional reprogramming in tomato fruits.

Importantly, PIF-dependent signaling is a central pathway for thermoresponsiveness under warmer but non-stressful ambient temperatures (Rosado et al., 2019; Vu et al., 2019;). Our findings thereby extend the role of the light signaling components in response to different temperature cues. The prevalence of phytochrome-signaling repressors, together with the suppression of *CNR* and *NOR*, may inhibit the ripening program in fruits experiencing heat stress at advanced ripening stages, which is in line with the down-regulation of genes involved in ethylene biosynthesis/signalling (*ACO1*, *ACS2*, *ACS4*, *E8*), cell wall degradation (*PG2a*, *EXP1*) (Li et al., 2019), and also with the persistence of thylakoid-like membranes (Figure 4, Figure S1). Moreover, consistent with previous studies in tomato (Mishra et al., 2002), fruit heat transcriptional response seems to be mediated by Hsfs (Figure 5). Finally, our transcriptome analysis suggested a role of epigenetic mechanisms mediating heat-induced transcriptional changes as chromatin remodelling and histone methylation were enriched among up-regulated genes in response to heat (Figure 5, Table S7). Epigenetic mechanisms as DNA methylation add another layer of regulation for the tomato ripening program. DNA methylation relies, in part, on the RNA directed DNA methylation (RdDM) pathway, dependent on small RNAs and the activity of DRM

and DRD1 (Gallusci et al., 2016). The up-regulation of tomato homologs *DRM1* and *DRD1* (Table S6) might contribute to rearrangements of epigenome landscape under high-temperature treatment and raises the possibility that plant adaptive responses to heat mediated by epigenetic mechanisms (Li et al., 2018a; Quadrana et al., 2019; Ohama et al., 2017) also operate in tomato fruit.

4.3 | Fruit primary metabolism changes in response to heat stress

The absence of signatures commonly shared through all stressed samples (Figure 2, Table S2) further supports the idea the thermoresponsive is highly dependent on fruit stage. Among the known osmoregulators, higher threonine levels at advanced ripening stages are in line with its conserved biomarker for abiotic stress, accumulating in Arabidopsis leaves under heat stress (Obata & Fernie, 2012). Notably, citric and malic acids that contribute most to the typical acidity of tomato fruit (Baldwin et al., 2008) remained unaffected.

Sucrose accumulation in heat-stressed fruit at early ripening stages (Figure 2) correlated with the sensitivity of sucrose metabolism to high temperatures previously reported in tomato male reproductive system and in fruits after pollination (Li et al., 2012; Liu et al., 2016; Sato et al., 2006). Indeed, enhanced LIN and VI activity in tomato has been associated with fruit thermotolerance at early developmental stages (Li et al., 2012; Liu et al., 2016). It is known that, at late-ripening stages, sucrose accumulation is limited since invertase activities intensify as ripening progresses, with VI controlling sucrose/hexose ratio (Biais et al., 2014; Klann et al., 1996; Qin et al., 2016; Yelle et al., 1991). In H$_B$ fruits, lower *VI* transcripts may explain why sucrose increased. However, control sucrose levels found at H$_{B3}$ did not correlate with down-regulation of *VI*, *LIN5* and *Susy6*. In this case, sugar metabolic fluxes at B3 stage may prevent sucrose from accumulating under heat. Lack of correlation between invertase activity and sucrose/hexose levels has been reported in tomato fruits of lines with increased LIN activity (Liu et al., 2016).

Effects on fruits seem to be minor compared to the vegetative system or pollen development, where altered carbon metabolism upon exposure to high temperatures can promote yield losses (Ruan et al., 2010; Rieu et al., 2017). Moreover, fluctuations in sugar levels are important, as cell signals, since they can act in crosstalk with hormones (e.g., auxin) and reactive oxygen species (ROS) signaling pathways during stress responses; sugars can also contribute to stress alleviation by facilitating production of HSP even in reproductive systems, property that correlates to high fruit sensitivity under stress (Liu et al., 2013). Molecule signaling and protective roles are, therefore, possible to intersect under heat stress in fruits.

4.4 | Lipid remodelling triggered by heat stress in fruits

Lipid remodelling was a pronounced feature of heat-stressed fruits, leading to TAG accumulation, and decreasing the level of membrane

lipid unsaturation (Figure 3, Table S3). Polyunsaturated acyl chains contribute to membrane fluidity and stability and, in response to higher temperatures, the degree of unsaturation is decreased to maintain optimal fluidity and integrity of membranes (Nishida & Murata, 1996; Murakami et al., 2000; Falcone et al., 2004; Zheng et al., 2011). Moreover, the observed changes in sterols might also be linked to membrane stability. Heat-induced β-sitosterol accumulation may contribute to control membrane permeability and membrane protein activity (Guo et al., 2019).

TAG accumulation associated with heat stress-induced lipid remodelling has been reported in photosynthetic organisms (Légeret et al. 2016; Mueller et al., 2015; Narayanan et al., 2016). The
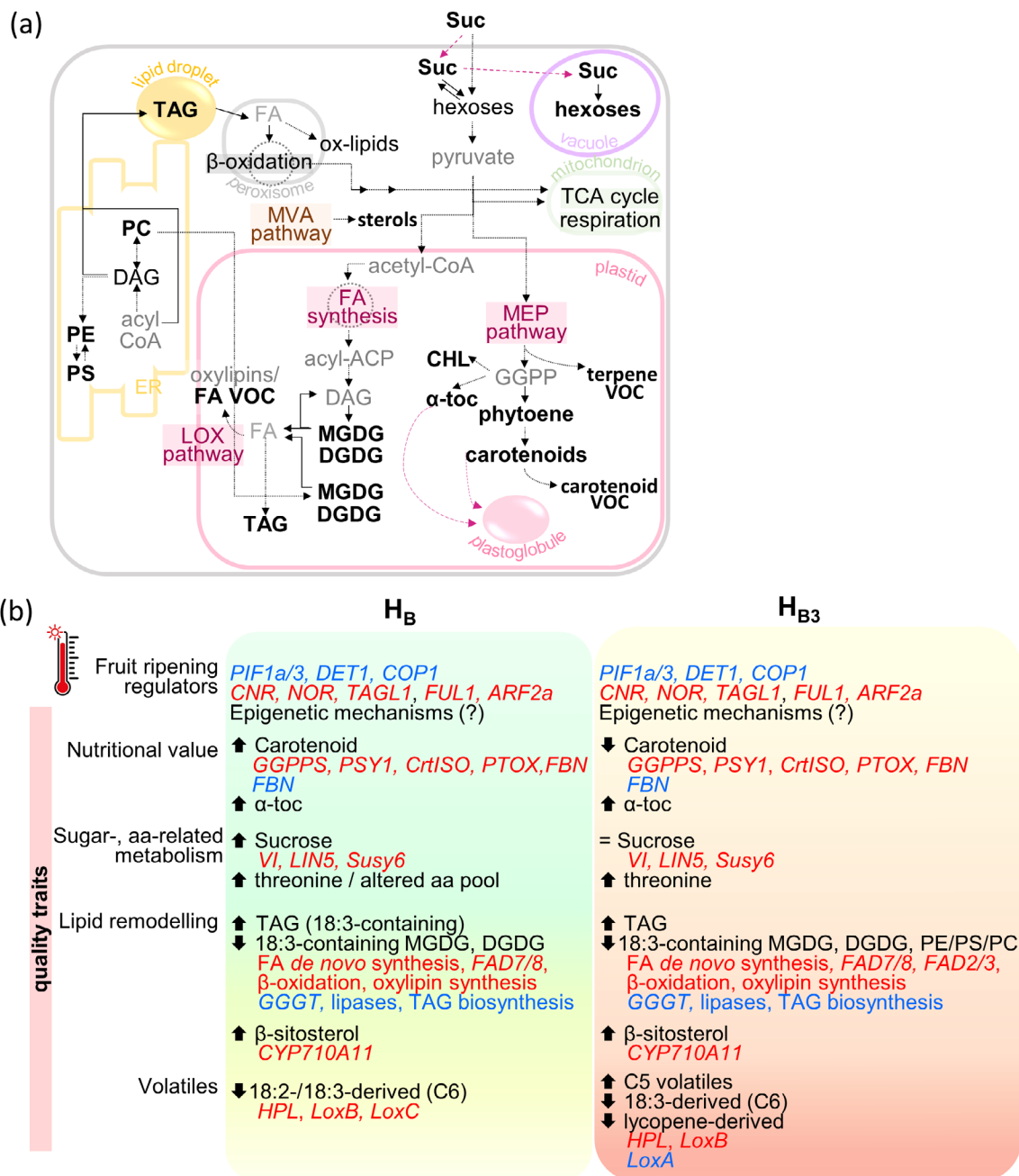

FIGURE 7 Impact of heat stress on tomato fruit. (a) Fruit metabolic processes affected by heat. Compounds targeted on metabolite profiling are shown in bold. Amino acid (aa) metabolism was omitted for simplicity. Dotted lines indicate multiple metabolic steps; (b) Summary of heat-induced metabolic changes and associated transcriptional regulation found in B and B3 fruits exposed to high-temperature treatment. Arrows indicate metabolite changes. Up-regulated and down-regulated genes (or pathways) are indicated in blue and red, respectively. ACP, acyl carrier protein; CHL, chlorophyll; DAG, diacylglycerol; FA, fatty acid; MVA, mevalonate; MEP, methyl-erythritol phosphate; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PC/PS, phosphatidylcholine/phosphatidylserine; PE, phosphatidylethanolamine; TAG, triacylglycerol; Suc, sucrose; α-toc, α-tocopherol; TCA, tricarboxylic acid; ox-lipids, oxidised phospholipids; VOC, volatile organic compound [Colour figure can be viewed at wileyonlinelibrary.com]

substantial heat-induced decrease in C18:3/C18:2 ratio of plastid membrane lipids in fruits is similar to previous reports in heat-stressed tomato leaves (Spicher et al., 2016). Besides the prevention of physical–chemical damages of membranes, it has been proposed that selective decline of trienoic acid acyl moieties might confer survival advantage imposed by cellular oxidative stress associated to excessive ROS generated under heat (Larkindale et al., 2007; Légeret et al., 2016). As galactolipids are highly enriched in polyunsaturated fatty acids, and thus easily prone to lipid peroxidation, photosynthetic organisms may transfer trienoic fatty acids from membrane lipids to TAG sequestered in lipid droplets as a strategy to control the extension of lipid oxidation (Du et al., 2018; Légeret et al., 2016).

Our transcriptome data provided insights into molecular mechanisms supporting lipid remodelling (Figure 6b, Table S9). The heat-induced decrease in the level of plastid lipid unsaturation coincided with the down-regulation of FADs, mainly the FAD7/FAD8 encoding the plastidial ω3 desaturase. Transcripts of the ER-counterpart desaturase (FAD3) were only significantly repressed in H_{B3}, following the temperature-induced decrease of extraplastidial 18:3-acyl-containing lipids specific to this stage (Figure 3). Lipid remodelling may also be supported for the up-regulation of (a) SFR2/GGGT, whose corresponding enzyme contributes to diminish the MGDG/oligogalactolipids ratio and to release DAG further used for TAG biosynthesis (Higashi & Saito, 2019; Moellering & Benning, 2011), and (b) DGAT and PDAT, which encode TAG biosynthetic-related enzymes (Fan et al., 2017). Together, these data suggest that the higher amounts of 18:3-acyl-containing TAGs upon heat, at B stage, may have been derived from C18:3 released from membrane lipids than from de novo synthesised fatty acids (Légeret et al., 2016), therefore reflecting the changes in the proportion of membrane glycerolipid composition (Higashi & Saito, 2019). By contrast, at B3 stage, 18:3 acyl moieties are likely redirected to lipid oxidation pathways (Schilmiller et al., 2007), for example, volatile production. In tomato, levels of C18:2 and C18:3 positively correlate to volatile derivatives hexanal and hexenal, respectively (Domínguez et al., 2010; Ties & Barringer, 2012). The lipid remodelling triggered by heat under our experimental conditions, affecting plastidial C18:3 availability, may explain the decrease in unsaturated C6 volatiles as hexenal. The increased C18:2 precursors may sustain non-stressed levels of C18:2-derived hexanal at H_{B3}, as opposed to lower levels found in H_B, even under a possible constraint in LOX/HPL-dependent pathway as suggested by heat-induced repression of HPL transcripts. C6-volatiles are synthesised via the action of 13-LOX and HPL enzymes, whilst C5-volatiles are LOX-dependent though HPL-independent (Shen et al., 2014). C5-volatiles accumulated only in H_{B3} as, in this case, 18:3-acyl-containing TAGs were unaffected. A decrease in HPL activity by heat might enhance the hydroperoxide pool, which can be redirected towards the C5 branch of LOX-pathway as proposed previously based on tomato HPL-deficient lines (Shen et al., 2014). Notably, the tomato fruit volatile profile is highly sensitive to heat exhibiting alterations even when the stress ceased.

In conclusion, our findings illustrated the impact of brief exposure to high-temperature events on tomato fruit quality and revealed potential molecular mechanisms associated with heat response (Figure 7). Depending on the ripening stage, heat may have underestimated yet significant effects on nutritionally value and other quality-related attributes in tomato, with sensitivity to high temperature increasing in more advanced ripening stages. Several heat-stress responsive genes, including fruit ripening regulators, have been identified from transcriptome analysis correlating with the metabolite changes. Collectively the data acquired provides a significant advancement to our understanding of fruit metabolic reprogramming associated with heat stress. It is now clear that cold storage is not the only stress affecting fruit quality but perturbations in heat will also alter quality attributes. These data provide an exploitable resource for the development of climate resilient crop varieties.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Juliana Almeida and Paul D. Fraser conceived the original research plan. Juliana Almeida performed the experimental programme, data analysis and statistics. Laura Perez-Fons carried out the volatile analysis and components of the metabolite analysis. Juliana Almeida and Paul D. Fraser wrote the manuscript. All authors contributed to data discussion and conclusions. Paul D. Fraser acquired the funding and accepts to serve as the contact point author for communication. All authors have read and approved the final manuscript.

ORCID

Paul D. Fraser   https://orcid.org/0000-0002-5953-8900

REFERENCES

Albert, E., Segura, V., Gricourt, J., Bonnefoi, J., Derivot, L., & Causse, M. (2016). Association mapping reveals the genetic architecture of tomato response to water deficit: Focus on major fruit quality traits. *Journal of Experimental Botany*, *67*(22), 6413–6430.

Almeida, J., Asís, R., Molineri, V. N., Sestari, I., Lira, B. S., Carrari, F., … Rossi, M. (2015). Fruits from ripening impaired, chlorophyll degraded and jasmonate insensitive tomato mutants have altered tocopherol content and composition. *Phytochemistry*, *111*, 72–83.

Anders, S., Pyl, P. T., & Huber, W. (2015). HTSeq-A python framework to work with high-throughput sequencing data. *Bioinformatics*, *31*(2), 166–169.

Baldwin, E. A., Goodner, K., & Plotto, A. (2008). Interaction of volatiles, sugars, and acids on perception of tomato aroma and flavor descriptors. *Journal of Food Science*, *73*(6), S294–S307.

Balogh, G., Péter, M., Glatz, A., Gombos, I., Török, Z., Horváth, I., … Vígh, L. (2013). Key role of lipids in heat stress management. *FEBS Letters*, *587*(13), 1970–1980.

Battisti, D. S., & Naylor, R. L. (2009). Historical warnings of future food insecurity with unprecedented seasonal heat. *Science*, *323*(5911), 240–244.

Bemer, M., Karlova, R., Ballester, A. R., Tikunov, Y. M., Bovy, A. G., Wolters-Arts, M., … de Maagd, R. A. (2012). The tomato FRUITFULL homologs TDR4/FUL1 and MBP7/FUL2 regulate ethylene-independent aspects of fruit ripening. *The Plant Cell*, *24*(11), 4437–4451.

Bergougnoux, V. (2014). The history of tomato: From domestication to biopharming. *Biotechnology Advances*, *32*(1), 170–189.

Biais, B., Bénard, C., Beauvoit, B., Colombié, S., Prodhomme, D., Ménard, G., … Gibon, Y. (2014). Remarkable reproducibility of enzyme activity profiles in tomato fruits grown under contrasting environments provides a roadmap for studies of fruit metabolism. *Plant Physiology*, *164*(3), 1204–1221.

Bita, C. E., & Gerats, T. (2013). Plant tolerance to high temperature in a changing environment: Scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science*, *4*, 1–18.

Cheng, Y., Bian, W., Pang, X., Yu, J., Ahammed, G. J., Zhou, G., … Wan, H. (2017). Genome-wide identification and evaluation of reference genes for quantitative RT-PCR analysis during tomato fruit development. *Frontiers in Plant Science*, *8*, 1–13.

Conesa, A., Götz, S., García-Gómez, J. M., Terol, J., Talón, M., & Robles, M. (2005). Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*, *21*(18), 3674–3676.

D'Andrea, L., Simon-Moya, M., Llorente, B., Llamas, E., Marro, M., Loza-Alvarez, P., … Rodriguez-Concepcion, M. (2018). Interference with Clp protease impairs carotenoid accumulation during tomato fruit ripening. *Journal of Experimental Botany*, *69*(7), 1557–1568.

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., … Gingeras, T. R. (2013). STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics*, *29*(1), 15–21.

Domínguez, T., Hernández, M. L., Pennycooke, J. C., Jiménez, P., Martínez-Rivas, J. M., Sanz, C., … Sanmartín, M. (2010). Increasing ω-3 desaturase expression in tomato results in altered aroma profile and enhanced resistance to cold stress. *Plant Physiology*, *153*(2), 655–665.

Du, Z. Y., Lucker, B. F., Zienkiewicz, K., Miller, T. E., Zienkiewicz, A., Sears, B. B., … Benning, C. (2018). Galactoglycerolipid lipase PGD1 is involved in thylakoid membrane remodeling in response to adverse environmental conditions in chlamydomonas. *The Plant Cell*, *30*(2), 447–465.

Enfissi, E. M. A., Barneche, F., Ahmed, I., Lichtlé, C., Gerrish, C., McQuinn, R. P., … Fraser, P. D. (2010). Integrative transcript and metabolite analysis of nutritionally enhanced DE-ETIOLATED1 down-regulated tomato fruit. *The Plant Cell*, *22*(4), 1190–1215.

Enfissi, E. M. A., Nogueira, M., Bramley, P. M., & Fraser, P. D. (2017). The regulation of carotenoid formation in tomato fruit. *The Plant Journal*, *89*(4), 774–788.

Exposito-Rodriguez, M., Borges, A. A., Borges-Perez, A., Perez, J., Expósito-Rodríguez, M., Borges-Pérez, A., & Pérez, J. (2008). Selection of internal control genes for quantitative real-time RT-PCR studies during tomato development process. *BMC Plant Biology*, *8*, 131.

Del Fabbro, C., Scalabrin, S., Morgante, M., & Giorgi, F. M. (2013). An extensive evaluation of read trimming effects on illumina NGS data analysis. *PLoS One*, *8*(12), e85024.

Falcone, D. L., Ogas, J. P., & Somerville, C. R. (2004). Regulation of membrane fatty acid composition by temperature in mutants of Arabidopsis with alterations in membrane lipid composition. *BMC Plant Biology*, *4*, 17.

Fan, J., Yu, L., & Xu, C. (2017). A central role for triacylglycerol in membrane lipid breakdown, fatty acid β-oxidation, and plant survival under extended darkness. *Plant Physiology*, *174*(3), 1517–1530.

Farmer, E. E., & Mueller, M. J. (2013). ROS-mediated lipid peroxidation and RES-activated signaling. *Annual Review of Plant Biology*, *64*(1), 429–450.

Feussner, I., & Wasternack, C. (2002). The lipoxygenase pathway. *Annual Review of Plant Biology*, *53*(1), 275–297.

Fragkostefanakis, S., Simm, S., Paul, P., Bublak, D., Scharf, K. D., & Schleiff, E. (2015). Chaperone network composition in *Solanum lycopersicum* explored by transcriptome profiling and microarray meta-analysis. *Plant, Cell and Environment*, *38*(4), 693–709.

Fraser, P. D., Romer, S., Shipton, C. a., Mills, P. B., Kiano, J. W., Misawa, N., … Bramley, P. M. (2002). Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(2), 1092–1097.

Fraser, P. D., Enfissi, E. M. A., Halket, J. M., Truesdale, M. R., Yu, D., Gerrish, C., & Bramley, P. M. (2007). Manipulation of phytoene levels in tomato fruit: Effects on isoprenoids, plastids, and intermediary metabolism. *The Plant Cell*, *19*(10), 3194–3211.

Gallusci, P., Hodgman, C., Teyssier, E., & Seymour, G. B. (2016). DNA methylation and chromatin regulation during fleshy fruit development and ripening. *Frontiers in Plant Science*, *7*, 1–14.

Gautier, H., Diakou-Verdin, V., Bénard, C., Reich, M., Buret, M., Bourgaud, F., … Génard, M. (2008). How does tomato quality (sugar, acid, and nutritional quality) vary with ripening stage, temperature, and irradiance? *Journal of Agricultural and Food Chemistry*, *56*(4), 1241–1250.

Giovannoni, J. J. (2007). Fruit ripening mutants yield insights into ripening control. *Current Opinion in Plant Biology*, *10*(3), 283–289.

Gray, S. B., & Brady, S. M. (2016). Plant developmental responses to climate change. *Developmental Biology*, *419*(1), 64–77.

Griffiths, A., Prestage, S., Linforth, R., Zhang, J., Taylor, A., & Grierson, D. (1999). Fruit-specific lipoxygenase suppression in antisense-transgenic tomatoes. *Postharvest Biology and Technology*, *17*(3), 163–173.

Guo, Q., Liu, L., & Barkla, B. J. (2019). Membrane lipid remodeling in response to salinity. *International Journal of Molecular Sciences*, *20*(17), 1–31.

Hao, Y., Hu, G., Breitel, D., Liu, M., Mila, I., Frasse, P., … Zouine, M. (2015). Auxin response factor SlARF2 is an essential component of the regulatory mechanism controlling fruit ripening in tomato. *PLoS Genetics*, *11*(12), e1005649.

Hernández, V., Hellín, P., Fenoll, J., & Flores, P. (2015). Increased temperature produces changes in the bioactive composition of tomato, depending on its developmental stage. *Journal of Agricultural and Food Chemistry*, *63*(9), 2378–2382.

Higashi, Y., Okazaki, Y., Myouga, F., Shinozaki, K., & Saito, K. (2015). Landscape of the lipidome and transcriptome under heat stress in *Arabidopsis thaliana*. *Scientific Reports*, *5*, 10533.

Higashi, Y., & Saito, K. (2019). Lipidomic studies of membrane glycerolipids in plant leaves under heat stress. *Progress in Lipid Research*, *75*, 100990.

Hirschberg, J. (2001). Carotenoid biosynthesis in flowering plants. *Current Opinion in Plant Biology*, *4*(3), 210–218.

Hölzl, G., & Dörmann, P. (2019). Chloroplast lipids and their biosynthesis. *Annual Review of Plant Biology*, *70*(1), 51–81.

Horton, D. E., Johnson, N. C., Singh, D., Swain, D. L., Rajaratnam, B., & Diffenbaugh, N. S. (2015). Contribution of changes in atmospheric circulation patterns to extreme temperature trends. *Nature*, *522*(7557), 465–469.

Hou, Q., Ufer, G., & Bartels, D. (2016). Lipid signalling in plant responses to abiotic stress. *Plant Cell and Environment*, *39*(5), 1029–1048.

IPCC (2013). Summary for policymakers. In T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, et al. (Eds.), *Climate change*

2013: The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge, England; New York, NY: Cambridge University Press.

Isaacson, T., Ronen, G., Zamir, D., & Hirschberg, J. (2002). Cloning of tangerine from tomato reveals a carotenoid Isomerase essential for the production of β-carotene and Xanthophylls in plants. *The Plant Cell*, 14(2), 333–342.

Karlova, R., Chapman, N., David, K., Angenent, G. C., Seymour, G. B., & De Maagd, R. A. (2014). Transcriptional control of fleshy fruit development and ripening. *Journal of Experimental Botany*, 65(16), 4527–4541.

Klann, E. M., Hall, B., & Bennett, A. B. (1996). Antisense Acid Invertase (TIV1) gene alters soluble sugar composition and size in transgenic tomato fruit. *Plant Physiology*, 112(3), 1321–1330.

Klee, H. J., & Giovannoni, J. J. (2011). Genetics and control of tomato fruit ripening and quality attributes. *Annual Review of Genetics*, 45(1), 45–59.

von Koskull-Döring, P., Scharf, K. D., & Nover, L. (2007). The diversity of plant heat stress transcription factors. *Trends in Plant Science*, 12(10), 452–457.

Kotak, S., Larkindale, J., Lee, U., von Koskull-Döring, P., Vierling, E., & Scharf, K.-D. (2007). Complexity of the heat stress response in plants. *Current Opinion in Plant Biology*, 10(3), 310–316.

Kruk, J., Szymańska, R., Cela, J., & Munne-Bosch, S. (2014). Plastochromanol-8: Fifty years of research. *Phytochemistry*, 108, 9–16.

Larkindale, J., Mishkind, M., & Vierling, E. (2007). Plant responses to high temperature. In M. A. Jenks & P. M. Hasegawa (Eds.), *Plant abiotic stress* (pp. 100–144). Oxford, England: Blackwell Publishing.

Lee, M. W., Padilla, C. S., Gupta, C., Galla, A., Pereira, A., Li, J., & Goggin, F. L. (2020). The FATTY ACID DESATURASE2 family in tomato contributes to primary metabolism and stress responses. *Plant Physiology*, 182(2), 1083–1099.

Légeret, B., Schulz-Raffelt, M., Nguyen, H. M., Auroy, P., Beisson, F., Peltier, G., ... Li-Beisson, Y. (2016). Lipidomic and transcriptomic analyses of *Chlamydomonas reinhardtii* under heat stress unveil a direct route for the conversion of membrane lipids into storage lipids. *Plant Cell and Environment*, 39(4), 834–847.

Leivar, P., & Monte, E. (2014). PIFs: systems integrators in plant development. *The Plant Cell*, 26(1), 56–78.

Lesk, C., Rowhani, P., & Ramankutty, N. (2016). Influence of extreme weather disasters on global crop production. *Nature*, 529(7584), 84–87.

Li, C., Schilmiller, A. L., Liu, G., Lee, G. I., Jayanty, S., Sageman, C., ... Howe, G. A. (2005). Role of β-oxidation in Jasmonate biosynthesis and systemic wound signaling in tomato. *The Plant Cell*, 17(3), 971–986.

Li, S., Chen, K., & Grierson, D. (2019). A critical evaluation of the role of ethylene and MADS transcription factors in the network controlling fleshy fruit ripening. *New Phytologist*, 221(4), 1724–1741.

Li, X. R., Deb, J., Kumar, S. V., & Østergaard, L. (2018a). Temperature modulates tissue-specification program to control fruit dehiscence in Brassicaceae. *Molecular Plant*, 11(4), 598–606.

Li, Y., Wang, H., Zhang, Y., & Martin, C. (2018b). Can the world's favorite fruit, tomato, provide an effective biosynthetic chassis for high-value metabolites? *Plant Cell Reports*, 37(10), 1443–1450.

Li, Z., Palmer, W. M., Martin, A. P., Wang, R., Rainsford, F., Jin, Y., ... Ruan, Y.-L. (2012). High invertase activity in tomato reproductive organs correlates with enhanced sucrose import into, and heat tolerance of, young fruit. *Journal of Experimental Botany*, 63(3), 1155–1166.

Liu, M., Pirrello, J., Chervin, C., Roustan, J.-P., & Bouzayen, M. (2015). Ethylene control of fruit ripening: Revisiting the complex network of transcriptional regulation. *Plant Physiology*, 169(4), 2380–2390.

Liu, Y., Roof, S., Ye, Z., Barry, C., Van Tuinent, A., Vrebalov, J., Bowler, C. & Giovannoni, J. (2004). Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. *Proceedings of the National Academy of Sciences of the United States of America*, 101(26), 9897–9902.

Liu, Y. H., Offler, C. E., & Ruan, Y. L. (2016). Cell wall invertase promotes fruit set under heat stress by suppressing ROS-independent cell death. *Plant Physiology*, 172(1), 163–180.

Liu, Y. H., Offler, C. E., & Ruan, Y. L. (2013). Regulation of fruit and seed response to heat and drought by sugars as nutrients and signals. *Frontiers in Plant Science*, 4, 1–12.

Llorente, B., D'Andrea, L., Ruiz-Sola, M. A., Botterweg, E., Pulido, P., Andilla, J., ... Rodriguez-Concepcion, M. (2015). Tomato fruit carotenoid biosynthesis is adjusted to actual ripening progression by a light-dependent mechanism. *The Plant Journal*, 85(1), 107–119.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15(12), 1–21.

Lu, T., Meng, Z., Zhang, G., Qi, M., Sun, Z., Liu, Y., & Li, T. (2017). Sub-high temperature and high light intensity induced irreversible inhibition on photosynthesis system of tomato plant (*Solanum lycopersicum* L.). *Frontiers in Plant Science*, 8, 1–16.

Lurie, S., Handros, A., Fallik, E., & Shapira, R. (1996). Reversible inhibition of tomato fruit gene expression at high temperature: Effects on tomato fruit ripening. *Plant Physiology*, 110(4), 1207–1214.

Manning, K., Tör, M., Poole, M., Hong, Y., Thompson, A. J., King, G. J., ... Seymour, G. B. (2006). A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nature Genetics*, 38(8), 948–952.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal*, 17, 1710–1712.

Massot, C., Bancel, D., Lauri, F. L., Truffault, V., Baldet, P., Stevens, R., & Gautier, H. (2013). High temperature inhibits ascorbate recycling and light stimulation of the ascorbate pool in tomato despite increased expression of biosynthesis genes. *PLoS One*, 8(12), 1–13.

Mishra, S. K., Tripp, J., Winkelhaus, S., Tschiersch, B., Theres, K., Nover, L., & Scharf, K. D. (2002). In the complex family of heat stress transcription factors, HsfA1 has a unique role as master regulator of thermotolerance in tomato. *Genes and Development*, 16(12), 1555–1567.

Moellering, E. R., & Benning, C. (2011). Galactoglycerolipid metabolism under stress: A time for remodeling. *Trends in Plant Science*, 16(2), 98–107.

Mueller, S. P., Krause, D. M., Mueller, M. J., & Fekete, A. (2015). Accumulation of extra-chloroplastic triacylglycerols in Arabidopsis seedlings during heat acclimation. *Journal of Experimental Botany*, 66(15), 4517–4526.

Mulholland, B. J., Edmondson, R. N., Fussell, M., Basham, J., & Ho, L. C. (2003). Effects of high temperature on tomato summer fruit quality. *Journal of Horticultural Science and Biotechnology*, 78(3), 365–374.

Murakami, Y., Tsuyama, M., Kobayashi, Y., Kodama, H., & Iba, K. (2000). Trienoic fatty acids and plant tolerance of high temperature. *Science*, 287(5452), 476–479.

Nakamura, S., Hondo, K., Kawara, T., Okazaki, Y., Saito, K., Kobayashi, K., ... Nishiguchi, M. (2016). Conferring high-temperature tolerance to non-transgenic tomato scions using graft transmission of RNA silencing of the fatty acid desaturase gene. *Plant Biotechnology Journal*, 14(2), 783–790.

Narayanan, S., Tamura, P. J., Roth, M. R., Prasad, P. V. V., & Welti, R. (2016). Wheat leaf lipids during heat stress: I. high day and night temperatures result in major lipid alterations. *Plant Cell and Environment*, 39(4), 787–803.

Neta-Sharir, I., Isaacson, T., Lurie, S., & Weiss, D. (2005). Dual role for tomato heat shock protein 21: Protecting photosystem II from oxidative stress and promoting color changes during fruit maturation. *The Plant Cell*, 17(6), 1829–1838.

Nishida, I., & Murata, N. (1996). Chilling sensitivity in plants and cyanobacteria: The crucial contribution of membrane lipids. *Annual Review of Plant Physiology and Plant Molecular Biology*, 47(1), 541–568.

Nogueira, M., Enfissi, E. M., Almeida, J., & Fraser, P. D. (2018). Creating plant molecular factories for industrial and nutritional isoprenoid production. *Current Opinion in Biotechnology*, 49, 80–87.

Nogueira, M., Mora, L., Enfissi, E. M. A., Bramley, P. M., & Fraser, P. D. (2013). Subchromoplast sequestration of carotenoids affects regulatory mechanisms in tomato lines expressing different carotenoid gene combinations. *The Plant Cell*, 25(11), 4560–4579.

Obata, T., & Fernie, A. R. (2012). The use of metabolomics to dissect plant responses to abiotic stresses. *Cellular and Molecular Life Sciences*, 69 (19), 3225–3243.

Ohama, N., Sato, H., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2017). Transcriptional regulatory network of plant heat stress response. *Trends in Plant Science*, 22(1), 53–65.

Peet, M. M., Sato, S., & Gardner, R. G. (1998). Comparing heat stress effects on male-fertile and male-sterile tomatoes. *Plant, Cell and Environment*, 21(2), 225–231.

Pertea, M., Pertea, G. M., Antonescu, C. M., Chang, T. C., Mendell, J. T., & Salzberg, S. L. (2015). StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nature Biotechnology*, 33(3), 290–295.

Picton, S., & Grierson, D. (1988). Inhibition of expression of tomato-ripening genes at high temperature. *Plant, Cell & Environment*, 11(4), 265–272.

Pressman, E., Peet, M. M., & Pharr, D. M. (2002). The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrate concentration in the developing anthers. *Annals of Botany*, 90 (5), 631–636.

Qin, G., Zhu, Z., Wang, W., Cai, J., Chen, Y., Li, L., & Tian, S. (2016). A tomato vacuolar invertase inhibitor mediates sucrose metabolism and influences fruit ripening. *Plant Physiology*, 172(3), 1596–1611.

Quadrana, L., Almeida, J., Otaiza, S. N., Duffy, T., Corrêa da Silva, J. V., de Godoy, F., … Rossi, M. (2013). Transcriptional regulation of tocopherol biosynthesis in tomato. *Plant Molecular Biology*, 81(3), 309–325.

Quadrana, L., Etcheverry, M., Gilly, A., Caillieux, E., Madoui, M., Guy, J., … Colot, V. (2019). Transposition favors the generation of large effect mutations that may facilitate rapid adaption. *Nature Communications*, 10(1), 3421.

Quinet, M., Angosto, T., Yuste-Lisbona, F. J., Blanchard-Gros, R., Bigot, S., Martinez, J.-P., & Lutts, S. (2019). Tomato fruit development and metabolism. *Frontiers in Plant Science*, 10, 1554.

Rambla, J. L., Alfaro, C., Medina, A., Zarzo, M., Primo, J., & Granell, A. (2015). Tomato fruit volatile profiles are highly dependent on sample processing and capturing methods. *Metabolomics*, 11(6), 1708–1720.

Rieu, I., Twell, D., & Firon, N. (2017). Pollen development at high temperature: From acclimation to collapse. *Plant Physiology*, 173(4), 1967–1976.

Rosado, D., Trench, B., Bianchetti, R., Zuccarelli, R., Rodrigues Alves, F. R., Purgatto, E., … Rossi, M. (2019). Downregulation of PHYTOCHROME-INTERACTING FACTOR 4 influences plant development and fruit production. *Plant Physiology*, 181(3), 1360–1370.

Ruan, Y.-L., Jin, Y., Yang, Y.-J., Li, G.-J., & Boyer, J. S. (2010). Sugar input, metabolism, and signaling mediated by Invertase: Roles in development, yield potential, and response to drought and heat. *Molecular Plant*, 3(6), 942–955.

Sato, S., Kamiyama, M., Iwata, T., Makita, N., Furukawa, H., & Ikeda, H. (2006). Moderate increase of mean daily temperature adversely affects fruit set of *Lycopersicon esculentum* by disrupting specific physiological processes in male reproductive development. *Annals of Botany*, 97(5), 731–738.

Sato, S., Peet, M. M., & Thomas, J. F. (2000). Physiological factors limit fruit set of tomato (*Lycopersicon esculentum* Mill.) under chronic, mild heat stress. *Plant, Cell and Environment*, 23(7), 719–726.

Sato, S., Tabata, S., Hirakawa, H., Asamizu, E., Shirasawa, K., Isobe, S., et al. (2012). The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*, 485(7400), 635–641.

Schilmiller, A. L., Koo, A. J. K., & Howe, G. A. (2007). Functional diversification of acyl-coenzyme A oxidases in jasmonic acid biosynthesis and action. *Plant Physiology*, 143(2), 812–824.

Seymour, G. B., Chapman, N. H., Chew, B. L., & Rose, J. K. C. (2013). Regulation of ripening and opportunities for control in tomato and other fruits. *Plant Biotechnology Journal*, 11(3), 269–278.

Shahbazi, M., Gilbert, M., Labouré, A.-M., & Kuntz, M. (2007). Dual role of the plastid terminal oxidase in tomato. *Plant Physiology*, 145(3), 691–702.

Shen, J., Tieman, D., Jones, J. B., Taylor, M. G., Schmelz, E., Huffaker, A., … Klee, H. J. (2014). A 13-lipoxygenase, TomloxC, is essential for synthesis of C5 flavour volatiles in tomato. *Journal of Experimental Botany*, 65 (2), 419–428.

Spicher, L., Almeida, J., Gutbrod, K., Pipitone, R., Dörmann, P., Glauser, G., … Kessler, F. (2017). Essential role for phytol kinase and tocopherol in tolerance to combined light and temperature stress in tomato. *Journal of Experimental Botany*, 68(21–22), 5845–5856.

Spicher, L., Glauser, G., & Kessler, F. (2016). Lipid antioxidant and galactolipid remodeling under temperature stress in tomato plants. *Frontiers in Plant Science*, 7, 1–12.

Tieman, D., Zhu, G., Resende, M. F. R., Lin, T., Nguyen, C., Bies, D., et al. (2017). A chemical genetic roadmap to improved tomato flavor. *Science*, 355(6323), 391–394.

Ties, P., & Barringer, S. (2012). Influence of lipid content and lipoxygenase on flavor volatiles in the tomato peel and flesh. *Journal of Food Science*, 77(7), C830–C837.

Tomes, M. L. (1963). Temperature inhibition of carotene synthesis in tomato. *Botanical Gazette*, 124(3), 180–185.

Uluisik, S., Chapman, N. H., Smith, R., Poole, M., Adams, G., Gillis, R. B., … Seymour, G. B. (2016). Genetic improvement of tomato by targeted control of fruit softening. *Nature Biotechnology*, 34(9), 950–952.

Vandesompele, J., De Preter, K., Filip, P., Poppe, B., Van Roy, N., De Paepe, A., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, 3(7), 0034.1-0034.11.

Viuda-Martos, M., Sanchez-Zapata, E., Sayas-Barbera, E., Sendra, E., Perez-Alvarez, J. A., & Fernandez-Lopez, J. (2014). Tomato and tomato byproducts. Human health benefits of lycopene and its application to meat products: A review. *Critical Reviews in Food Science and Nutrition*, 54(8), 1032–1049.

Vrebalov, J., Pan, I. L., Arroyo, A. J. M., McQuinn, R., Chung, M., Poole, M., … Irish, V. F. (2009). Fleshy fruit expansion and ripening are regulated by the tomato SHATTERPROOF gene TAGL1. *The Plant Cell*, 21(10), 3041–3062.

Vu, L. D., Xu, X., Gevaert, K., & De Smet, I. (2019). Developmental plasticity at high temperature. *Plant Physiology*, 181(2), 399–411.

Wahid, A., Gelani, S., Ashraf, M., & Foolad, M. R. (2007). Heat tolerance in plants: An overview. *Environmental and Experimental Botany*, 61(3), 199–223.

Welsch, R., Zhou, X., Yuan, H., Álvarez, D., Sun, T., Schlossarek, D., … Li, L. (2018). Clp protease and OR directly control the proteostasis of phytoene synthase, the crucial enzyme for carotenoid biosynthesis in Arabidopsis. *Molecular Plant*, 11(1), 149–162.

van Wijk, K. J., & Kessler, F. (2017). Plastoglobuli: Plastid microcompartments with integrated functions in metabolism, plastid developmental transitions, and environmental adaptation. *Annual Review of Plant Biology*, 68(1), 253–289.

Yelle, S., Chetelat, R. T., Dorais, M., Deverna, J. W., & Bennett, A. B. (1991). Sink metabolism in tomato fruit. *Plant Physiology*, 95, 1026–1035.

Yu, C., Wang, H.-S., Yang, S., Tang, X.-F., Duan, M., & Meng, Q.-W. (2009). Overexpression of endoplasmic reticulum omega-3 fatty acid desaturase gene improves chilling tolerance in tomato. *Plant Physiology and Biochemistry*, 47(11–12), 1102–1112.

Zhang, H., Lang, Z., & Zhu, J. K. (2018). Dynamics and function of DNA methylation in plants. *Nature Reviews Molecular Cell Biology*, 19(8), 489–506.

Zhang, R., Wise, R. R., Struck, K. R., & Sharkey, T. D. (2010). Moderate heat stress of *Arabidopsis thaliana* leaves causes chloroplast swelling and plastoglobule formation. *Photosynthesis Research*, 105(2), 123–134.

Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D. B., Huang, Y., … Asseng, S. (2017). Temperature increase reduces global yields of major crops in four independent estimates. *Proceedings of the National Academy of Sciences of the United States of America*, *114* (35), 9326–9331.

Zheng, G., Tian, B., Zhang, F., Tao, F., & Li, W. (2011). Plant adaptation to frequent alterations between high and low temperatures: Remodelling of membrane lipids and maintenance of unsaturation levels. *Plant, Cell and Environment*, *34*(9), 1431–1442.

Zouine, M., Maza, E., Djari, A., Lauvernier, M., Frasse, P., Smouni, A., … Bouzayen, M. (2017). TomExpress, a unified tomato RNA-Seq platform for visualization of expression data, clustering and correlation networks. *The Plant Journal*, *92*(4), 727–735.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Almeida J, Perez-Fons L, Fraser PD. A transcriptomic, metabolomic and cellular approach to the physiological adaptation of tomato fruit to high temperature. *Plant Cell Environ*. 2021;44:2211–2229. https://doi.org/10.1111/pce.13854