



OPEN ACCESS

EDITED BY
Andrea Miyasaka Almeida,
Universidad Mayor, Chile

REVIEWED BY
Silvia Guidoni,
University of Turin, Italy
Eleonora Nistor,
University of Life Sciences "Regele Mihai
I", Romania

*CORRESPONDENCE
Jeffrey S. Bennett
✉ jeff.bennett@plantandfood.co.nz

RECEIVED 09 September 2025
REVISED 06 December 2025
ACCEPTED 12 December 2025
PUBLISHED 11 February 2026

CITATION

Bennett JS, Albright AL, Friend AP,
Boldingh H, Cooney J, Elborough C,
Kirk RA, Nguyen HM, Deng C,
Plunkett BJ, Martin D and Espley RV
(2026) Effects of bunch heating on Pinot
noir grape ripening: changes in berry
anthocyanin and malic acid
concentrations and their regulation.
Front. Plant Physiol. 3:1701827.
doi: 10.3389/fphys.2025.1701827

COPYRIGHT

© 2026 Bennett, Albright, Friend,
Boldingh, Cooney, Elborough, Kirk,
Nguyen, Deng, Plunkett, Martin and
Espley. This is an open-access article
distributed under the terms of the
[Creative Commons Attribution License
\(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original publication
in this journal is cited, in accordance
with accepted academic practice. No
use, distribution or reproduction is
permitted which does not comply with
these terms.

Effects of bunch heating on Pinot noir grape ripening: changes in berry anthocyanin and malic acid concentrations and their regulation

Jeffrey S. Bennett^{1*}, Abigail L. Albright², Adam P. Friend¹,
Helen Boldingh³, Janine Cooney³, Caitlin Elborough⁴,
Rebecca A. Kirk⁴, Han M. Nguyen⁴, Cecilia Deng⁴,
Blue J. Plunkett⁴, Damian Martin² and Richard V. Espley⁴

¹Plant & Food Research Group, New Zealand Institute for Bioeconomy Science Limited, Motueka, New Zealand, ²Plant & Food Research Group, New Zealand Institute for Bioeconomy Science Limited, Blenheim, New Zealand, ³Plant & Food Research Group, New Zealand Institute for Bioeconomy Science Limited, Hamilton, New Zealand, ⁴Plant & Food Research Group, New Zealand Institute for Bioeconomy Science Limited, Auckland, New Zealand

Cool-climate wine regions will face significant impacts from climate change over the coming decades. To predict the likely effects of climate change-associated high temperatures on berry ripening, a series of field-based grape bunch heating experiments were conducted on Pinot noir (*Vitis vinifera* L.) berries in Marlborough, New Zealand. Pinot noir bunches were heated for 7 days at the beginning of ripening (véraison) to a diurnal temperature range of 20 °C–39 °C, while unheated control bunches experienced an ambient diurnal temperature range of 11 °C–31 °C. This heating resulted in changes to several key quality criteria. The organic acid composition was altered by heat in favour of hastened reduction in malic acid concentration for a given total sugar concentration post-véraison. The sensitivity of anthocyanin accumulation in berry skins to heating varied depending on season: major inhibition in season 1 and minor inhibition in season 2. Abscissic acid (ABA) plays a pivotal role in the initiation of berry ripening, including the onset of anthocyanin accumulation in berry skins. Seasonal variation in peak berry ABA concentration was associated with seasonal drought, suggesting that the higher ABA concentrations in the drier second season of experimentation may have masked the heating effect on anthocyanin accumulation. While perception of heat (expression of heat shock factor *HsfA2*) by the berries was found to occur as quickly as 4 hours after heating, changes in berry composition and the expression of genes and transcription factors regulating malic acid and anthocyanins took two or more days to respond in a manner that was mostly in proportion to the concentrations observed. The study illustrates that hot periods, with daily maximum temperatures up to 40 °C over periods of time (7 days), may have direct impacts on the ripening of Pinot noir grapes, reducing organic acid and anthocyanins.

KEYWORDS

anthocyanins, coolclimate, gene expression, grape berry, heatwave, malic acid, pinot noir

1 Introduction

The wine regions of New Zealand, like many around the world, face the threat of climate change on a scale and pace not previously witnessed (Gladstones, 2011; Jones et al., 2005). Although climate change is predicted to raise the average temperature, the threat of change in the frequency and intensity of extreme heat, such as summer heatwaves that last days or weeks, is of greatest concern to premium Pinot noir cool-climate wine production (Jones et al., 2005; Jones and Schultz, 2016).

Numerous studies have demonstrated the influence and impact of temperature on grape berry ripening, composition, and maturity, although the effects are often complex manifestations of direct and indirect factors on individual berry and whole vine physiology (Bergqvist et al., 2001; Friedel et al., 2015; Keller et al., 2005; Mori et al., 2005; Pereira et al., 2006; Sweetman et al., 2014). Isolating the direct impacts of temperature on grape berries requires a controlled approach where only grape bunches are subject to heat treatment (Tarara et al., 2000). Of those studies that have directly manipulated the temperature of berries, most have illustrated that bunch heating may delay the onset of véraison, reduce the acidity of berries, inhibit or reduce the accumulation of anthocyanins and phenolics, and change the amino acid composition of berries (Cohen et al., 2008, 2012a, b; Lecourieux et al., 2017; Rienth et al., 2016; Spayd et al., 2002). Understanding such impacts on the synchrony of primary and secondary metabolite accumulation in grape berries during ripening, and hence the balance of maturity parameters at harvest, is vital to anticipating and managing the influence of climate change on wine quality (Venios et al., 2020).

Grape sugars and organic acids are critical to the production of quality wine, and their accumulation and regulation in grapes have been the subject of study for many decades. The rapid accumulation of sugar in the berry relies on sucrose translocated from the leaves to the berries and then its subsequent breakdown into glucose and fructose by the action of invertase (Düring and Alleweldt, 1984; Mullins et al., 1992). Specifically, heating grape bunches has revealed that sugar accumulation is generally unaffected (Lecourieux et al., 2017; Spayd et al., 2002). However, berry organic acids, in particular malic acid concentration, are influenced by heating. Lecourieux et al. (2017) determined that 14 days of bunch heating at or after véraison significantly reduced malic acid concentrations at harvest, but did not influence tartaric acid. Whole vine heating studies (Rienth et al., 2016; Sweetman et al., 2014) have also shown that hot temperatures (30 °C) during the pre-véraison to véraison stage of development accelerated a reduction in malic acid concentration. The regulatory mechanisms responsible for this are only partially understood, although it has been known for some time that malic acid is respired at an elevated rate at high temperatures (Lakso and Kliwer, 1975; Sweetman et al., 2014).

The synthesis and regulation of anthocyanins via the phenylpropanoid pathway in red grapes have been the subject of many studies and reviews (Boss et al., 1996; Mazza, 1995; Mazza et al., 1999; Springob et al., 2003; He et al., 2010; Shahab et al., 2023). There is considerable knowledge on the molecular regulation of anthocyanin synthesis from the structural genes in the pathway to

the key transcription factor families such as MYB, MYC, and WD40 associated with the regulation of structural genes. Among the MYB transcription factors, some have been characterised to elevate or repress anthocyanin synthesis (Azuma, 2018; Deluc et al., 2006, 2008; Kobayashi et al., 2005), and some have been shown to respond to heat (Azuma et al., 2012). In red grapes, high night temperatures may inhibit the gene expression of several structural genes in the phenylpropanoid pathway during the early stages of ripening, resulting in the poor production of anthocyanins (Mori et al., 2005; Yamane et al., 2006).

In contrast to many red grape varieties, Pinot noir lacks the ability to synthesise acylated anthocyanins (Mazza et al., 1999). This is because Pinot noir contains a nonsense mutation in the gene anthocyanin 3-O-glucoside-6"-O-acyltransferase (*Vv3AT*), which is responsible for synthesising acylated anthocyanins in red grapes (Rinaldo et al., 2015). A key feature of acylated anthocyanins is their increased stability to factors like light, heat, pH, and the rigours of the fermentation process (Mazza et al., 1999; Rinaldo et al., 2015). Without the ability to synthesise stable acylated anthocyanins, Pinot noir berry anthocyanins may be more vulnerable to environmental heat stress than other red grapes.

To investigate the direct impact of high temperatures on berry ripening, a series of field-based grape bunch heating experiments were carried out on Pinot noir. Our experimental aim was to simulate "heatwave" conditions by elevating berry temperature for a period of 7 days at the key stage of ripening (onset of véraison) using a fan-assisted heating system and determine the short- to long-term influence of heating on anthocyanin accumulation in berry skins and changes in berry organic acids, and other parameters associated with the ripening of grape berries. The design of the experiment allowed us to clearly determine if heating Pinot noir grapes under New Zealand conditions would result in a negative impact on malic acid and anthocyanin concentrations, as reported more extensively in other red grape varieties around the world.

2 Materials and methods

2.1 Field trial set-up and heating system

The grapevines used in the study were grown in a vineyard located at the Marlborough Research Centre, Blenheim, New Zealand (latitude -41.50379°, longitude 173.96664°). The north-south-orientated row of mature 20-year-old vines (*Vitis vinifera* L. Pinot Noir clone UCD5 grafted onto 3309 rootstock) was trained to two to four canes per vine using a vertical shoot position canopy ideotype. The vineyard soil type is described as a fertile deep clay loam with a shallow water table. Vines received no summer irrigation but were canopy-trimmed several times (canopy top and sides), and the fruit zone was leaf-plucked, removing approximately 50% of the leaves prior to véraison.

An air delivery system designed for the measurement of whole-tree gas exchange (Wünsche and Palmer, 1997) was modified to include an in-line heater so that warm air could be directed

selectively onto eight individual bunches of grapes using 10-cm-diameter flexible ducts (Supplementary Figure 1A). Each duct was positioned directly under a bunch of grapes and held in position using a laboratory clamp stand attached to a weighted base (Supplementary Figures 1B,C). A target temperature differential of 10 °C between heated and unheated ambient bunches was achieved by adjusting the number of heater elements turned on and the air flow through the heater system. Three identical heated air delivery systems were set up to service three vineyard bays (training post to training post) of four grapevines each (Supplementary Figure 1A). The bay and its associated heating unit represented the experimental unit, which was replicated three times. Each heating system and its bay of four vines were separated by a buffer bay along the row of grapevines. The directed heating enabled the specific and selective temperature modification of eight bunches per bay of vines (a total of 24 bunches per experiment), with minimal disturbance to surrounding unheated bunches on the same vines, eight of which were randomly selected as the unheated bunches (control treatment) per replicate heating system. The unheated bunches had no equivalent air flow (at ambient temperature) over them, as all 24 fan ports from the three heaters were used to heat. Heating was applied continuously over a 7-day period when grapes had reached mid-véraison (50% berries per bunch showing signs of colour change). In 2014, heating commenced from midday 11 February until midday 18 February and, in 2015, from midday 16 February until midday 23 February.

Berry temperatures were monitored using 42 SWG copper/constantan thermocouples monitored by a Delta-T data logger (Type DL2; Delta-T Devices, Burwell, Cambridge, UK). Six bunches, three heated and three unheated controls, were randomly selected from the 48 bunches being used in the trial for thermocouple temperature monitoring. One berry on the east and west side of each of the six bunches had a thermocouple inserted just under the berry skin (exocarp). Temperature readings were recorded by the data logger every 5 min. To determine the effect of heating on temperature, the readings of each thermocouple were averaged for heated and unheated controls.

2.2 Berry collection and sample preparation

Berry sampling consisted of collecting a composite sample of 24 berries from each of the three experimental bays for both unheated and heated treatments. The composite berry sample consisted of three berries from each of the eight individual heated and unheated bunches per experimental bay. The three berries per bunch were sampled from the top, middle, and bottom positions of a bunch. The composites were immediately frozen in a −80 °C freezer. In 2014, the berry sampling had two aims: firstly, to determine the short-term diurnal effects of heating and, secondly, to assess the long-term effects until maturity (harvest time). For the first 48 hours of heating in 2014, composite samples were collected from heated and unheated bunches every 4 hours starting at the beginning of heating. After the first 48 hours of heating, samples were collected on days 4 and 7 after 50% véraison (during heating) and also on days 14 and 34 after 50% véraison (after heating). In 2015, berry

sampling occurred once a day during heating and then every 4–7 days after heating until maturity (harvest time). All composite berry samples (excluding the diurnal period in 2014) were collected at midday during both experiments. Composite samples of 24 berries were also collected from the experimental bays before heating in both years. Two to three samples were collected from approximately 16 days before 50% véraison until the start of heating. This “pre-sampling” enabled us to determine the point of 50% véraison each year.

Berries were removed from the −80 °C freezer, and while frozen, the berry skin was gently scored with a scalpel from tip to base in two planes at 90° to each other, resulting in four quarters of skin. Each quarter was gently but quickly lifted off the berry pulp with tweezers and re-frozen in liquid nitrogen. The remaining tissue of each berry was sliced in half, de-seeded, and stored at −80 °C. The skin and pulp samples were separately weighed and then ground to a powder using an IKA® A 11 analytical mill (Staufen, Germany; <https://www.ika.com/en/Products-LabEq/Mills-pg194/>) using liquid nitrogen and dry ice to keep samples and handling implements frozen. The powdered skin and pulp tissues were then subsampled into aliquots for chemical analysis. This included one aliquot for anthocyanin analysis (skin only), and three aliquots for juice-based analysis (soluble solids, pH, titratable acidity, and organic acid), and sugar and phytohormone (abscisic acid) analyses. The remaining skin and pulp tissues were pooled across replicates to provide a sufficient sample for q-PCR analysis and RNA transcriptome sequencing for heated versus unheated treatments.

2.3 Soluble solids, pH, titratable acidity, organic acid, and sugar analyses

Approximately 30 g of frozen powdered berry pulp was thawed at room temperature and then centrifuged at 4,500 rpm for 5 min to yield a clear juice supernatant ready for analysis. Aliquots of juice were measured for soluble solids using an Atago PAL-1 digital pocket refractometer (Atago USA Inc., Bellevue, WA, USA), pH was measured using a Metrohm 744 pH meter (Metrohm AG, Herisau, Switzerland), and titratable acidity was measured using a Mettler Toledo DL50 autotitrator (A. Daigger & Company, Vernon Hills, IL, USA). A 5-mL aliquot of juice was diluted with 50 mL of distilled water and titrated using 0.1 mol NaOH to an endpoint pH of 8.2. The titratable acidity was expressed as g/L tartaric acid equivalent.

Further aliquots of juice were taken for organic acid analysis. Tartaric and malic acids were quantified using a method adapted from Shi et al. (2011) on a Shimadzu Prominence HPLC system (Kyoto, Japan) using isocratic elution with a phosphate buffer (140 mM, pH 2.4) on an Allure Organic Acids Restek column (Bellefonte, PA, USA) (5 µm, 240 × 4.6 mm). All samples were diluted 10-fold in a solution containing thiourea as an internal standard and filtered through a 0.45-µm syringe filter prior to injection. Samples were run in duplicate and quantified on a 5-point standard curve ($R^2 > 0.98$).

Pulp sugar was analysed using an aliquot of frozen tissue. Approximately 200 mg of the frozen homogenised powdered

grape pulp tissue was extracted using 5 mL of 80% ethanol with the addition of adonitol (02240, Sigma-Aldrich, St. Louis, MO, USA) as an internal standard and incubated for 1 hour at 60 °C. The samples were mixed and then centrifuged. A subsample of the supernatant was taken and dried using a CentriVap (Scanvac Coolsafe, LaboGene, Lillerød, Denmark). The sample was then re-dissolved in ultrapure water. The soluble sugars (fructose, glucose, and sucrose) were analysed using a DIONEX ICS-5000 Reagent-FreeTM IC (RFICTM) system with a CarboPac PA20 column.

2.4 Anthocyanin analysis

An aliquot of the sample (200 ± 40 mg) was weighed and recorded. The sample was then extracted with acidified aqueous ethanol (10.0 mL, 50% v/v, pH 2.0) by sonication (1 hour, cool water). The resultant extract was centrifuged (10 min, 4,600 rpm) and decanted. Anthocyanins were quantified on an Agilent 1200 Series HPLC equipped with a Hypersil Gold C18 column using a method adapted from Rodríguez-Delgado et al. (2001). A malvidin-3-O-glucoside (Sigma-Aldrich, St. Louis, MO, USA) stock standard was prepared in acidified aqueous ethanol (50% v/v, pH 2.0) and frozen in small-volume aliquots to avoid repeated freeze-thaw cycles. The stock standard was serially diluted and injected in duplicate to generate a 5-point standard curve ($R^2 > 0.98$). A freshly prepared malvidin-3-O-glucoside standard solution was run against the frozen standard to ensure accuracy. All samples were analysed in duplicate and filtered through a 0.45- μ m syringe filter prior to injection. The concentrations of anthocyanins in the extracted sample aliquots were determined using the standard curve and were expressed in mg of anthocyanin per g of skin fresh weight (FW).

2.5 Absciscic acid analysis

Phytohormone concentrations [abscisic acid (ABA) in free active and storage forms] were determined via liquid chromatography–mass spectrometry (LC–MS) on a 7500 QTrap triple quadrupole/linear ion trap (QqLIT) mass spectrometer equipped with a TurboIon-SprayTM interface (AB Sciex, ON, Canada) coupled to a Shimadzu Nexera UHPLC (Kyoto, Japan) as described by Wurms et al. (2023) and Bulley et al. (2021). Aliquots of frozen powdered grape berry material were weighed (100 mg), and to each were added chilled (4 °C) extraction solvent [acetonitrile + 0.01% trifluoroacetic acid (TFA)] (1 mL), labelled internal standard mix, and stainless steel beads 0.9–2 mm (0.8 g) (Next Advance Inc., Raymertown, NY, USA). Samples were bead-beaten for 5 min (Bullet Blender 24 Gold, Next Advance Inc., NY, USA) before being extracted overnight at 4 °C using an end-over-end rotator at 30 rotations/min. After centrifugation at $16,000 \times g$ for 5 min, supernatant from each sample was transferred into a well in a 96-well collection plate (Phenomenex, Torrance, CA, USA). The remaining pellet was re-extracted with the chilled (4 °C) extraction solvent (0.5 mL), combined with the first supernatant, and evaporated to dryness using a CentriVap concentrator (Labconco, Kansas City, MO, USA) at -4 °C. Sample clean-up employed graphitised carbon following a method described by Cai

et al. (2016), with modifications to adapt to a 96-well plate format. Briefly, samples were reconstituted in chilled (4 °C) 80:20 acetonitrile:water (1 mL) and shaken for 20 min on a flat-bed orbital shaker before SPE clean-up on a Hypersep Hypercarb 96-well plate (25 mg/1 mL; Thermo Scientific, CA, USA). Plates were conditioned using acetonitrile (1 mL) followed by water (1 mL). After conditioning, samples were loaded, and the acidic phytohormones were eluted with acetonitrile (0.5 mL) and evaporated to dryness using a CentriVap concentrator at -4 °C. Samples were reconstituted in 10:90 acetonitrile:water (0.2 mL) for analysis by LC–MS. The concentrations of hormones were calculated on the basis of the LC–MS peak area for the endogenous compounds relative to those determined for the internal standards and were expressed in ng/g berry fresh weight.

2.6 RNA extraction, qPCR, and RNA-seq analysis

A 200–400-ng berry tissue aliquot was used for total RNA isolation using a SpectrumTM RNA extraction kit (Sigma-Aldrich) following the manufacturer's protocol. RNA integrity was assessed via gel electrophoresis, and quantification was performed using a NanoDropTM ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Thermo Scientific, USA). First-strand cDNA synthesis and DNase treatment were carried out using the QuantiTect[®] Reverse Transcription kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). Gene-specific primers were designed to a stringent set of criteria to enable the application of universal reaction conditions (Supplementary Table 1). qPCR DNA amplification and analysis were carried out using the LightCycler[®] 480 System (Roche Diagnostics Indianapolis, Indiana, USA) with LightCycler 480 software v1.5. All reactions were performed using the LightCycler 480 SYBR Green I Master Mix (Roche). Reactions were 5 min at 95 °C, followed by 40 cycles of 5 s at 95 °C, 5 s at 60 °C, and 10 s at 72 °C, followed by a 65 °C to 95 °C melting curve detection. Four replicates were performed using 2.5 μ L Master Mix, 0.25 μ L of each primer (10 μ M), 1.25 μ L diluted cDNA (1:50), and nuclease-free water to a final volume of 5 μ L. A no-template control was included in each run. Standard curves for each gene were generated using a cDNA serial dilution, and resultant PCR efficiency calculations were imported into the expression analysis. For relative quantification, *Actin* and *GAPDH* were selected as the reference genes due to their consistent transcript level throughout all samples.

mRNA sequencing, including library preparation, sequencing, and quantification analysis, was performed by Australian Genome Research Facility (AGRF) Limited (Brisbane, Australia) using Illumina HiSeq sequencing with 100-bp paired-end reads in two HiSeq lanes with a minimum of 200 million passed filter reads per lane.

2.7 Data handling and statistical analysis

For both years, berry temperatures were averaged across all thermocouples on a per-treatment basis (heated and unheated), and accumulated growing degree days (base temperature 10 °C) were

calculated using temperature means generated every 5 min. Treatment effects were statistically analysed by one-way ANOVA using Genstat 22nd edition (VSN International Ltd., Hemel Hempstead, England). The variation of each sample point was expressed as the standard deviation in the result graphs. Where samples were analysed in duplicate via q-PCR (technical replicates), the relative standard deviation (RSD) was calculated and reported in the result graphs. For generating gene expression heatmaps, Transcripts per million (TPM) data were used for a z-score analysis using the gplots R package. Gene Ontology (GO) over-representation analysis of highly differentially expressed genes ($|\log_2\text{FoldChange}| > 2$ and adjusted p-value < 0.05) was performed using the clusterProfiler R package v4.10.1 (Wu et al., 2021). GO terms with p-values < 0.05 were considered significantly enriched.

3 Results

3.1 Berry temperatures

The heating apparatus raised the mean berry temperature quickly, and within 60 min, the temperature increased by 7.5 °C compared to the unheated treatment. General trends indicated that the temperature differential between heated and unheated bunches increased at night by 10 °C–12 °C and during the day by 8 °C–10 °C (Supplementary Figure 2). Heated bunches never fell below 17 °C and peaked at almost 40 °C during the hottest part of the day, while unheated bunches ranged from 8 °C to 30 °C. Calculation of growing degree days (GDD) revealed the significance of heating with a 97%–117% increase in accumulated GDD during the experimental period for the heated bunches (Supplementary Figure 2).

3.2 Heating effects on organic acids, sugars, and abscisic acid

Heating during the véraison periods of 2014 and 2015 resulted in a small but consistent increase in juice soluble solids. In 2014, this effect was no longer evident 1 week after heating, but in 2015, a small elevation in soluble solids was still apparent several weeks after heating (Supplementary Figures 3A, B). In contrast, in 2014, titratable acidity was significantly lower in heated berries for a period of time during and after heating (4–14 days after 50% véraison) compared with that in unheated berries. In 2015, the same effect was observed: titratable acidity was significantly lower for a period of time during and after heating (4–20 days after 50% véraison) compared with that in unheated berries. However, by maturity (harvest), the influence of heating on titratable acidity had all but disappeared (Supplementary Figures 3C, D). The reduction in titratable acidity mirrored the increase in juice pH (Supplementary Figures 3E, F).

Analysis of berry sugar and organic acid concentrations revealed that increased temperatures had little impact on glucose, fructose, and sucrose concentrations (Supplementary Figures 4A–F). Tartaric acid concentration was not consistently altered by

heating (Figures 1A, B), but malic acid concentration was significantly reduced by heating during treatment and for a while after treatment compared with no heating. Malic acid concentrations were 25% and 16% lower than those of unheated bunches in 2014 and 2015, respectively, at day 6 (Figures 1C, D). However, differences in malic acid concentration had all but disappeared by harvest time. No significant heating effect could be seen in the first 48 hours on tartaric or malic acid concentrations (Supplementary Figure 5).

Free ABA concentrations rapidly reduced from mid-véraison to harvest, but no effect of heating was seen in either year (Figures 1E, F). In contrast, the storage form of ABA (conjugated abscisic acid glucose ester) increased in concentration as the berries ripened and also increased in response to heating in both years (Figures 1G, H). ABA-GE concentrations measured for heated berries increased by as much as 40% relative to those measured for unheated berries by the end of the heating period and remained elevated after heating (Figures 1G, H).

3.3 Heating effects on grape skin anthocyanins

Five anthocyanin species [malvidin-3-O-glucoside (M3G), peonidin-3-O-glucoside (P3G), petunidin-3-O-glucoside (Pt3G), delphinidin-3-O-glucoside (D3G), and cyanidin-3-O-glucoside (C3G)] were detected as early as 7 days prior to 50% véraison (Figure 2). No acylated anthocyanins were detected during analysis, as expected, in Pinot noir. As berries progressed towards full pigmentation, anthocyanin concentrations increased until 15 days after 50% véraison (M3G, Pt3G, and D3G), while P3G, C3G, and total anthocyanins continued to increase in concentration until harvest. M3G was the most abundant anthocyanin found in the skins throughout the ripening period, accounting for approximately half of the anthocyanin glucosides (Figure 2). In 2014, heating significantly reduced the concentrations of M3G, P3G, Pt3G, and total anthocyanins at day 4 of heating and after treatment until harvest compared with those in unheated berries (Figures 2A, C, E). The concentrations D3G and C3G, in contrast, were only significantly reduced by heating after the treatment period until harvest (Figures 2H, J). However, no effect of heating on anthocyanin concentrations was observed in the first 48 hours of heating in 2014 (Supplementary Figure 6). In 2015, heating had less impact on anthocyanin accumulation, with the concentrations of M3G, P3G, and C3G showing little response to heat, although Pt3G and D3G concentrations were significantly suppressed by heating until harvest, compared with unheated treatment (Figures 2F, I).

3.4 Heating effects on the expression of genes and transcription factors using qPCR analysis

Gene expression was measured using qPCR and RNA-seq analysis. To gain insight into the expression of key genes of interest over the course of heating and after heating, we used qPCR analysis on a select number of genes. To observe a more

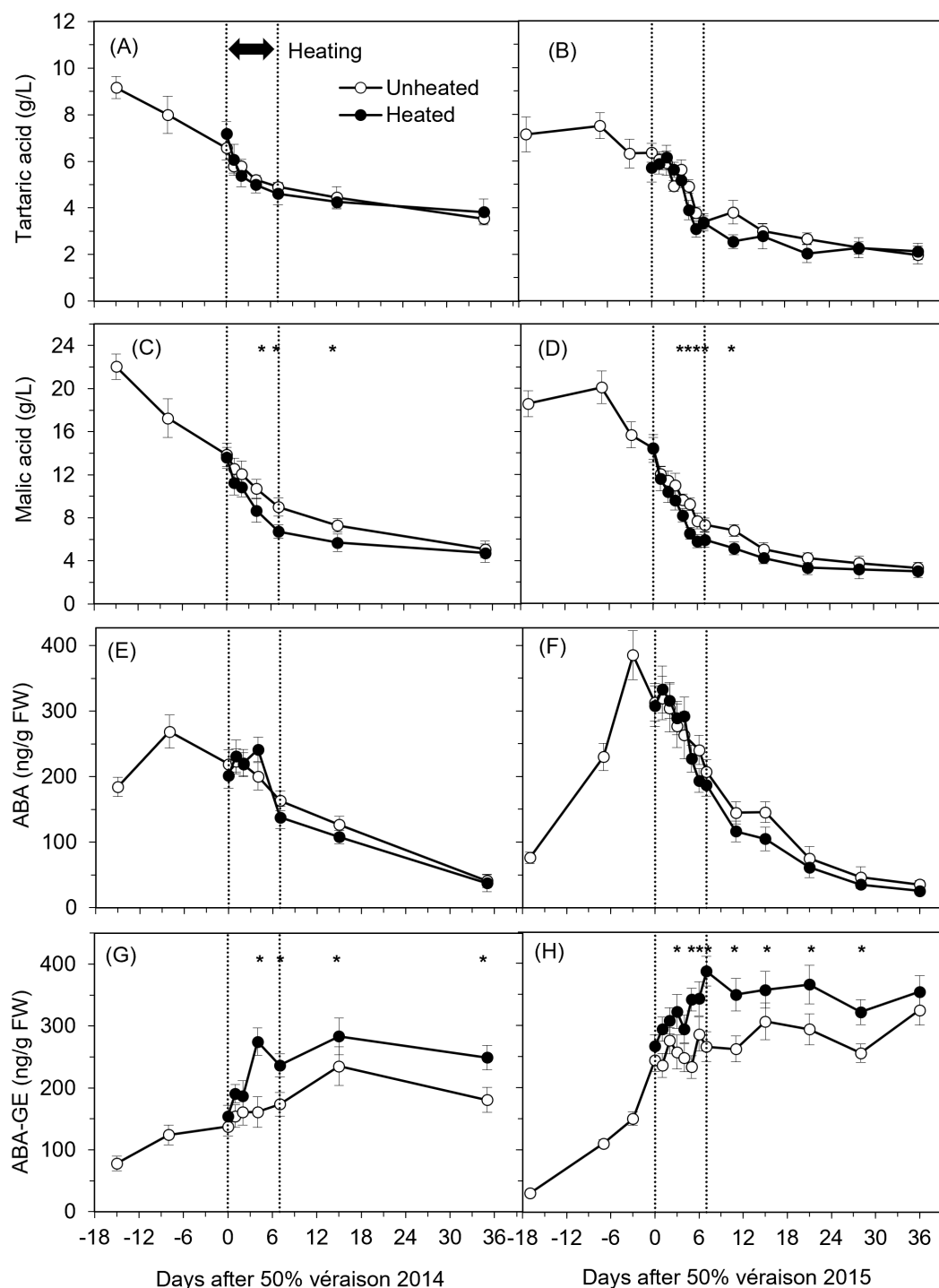


FIGURE 1

The effect of bunch heating for 7 days at mid-véraison on the concentrations of tartaric acid (A, B), malic acid (C, D), free abscisic acid (ABA) (E, F), and ABA-glucose ester (ABA-GE) (G, H) in midday samples of Pinot noir grape berries during ripening in 2014 and 2015. Organic acid concentrations expressed in g/L grape juice. Phytohormone concentrations expressed in ng/g berry pulp fresh weight (FW). *Sample points where a significant difference by one-way ANOVA was determined ($p \leq 0.05$). Error bars represent standard deviation.

global view of gene responses, we used RNA-seq analysis on samples collected on day 4 of heating in 2015. We chose day 4 based on observed heating responses to our marker gene *HsfA2* as described below.

To test the effect of heat on a known gene marker, we measured the gene expression of heat shock transcription factor *HsfA2*, which

showed a strong elevation in transcript abundance in the heated berries during the first 3–4 days of heating in 2014 and 2015 (Figures 3A, B). Results from our diurnal sampling regime during the first 2 days of heating in 2014 further revealed that heat shock factor expression increased 4 hours after heating commenced and peaked in expression around midday (Supplementary Figure 7D).

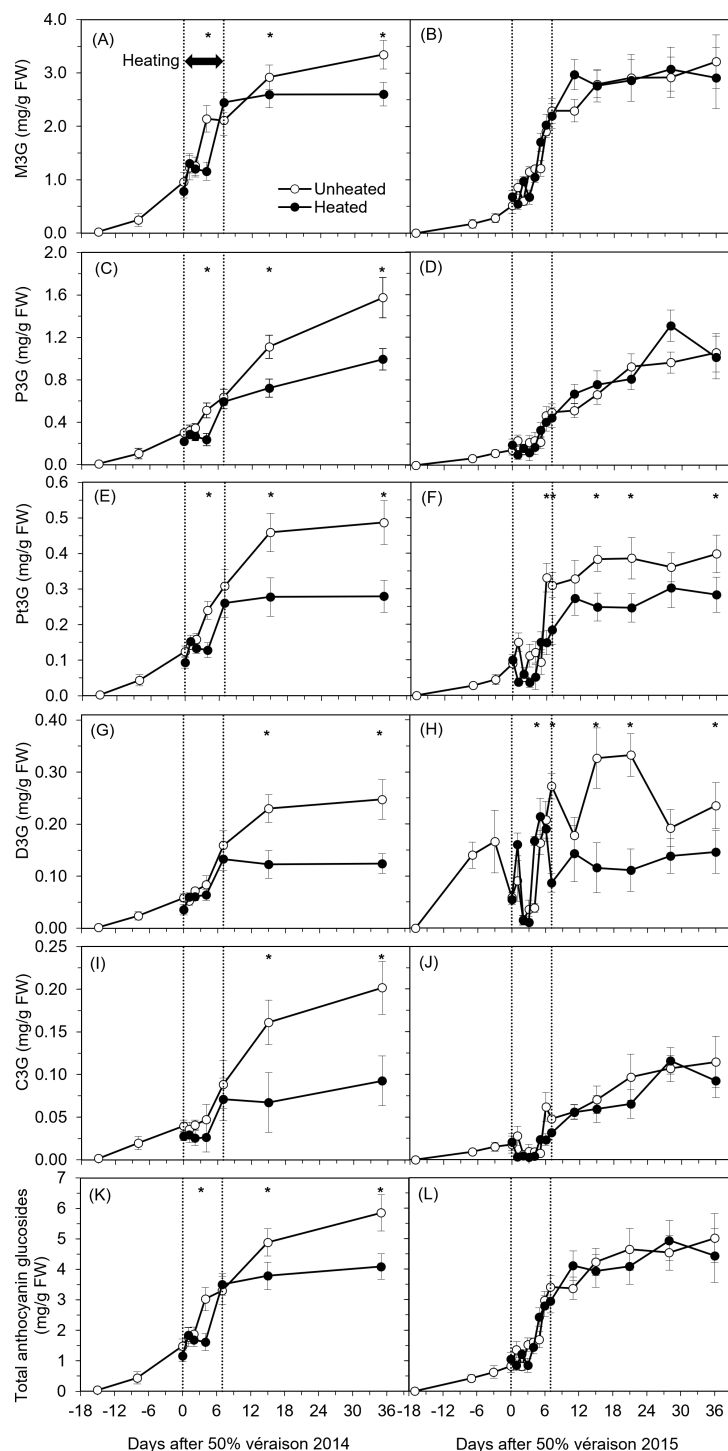


FIGURE 2

The effect of bunch heating for 7 days at mid-véraison on the concentrations of malvidin-3-O-glucoside (M3G) (A, B), peonidin-3-O-glucoside (P3G) (C, D), petunidin-3-O-glucoside (Pt3G) (E, F), delphinidin-3-O-glucoside (D3G) (G, H), and cyanidin-3-O-glucoside (C3G) (I, J) and total anthocyanins (K, L) in midday samples of Pinot noir grape berries during ripening in 2014 and 2015. Anthocyanin concentrations expressed in mg/g berry skin fresh weight (FW). *Represent samples points where a significant difference by one-way ANOVA was determined ($p \leq 0.05$). Error bars represent standard deviation.

Expression during the remainder of the heating period (after day 4) was similar to that in unheated berries and thereafter until harvest in 2014 and 2015 (Figures 3A, B).

We tested the expression patterns of functional phenylpropanoid genes and associated transcription factors involved in anthocyanin synthesis. The expression of *PAL* showed no clear trends, although

expression was lower in the heated berries at harvest (Figures 3C, D). *CHS* transcription was clearly suppressed by the heating treatment for both years (Figures 3E, F). Similarly, the transcription of *DFR* and *UFGT* was reduced by heating in 2014, although in both cases, the reduction in expression was not evident until the end of day 2 of heating (Supplementary Figures 7A, B). In 2015, however, the

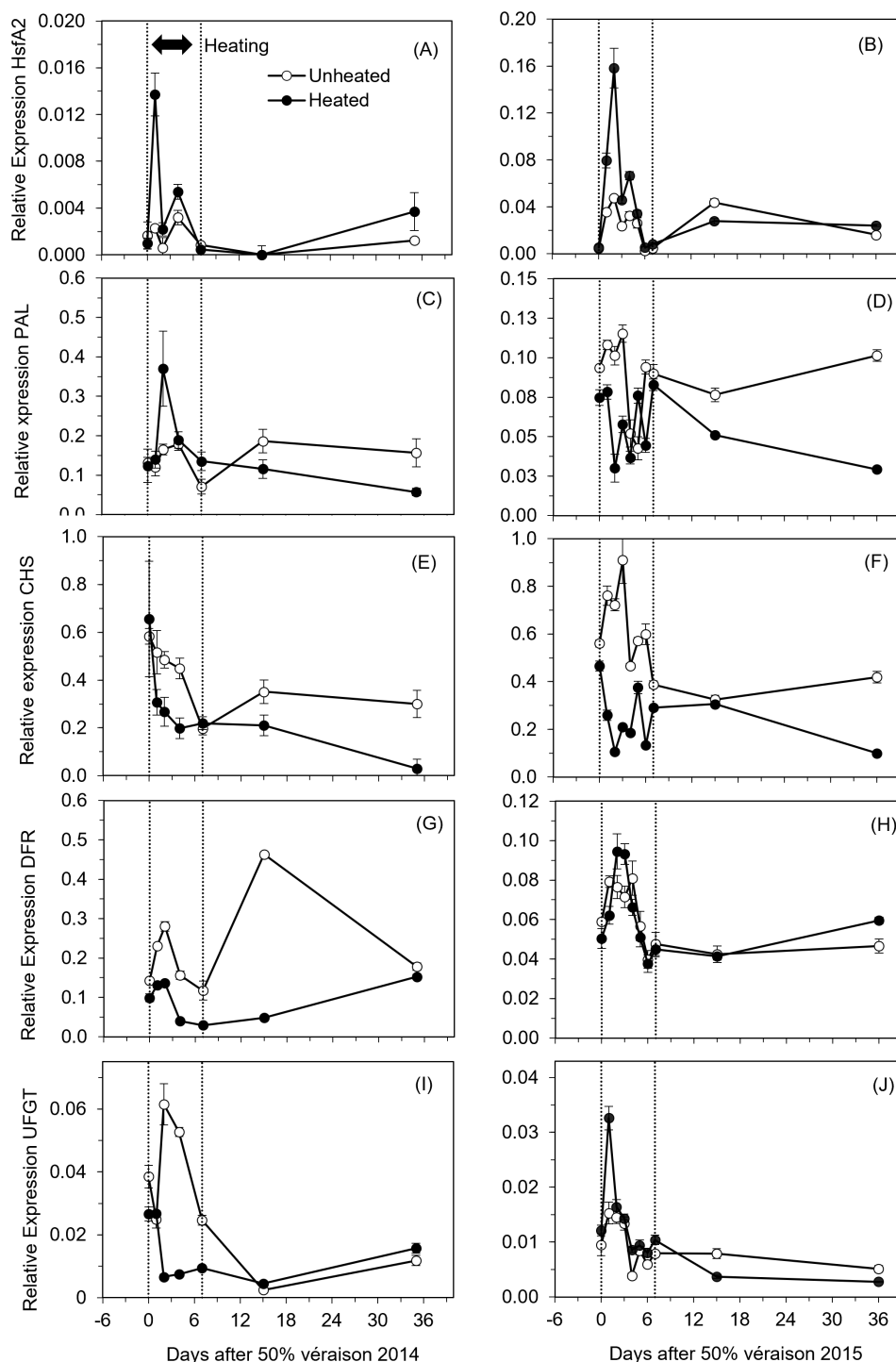


FIGURE 3

The effect of bunch heating for 7 days at mid-véraison on midday Pinot noir grape berry gene expression determined via q-PCR for heat shock factor (*HsfA2*) (A, B), phenylalanine lyase (*PAL*) (C, D), chalcone synthase (*CHS*) (E, F), dihydroflavanol reductase (*DFR*) (G, H), and UDP-glucose: flavonoid 3-O-glucosyltransferase (*UFGT*) (I, J) in 2014 and 2015. Error bars represent relative standard deviation.

expression of *DFR* or *UFGT* was unaffected by heating (Figures 3H, J).

There was no consistent effect of heating on the expression of transcription factor *MYBA1* during the first 2 days of heating (Supplementary Figure 7C) or during the remainder of the experiment in 2014 nor in 2015 (Supplementary Figures 8E, F). We did observe a pattern of mirrored expression between heat

shock factor *HsfA2* and anthocyanin repressor *MYB4a* during the first 2 days of heating in 2014. A spike in the expression of *HsfA2* was followed by a spike in the expression of *MYB4a* 4 hours later during the first and second days of heating (Supplementary Figures 7D, E). However, the expression of *MYB4a* after this period was generally reduced by heating in 2014 (Supplementary Figure 8A). In 2015, *MYB4a* expression was higher in heated grapes

from day 2 to day 4 of heating, but generally lower in heated grapes after heating (Supplementary Figure 8B). The expression of anthocyanin activator *MYB5a* was consistently lower in heated grapes from 8 pm on the second day of heating in 2014 (Supplementary Figure 7F) and for the remainder of the experiment in 2014 (Supplementary Figure 8C). However, in

2015, no influence of heating on *MYB5a* expression was observed (Supplementary Figure 8D).

The analysis of genes associated with malic acid synthesis and metabolism revealed that heating in both years increased the expression of *FH* (Figures 4A, B). The expression of cytosolic malate dehydrogenase (*MDHc*) was not consistently altered by

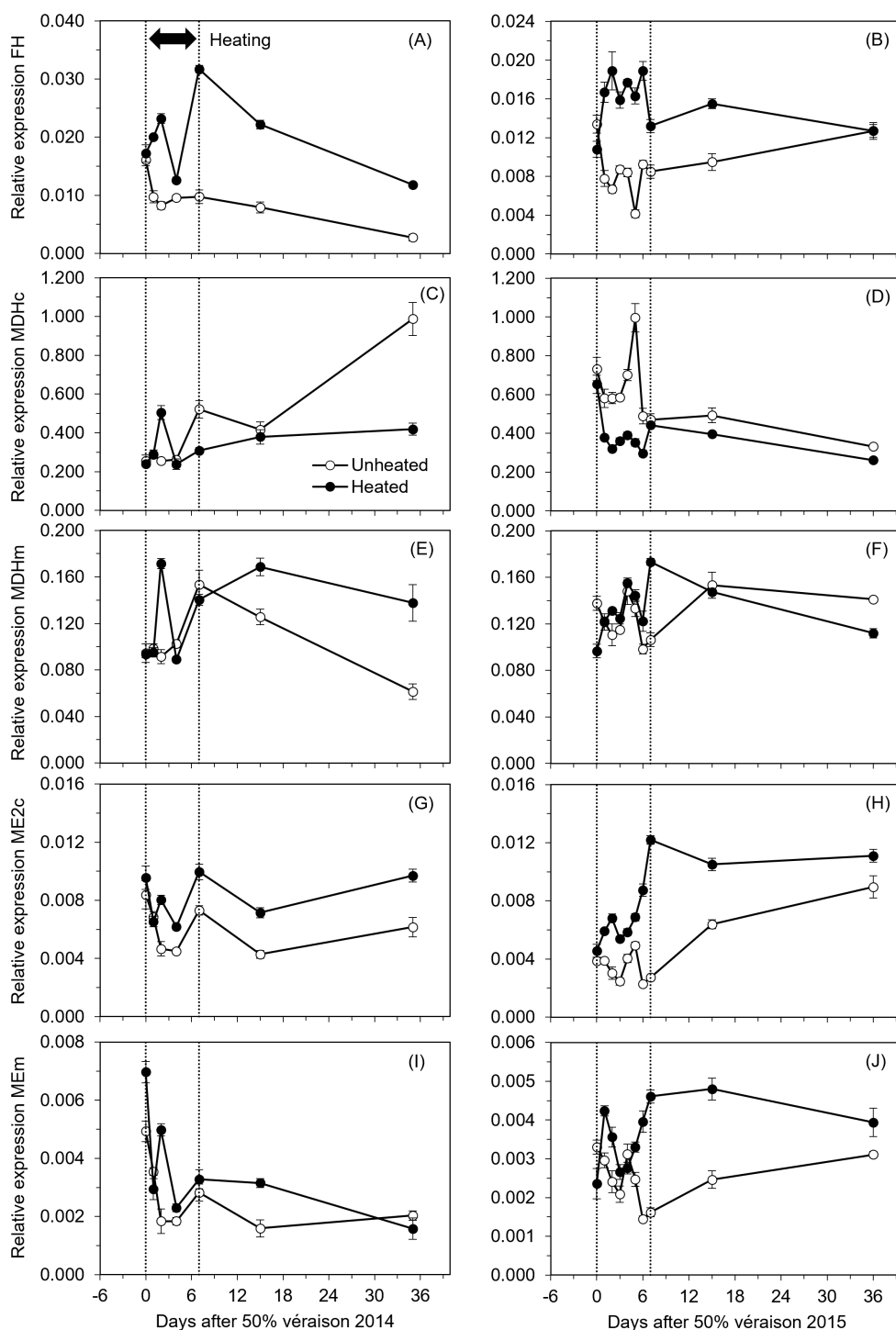


FIGURE 4

The effect of bunch heating for 7 days at mid-véraison on midday Pinot noir grape berry gene expression via q-PCR for fumarate hydratase (*FH*) (A, B), cytosolic malate dehydrogenase (*MDHc*) (C, D), mitochondrial malate dehydrogenase (*MDHm*) (E, F), cytosolic malic enzyme (*ME2c*) (G, H), and mitochondrial malic enzyme (*MEM*) (I, J) in 2014 and 2015. Error bars represent relative standard deviation.

heating in 2014 (Figure 4C), but was reduced during the heating period in 2015 (Figure 4D). Mitochondrial malate dehydrogenase (*MDHm*) expression was not consistently altered by heating in 2014 or 2015 (Figures 4E, F). Cytosolic malic enzyme (*ME2c*) expression was reduced by heating in both 2014 and 2015 (Figures 4G, H): its mitochondrial counterpart (*MEM*) was not consistently altered by heating in 2014, but in 2015, the expression of *MEM* increased towards the end of heating and for a while after heating (Figure 4J).

3.5 Global gene expression using RNA-seq analysis

To evaluate for global gene expression, we selected a representative sample (day 4 of heating in 2015) for RNA sequencing. Gene Ontology enrichment analysis provided some insights into the patterns of differentially expressed genes in heated versus unheated berries in relation to biological processes. Genes associated with hypoxia, cellular oxygen, heat response, and secondary metabolism all showed highly significant responses. Similarly, genes associated with phenylpropanoid biosynthesis and metabolism, organic hydroxy biosynthesis, and protein folding showed significant responses, too (Supplementary Figure 9). Narrowing our focus to anthocyanins, organic acids, ABA, and sugar, we examined transcriptional activity with a TPM data z-score heatmap analysis. The analysis revealed that at least four variants of *PAL* were downregulated by heating, while another two were upregulated (Figure 5). Similarly, one variant of *CHS* was upregulated, and another downregulated. Flavonoid hydroxylase

genes *F3'H* and *F3'5'H* were downregulated by heating. In contrast, genes involved with the final stages of anthocyanin synthesis (*ANS*, *UFGT*, and *OMT*) were upregulated by heating, while *ANR*, which converts anthocyanidins towards flavan-3-ol production, was downregulated by heating (Figure 5). Representatives of both anthocyanin activators (*MYBA1*, *MYBA3*, and *MYB15*) and repressors (*MYB44*, *MYBC2-L1*, and *MYB4a*) were upregulated by heating, but the anthocyanin-related bHLH transcription factor (*MYC1*) was downregulated by heating (Figure 5).

Key genes associated with sugar, malic acid, and ABA regulation revealed that sucrose biosynthesis (*SPP* and *SPS*), sugar transport (*SWEET10* and *15*, *HT3*, and *TMT1*), and sugar catabolism (*GIN1*) genes were all downregulated by heating, but genes associated with sugar sensing/signalling (*HXX2* and *SuSy*) tended to be upregulated (Figure 6). ABA-related genes showed consistent patterns, indicating that ABA signalling (*NAC17*, *ABF2*, *NAC26*, and *SnRK2F*) was downregulated by heating, and likewise, two variants of the ABA biosynthesis gene (*NCED*) were downregulated, too. Genes associated with the regulation of ABA-glucose ester were mostly upregulated in the case of ABA-glucosyltransferase (*ABA-UGT*) and downregulated for beta-glucosidase (*BGLU*) (Figure 6).

The regulation patterns of a range of malic acid genes showed that fumarate hydratase (*FH*) was upregulated by heating, along with cytosolic malic enzyme (*ME2c*), but mitochondrial *MEM* was downregulated by heating. One variant of malate dehydrogenase (*MDH*) was also downregulated. Other regulatory genes associated with malic acid regulation, including pyruvate kinase (*PK*),

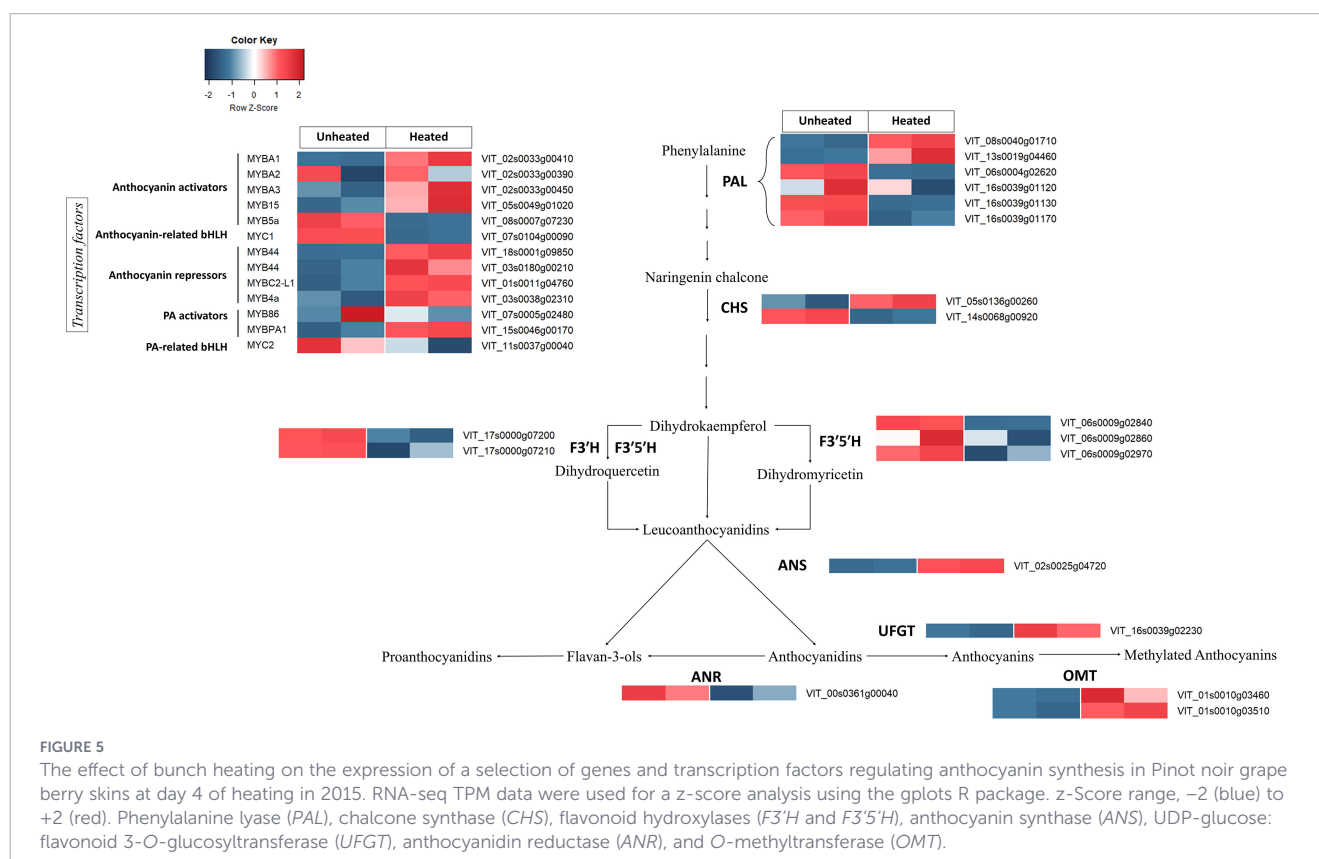


FIGURE 5

The effect of bunch heating on the expression of a selection of genes and transcription factors regulating anthocyanin synthesis in Pinot noir grape berry skins at day 4 of heating in 2015. RNA-seq TPM data were used for a z-score analysis using the gplots R package. z-Score range, -2 (blue) to +2 (red). Phenylalanine lyase (*PAL*), chalcone synthase (*CHS*), flavonoid hydroxylases (*F3'H* and *F3'5'H*), anthocyanin synthase (*ANS*), UDP-glucose: flavonoid 3-O-glucosyltransferase (*UFGT*), anthocyanidin reductase (*ANR*), and O-methyltransferase (*OMT*).

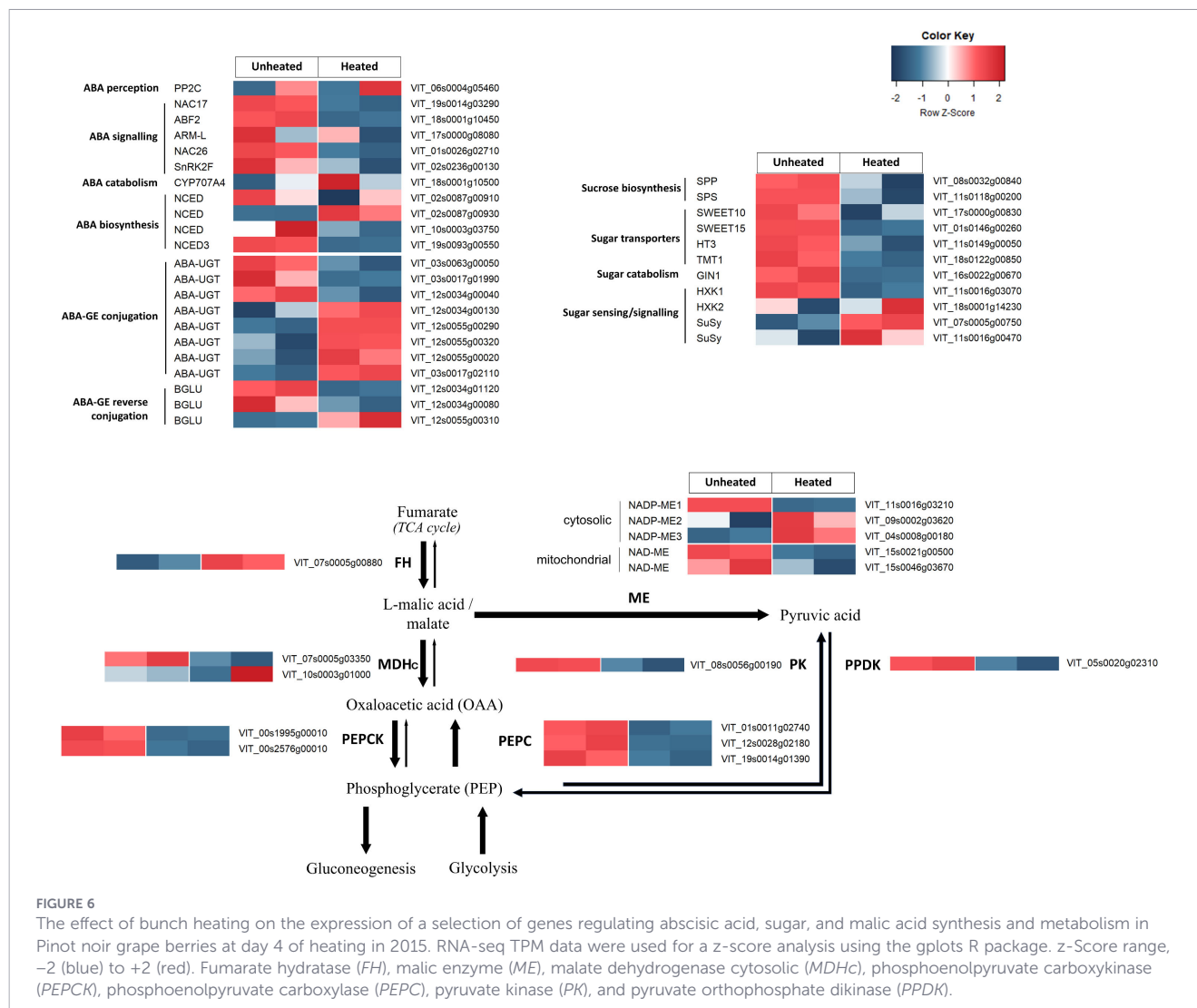


FIGURE 6

The effect of bunch heating on the expression of a selection of genes regulating abscisic acid, sugar, and malic acid synthesis and metabolism in Pinot noir grape berries at day 4 of heating in 2015. RNA-seq TPM data were used for a z-score analysis using the gplots R package. z-Score range, -2 (blue) to +2 (red). Fumarate hydratase (FH), malic enzyme (ME), malate dehydrogenase cytosolic (MDHc), phosphoenolpyruvate carboxykinase (PEPCK), phosphoenolpyruvate carboxylase (PEPC), pyruvate kinase (PK), and pyruvate orthophosphate dikinase (PPDK).

pyruvate orthophosphate dikinase (PPDK), phosphoenolpyruvate carboxykinase (PEPCK), and phosphoenolpyruvate carboxylase (PEPC), were all found to be downregulated by heating (Figure 6).

4 Discussion

Our method of heating was similar to that employed by Tarara et al. (2008), using a flow of heated air to warm specific grape bunches on a grapevine. Our experimental aim was to simulate a short-moderate term heat event (akin to a “heatwave”), rather than to dissect the effect of ambient temperature from sunlight exposure on Pinot noir grape berry ripening. In many cool-climate Pinot noir vineyards, fruit-zone leaf removal is carried out before or during véraison to increase the penetration of spraying regimes and sunlight exposure to mitigate post-véraison berry grey mould (*Botrytis*). In our experiment, to be consistent with vineyard practices, most of the leaves were removed from the fruit zone prior to véraison (Supplementary Figure 1); thus, we focused on the net effect of temperature on ripening in the form of a 7-day simulated heat event. The diurnal temperature of unheated bunches ranged from 10 °C to

32 °C, while heated bunches experienced a significantly warmer diurnal range of 20 °C–39 °C. A concomitant doubling of accumulated heat, as determined by growing degree days, was experienced by heated bunches versus unheated bunches (Supplementary Figure 2). In the context of global warming, the elevated diurnal temperature range of 20 °C–39 °C for 7 days, we believe, simulates a summer heatwave in New Zealand’s temperate cool-climate, where summer diurnal temperatures during véraison (January–February) are typically much cooler, ranging from 11 °C to 28 °C (Chappell, 2016).

4.1 Berry composition: sugar, organic acids, and abscisic acid

Our berry sampling regime enabled analysis of short- and long-term responses to heating during and after treatment. This is important in determining whether short-term heat events, which may occur over a period of just days, have a lasting impact on grape ripening. Time series samples indicated that soluble solids (a proxy for sugar content) were not significantly altered by heating (Supplementary Figure 3), which was supported by berry sugar

concentrations showing that glucose and fructose (the primary hexoses accumulated during grape ripening) and sucrose were not altered by heating (Supplementary Figure 4). Previous research has shown that moderate warming of whole vines in controlled or field conditions generally has little or no effect on sugar accumulation, but high-temperature heat stress has been illustrated to slow down or inhibit sugar accumulation, with the effect manifested by changes in photosynthesis and carbon partitioning within the vine (Crippen and Morrison, 1986; Greer & Weston, 2010; Pereira et al., 2006; Reynolds et al., 1986; Rienth et al., 2016; Sepúlveda and Kliewer, 1986; Soar et al., 2009). Studies where individual grape bunches have been heated have reported little or no influence on berry soluble solids and sugar (Spayd et al., 2002). Lecourieux et al. (2017), for example, demonstrated that 14 days of bunch heating at véraison only slightly increased hexose concentrations at harvest.

In terms of grape acidity, a transient (1–3-week period) increase in grape pulp juice pH and corresponding reductions in titratable acidity were observed in response to véraison bunch heating (Supplementary Figure 3). The hastened drop in titratable acidity was primarily driven by a corresponding reduction in malic acid rather than tartaric acid (Figures 1A–D). Similar responses have been reported as a consequence of sunlight warming of exposed grape bunches, where increases in bunch temperature induced a reduction in malic acid concentration (Bergqvist et al., 2001; Friedel et al., 2015; Keller et al., 2005; Reynolds et al., 1986). Lecourieux et al. (2017) determined that 14 days of bunch heating at or after véraison significantly reduced malic acid concentrations at harvest, but did not influence tartaric acid. A whole-vine temperature study (Rienth et al., 2016) also found that hot temperatures (30 °C) during the pre-véraison to véraison stage of development accelerated a reduction in malic acid concentration. Passive vine heating of Shiraz grapevines by Sweetman et al. (2014) found that heating only at véraison and post-véraison stages of berry development accelerated a reduction in malic acid concentration. The timeframe for a heating effect to manifest, however, has not been clearly determined. The current study illustrated that for an increase in berry temperature of 10 °C above ambient temperatures (day and night), it takes 3 to 4 days for a hastened reduction in malic acid concentration to become apparent in Pinot noir grapes (Figure 1). Taken together, the primary metabolite results have illustrated that synchrony of berry sugar, organic acid (relative changes in concentrations over time), was altered by the impact of heat on reducing malic acid. Similar desynchronisation patterns between sugar and malic acid, as a consequence of bunch heating, have been previously illustrated by Rienth et al. (2016).

4.2 Skin anthocyanins

Numerous studies on a range of red grape varieties have illustrated that heating or high temperatures to a lesser or greater extent inhibit anthocyanin accumulation and alter the proportions of glycosylated and acylated anthocyanins according to cultivar (de Rosas et al., 2022) and the ratio of di-hydroxylated and tri-hydroxylated anthocyanins (Chorti et al., 2010). Pinot noir is also one of the few red grape varieties that do not synthesise acylated anthocyanins, which are reportedly more heat-stable (Mazza et al.,

1999). Furthermore, many studies have illustrated that the accumulation of anthocyanins in different grape varieties has contrasting or varied responses to high temperatures depending on the nature of the heat stress (timing, period, and diurnal temperature range) and how heat stress is applied (passive or active heating, and bunch versus whole vine heat) (Azuma et al., 2012; Bonada and Sadras, 2015; Buttrose et al., 1971; Chorti et al., 2010; Cohen et al., 2008, 2012a, b; Kliewer and Torres, 1972; Kliewer, 1977; Koshita et al., 2015; Mori et al., 2005, 2007a, b; Sadras and Moran, 2012; Spayd et al., 2002; Yamane et al., 2006; Yamane and Shibayama, 2006). Consequently, comparisons between studies are arguably limited (e.g. confounded methods) or are circumstantially complex.

Anthocyanin accumulation in grape skins is characterised by a rapid increase in concentration at the onset of véraison, and in many red grapes, the accumulation begins to plateau and/or cease at soluble solids of approximately 18°Brix, often well before the rest of the berry is considered to reach full physiological and commercial maturity (Mullins et al., 1992). Furthermore, the synchrony of berry sugar and skin anthocyanin accumulation can be altered by inter-seasonal environmental conditions, agronomical practices (leaf removal and cluster thinning), and interactions between these factors (Guidoni et al., 2008; Reynolds et al., 2005). Anthocyanins in our Pinot noir berries began to accumulate 7 days prior to the commencement of heating treatment at the stage of 50% véraison in both years (Figure 2). In unheated grapes, the concentrations of Pt3G and D3G peaked at 15 days after véraison, or at harvest time in the case of M3G, P3G, C3G, and total anthocyanins (Figure 2). In heated grapes, all the anthocyanins measured were reduced by heating in 2014, showing little or no increase in concentration from 0 to 4 weeks after heating (harvest) (Figure 2). This agrees with Spayd et al. (2002), who found that total monomeric anthocyanins in Merlot grapes were reduced by heating. Likewise, Tarara et al. (2008) showed that four out of the five anthocyanin species (excluding malvidin) were reduced in Merlot grapes by bunch heating. Tarara et al. (2008) also noted that heating increased the proportion of anthocyanins in acylated form (up to 28% of total anthocyanins). In 2015, heating had no influence on the accumulation of M3G, P3G, C3G, and total anthocyanins, while the concentrations of Pt3G and D3G were significantly reduced, as they were in 2014. This response hints at a possible disparity in the sensitivity of Pt3G and D3G to heat stress compared with other anthocyanins. This observation is supported by Mori et al. (2005), who found that increased night-time temperature of pot-grown Pinot noir vines had a much greater effect on D3G, C3G, and Pt3G than it did on M3G and P3G. The concentrations of the former were reduced by 50%–75% compared with a 17%–30% reduction for the latter. In 2014, anthocyanin concentrations, although variable when measured at 4-hourly intervals, did show an upward trend by the end of the first 48 hours of heating. However, no clear influence of heating was observed during this period (Supplementary Figure 6). The lag time with respect to heating influence suggests that the accumulation of anthocyanins, measured by skin concentration, takes a while (up to 4 days) to become evident, even if the berries quickly perceive heat stress as indicated by heat shock factor gene expression (Figures 3A, B and

Supplementary Figure 7D). However, some of the genes regulating anthocyanin biosynthesis did show responses before day 4 of heating (see Section 4.4).

The differing seasonal responses of anthocyanins to heating suggest that other factors may interfere with or mask the heating effect. In contrast to malic acid, which responded to heating in an identical manner in both seasons, anthocyanin accumulation was less affected in 2015 despite heated grape berries experiencing the same amount of heat increase (as measured by GDD) in each experiment (Supplementary Figure 2). Key to triggering grape ripening and anthocyanin accumulation in the non-climacteric grape berry is a notable uplift in ABA concentration at the onset of véraison (Bennett et al., 2023; Coombe, 1987; Ferrara et al., 2015; Gagné et al., 2006; Gambetta et al., 2010; Hiratsuka et al., 2001; Koyama et al., 2018; Wheeler et al., 2009). In addition, ABA is classically associated with plant water stress responses. Grapevines experiencing a water deficiency have been shown to exhibit increased ABA concentration in various parts of the vine (Deis et al., 2011; Niculcea et al., 2014).

Measurement of active ABA showed that peak concentrations at the onset of véraison were 30% higher in 2015 than in 2014 (Figures 1E, F). Results also showed that the conjugated storage form of ABA (ABA-glucose-ester) was at a greater concentration in 2015 and was elevated by heating, but this did not translate into an increase in active ABA in heated berries versus unheated berries. The 2015 season was characterised as being notably drier with significantly less rainfall before and during véraison than 2014 (Supplementary Table 2, Supplementary Figure 10). This dryness may have induced a mild-to-moderate water stress response within the vine, which manifested as higher peak berry active ABA concentrations (Figures 1E, F) as seen previously in other red grapes (Deis et al., 2011; Niculcea et al., 2014). Given ABA's role in triggering anthocyanin accumulation, we speculate that elevated ABA concentrations stemming from a mild-to-moderate whole-plant water stress response were sufficient to augment anthocyanin accumulation in 2015 and hence masked the inhibitory effect of high temperature previously observed in the 2014 season. Evidence to support the mitigating influence of ABA is shown by Mori et al. (2005), who illustrated that exogenous applications of ABA to Pinot bunches at véraison could alleviate the negative effect of night heating on Pinot noir anthocyanins. Mori et al. (2005) illustrated that the concentrations of anthocyanins found in night-heated grapes (12–36 days after véraison) treated with ABA were similar to those in grapes exposed to low night temperatures without ABA treatment, while anthocyanin concentrations in night-heated grapes without ABA application were significantly reduced during ripening.

4.3 Absciscic acid and malic acid regulation

The concentration of ABA in plants is tightly regulated by the expression of genes involved in both its synthesis and catabolism. In our study, genes associated with ABA regulation, including biosynthesis (*NCEDs*), perception (*PP2C*), signalling (*NAC17*, *NAC26*, *ABF2*, *ARM-L*, and *SnRK2F*), and catabolism (*CYP707A4*), were generally downregulated by heating treatment (Figure 6). Only one out of four *NCEDs* assessed showed an

upregulation in response to heating. Despite the overall pattern of downregulation, the concentration of free or active ABA did not vary between heated and unheated (Figure 6). This stasis in concentration may have been related to the one *NCED* gene that showed increased expression versus the other three *NCED* genes that were downregulated by heating (Figure 6). However, of interest was the upregulation of a subset of *ABA-UGT* genes in response to heating (Figure 6). *ABA-UGT* catalyses the conjugation of ABA to form ABA-glucose ester, a key storage form of ABA in grape and other berry fruits (Guo et al., 2022; Günther et al., 2022). In contrast, the expression of *BGLU*, which reverses the conjugation of ABA (Liang et al., 2020), was largely downregulated by heating (Figure 6). Thus, the enhanced conjugation of ABA was favoured by heating, as clearly evidenced by increases in the concentrations of ABA-glucose ester during and after heating in both years (Figures 1G, H). The increased abundance of ABA-GE was not reflected in a decrease in free ABA in heated grapes; thus, the increased accumulation of ABA-GE was in addition to the free form of ABA (Figures 1E, F). The increase in the pool of ABA-GE in heated grape berries and the associated upregulation of *ABA-UGT* have not been previously reported, but heat-induced expression of *ABA-UGT* in grape leaves was illustrated when potted 'Kyoho' grapevines were heated for 24 hours or more (Guo et al., 2022). To gain further insight into the dynamics of the ABA forms in the berry, a more detailed metabolite and gene expression profile would need to be undertaken.

The synthesis and metabolism of malic acid in grape berries involve a complex process of equilibrium and feedback loops between the Tricarboxylic Acid Cycle (TCA) cycle via fumarate and pyruvic acid metabolism, and also the processes of gluconeogenesis and glycolysis via phosphoglycerate and oxaloacetic acid (Figure 6) (Sweetman et al., 2009). Malic enzyme genes (*ME*), which operate in both cytoplasm and mitochondria, are responsible for the breakdown of malic acid into pyruvate. Cytosolic NADP-dependent *ME2* and *ME3* gene expression were upregulated by heating. This was confirmed by q-PCR expression patterns, which indicated that upregulation of *ME2c* began within the first few days of heating in both seasons (Figures 4G, H), and was consistent with results reported by Sweetman et al. (2014), who found increased *NAD-ME* expression in heated Shiraz grapes. However, mitochondrial *NAD ME_m* expression was downregulated by heating (Figure 6). This contrasted with q-PCR results, which indicated that *NAD ME_m* expression was either unaffected or raised by heating (Figures 4I, J). Fumarate hydratase (*FH*), which can synthesise malic acid, was upregulated by heating (Figures 4A,B, 6), while both cytosolic and mitochondrial *MDH* expression showed no consistent patterns (Figures 4C–F). Any contribution to malic acid synthesis via glycolysis appeared to be negated as *PEPC* expression was downregulated by heating (Figure 6). This contrasts with Sweetman et al. (2014), who found that *PEPC* tended to be upregulated in heated Shiraz grapes. Conversely, any potential dissimilation of malic acid via gluconeogenesis appeared to be negated as *PEPCK* expression was downregulated by heating (Figure 6). Based on these observations, we surmise that any potential increase in malic acid synthesis via *FH* expression was counteracted by a corresponding increase in

ME2c expression and hence breakdown of malic acid in heated grapes. This leads us to conclude that changes in malic acid concentration are altered via malic enzyme, gluconeogenesis, and glycolysis, with the net effect of these processes resulting in a temporary reduction of malic acid in heated Pinot noir grapes.

4.4 Anthocyanin regulation

RNA-seq and q-PCR results revealed that heating altered the expression of several genes and transcription factors associated with anthocyanin synthesis. Within the structural genes analysed via q-PCR in 2014, *CHS*, *DFR*, and *UFGT* were all found to be downregulated during heating and/or after heating (Figure 3). The reduction in the expression of *UFGT*, the gene controlling the final step in anthocyanin synthesis, took at least 2 days to occur (Figure 3I). Similar observations of reduced *UFGT* expression in response to high temperature have been reported by Azuma et al. (2012) in cultured 'Pione' grapes, Mori et al. (2005) in 'Darkridge' grapes, and Yamane et al. (2006) in 'Aki Queen' grapes. Concurrent with these results, we found that the expression of transcription factor *MYB5a*, a general activator of structural phenylpropanoid genes (Azuma, 2018), was reduced 1 day after heating (Supplementary Figures 8C, 9F). In contrast, the anthocyanin repressor *MYB4a* was upregulated quickly in response to heat stress perception by *HsfA2* (within 8–16 hours of heating) in 2014 (Figures 9D, E). Furthermore, an emerging pattern of elevated expression of *MYB4a* during the first 2 days of heating in 2014 and on days 2–4 of heating in 2015 suggests that *MYB4a* expression may be triggered by the upregulation of *HsfA2*. The altered regulation of said structural genes and transcription factors under the influence of heating was sufficient to explain the significant reduction in anthocyanin concentration observed in 2014.

In 2015, heating had less influence on anthocyanin concentrations, with only Pt3G and D3G showing a reduction in concentration at the end of heating and after (Figure 2). Total anthocyanins, M3G, P3G, and C3G were not reduced by heating. Consistent with this, the q-PCR analysis in 2015 showed that the expression of *DFR*, *UFGT*, *MYB5a*, and *MYB4a* was unaffected by heating (Figure 3, Supplementary Figure 8). However, *CHS* was still downregulated during heating (Figure 3). The RNA-seq data, which provided a snapshot of transcriptional activity on day 4 of heating in 2015, further confirmed the downregulation of *CHS* (Figure 5). However, in contrast to the q-PCR results, the RNA-seq analysis indicated the upregulation of *UFGT* as well as anthocyanin regulator *MYB1* (Figure 5). This suggests two things: measurement of gene expression over a period of time gives more informative insight into the impact of heating, and different parts of the anthocyanin pathway responded to heat in different ways.

Using the RNA-seq data to look at a wider range of genes in 2015, we found that a number of variants of *PAL*, along with *F3'H* and *F3'5'H*, were downregulated by heating (Figure 5), while other anthocyanin activators (*MYB3* and *MYB15*) and anthocyanin repressor *MYB44* were upregulated. Despite changes in the expression of this group of genes, the concentrations of most anthocyanins measured were not affected in 2015. Interestingly,

though, Pt3G and D3G were reduced from the end of heating onwards (Figure 2). The synthesis of Pt3G relies on a further step involving *O*-methyltransferase (*OMT*), the expression of which was found to be upregulated at day 4 of heating, clearly in contrast to the reduced abundance of Pt3G. We speculate that other transcription factors may be responsible for reduced Pt3G and D3G.

5 Conclusion

The elevated temperature range and doubling of accumulated heat (GGD) during treatment resulted in a range of changes in Pinot noir berry maturity and pulp and skin metabolite composition, including a decrease in titratable acidity and malic acid and reductions in skin anthocyanins, along with changes in abscisic acid forms. The compositional changes often remained for days or weeks after the heating treatment had ceased and altered the synchrony of primary and secondary metabolite accumulation and hence berry maturity balance. Many of the observed changes in pulp and skin metabolites, for example, anthocyanins, could be linked to changes in the expression of genes and transcription factors associated with their regulation. This study suggests that periods of high summer temperatures (diurnal min–max range of 20 °C–40 °C) are likely to have a significant direct impact on the ripening of cool-climate Pinot noir grapes.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

JB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing, Software. AA: Data curation, Formal analysis, Methodology, Writing – review & editing. AF: Data curation, Investigation, Methodology, Resources, Writing – review & editing. HB: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. JC: Formal analysis, Investigation, Methodology, Writing – review & editing. CE: Formal analysis, Investigation, Methodology, Writing – review & editing. RK: Formal analysis, Investigation, Methodology, Writing – review & editing. HN: Data curation, Formal analysis, Investigation, Methodology, Software, Writing – review & editing. CD: Data curation, Formal analysis, Writing – review & editing. BP: Data curation, Formal analysis, Investigation, Methodology, Resources,

Writing – review & editing. DM: Funding acquisition, Writing – review & editing. RE: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This work is part of the Plant & Food Group, New Zealand Institute for Bioeconomy Science Limited Wine Research programme, funded by the MBIE Strategic Science Investment Fund (SSIF), delivered by Plant & Food Research in consultation with New Zealand Winegrowers.

Acknowledgments

The authors acknowledge the laboratory assistance of Sharlene Haycock, Rafidah Horner, and Rachel Bishell and the in-kind support of the Nelson Marlborough Institute of Technology for the use of grapevines in their vineyard at 85 Budge Street, Blenheim, New Zealand.

Conflict of interest

All authors were employed by the company New Zealand Institute for Bioeconomy Science Limited.

References

- Azuma, A. (2018). Genetic and environmental impacts on the biosynthesis of anthocyanins in grapes. *Horticulture J.* 87, 1–17. doi: 10.2503/hortj.OKD-IR02
- Azuma, A., Yakushiji, H., Koshita, Y., and Kobayashi, S. (2012). Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. *Planta* 236, 1067–1080. doi: 10.1007/s00425-012-1650-x
- Bennett, J., Meiyalaghan, S., Nguyen, H. M., Boldingh, H., Cooney, J., Elborough, C., et al. (2023). Exogenous abscisic acid and sugar induce a cascade of ripening events associated with anthocyanin accumulation in cultured Pinot Noir grape berries. *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1324675
- Bergqvist, J., Dokoozlian, N., and Ebisuda, N. (2001). Sunlight exposure and temperature effects on berry growth and composition of cabernet sauvignon and grenache in the central San Joaquin valley of California. *Am. J. Enol. Viticulture* 52, 1–7. doi: 10.5344/ajev.2001.52.1.1
- Bonada, M., and Sadras, V. O. (2015). Review: critical appraisal of methods to investigate the effect of temperature on grapevine berry composition. *Aust. J. Grape Wine Res.* 21, 1–17. doi: 10.1111/ajgw.12102
- Boss, P. K., Davies, C., and Robinson, S. P. (1996). Analysis of the expression of anthocyanin pathway genes in developing *Vitis vinifera* L. cv Shiraz grape berries and the implications for pathway regulation. *Plant Physiol.* 111, 1059–1066. doi: 10.1104/pp.111.4.1059
- Bulley, S. M., Cooney, J. M., and Laing, W. (2021). Elevating ascorbate in arabidopsis stimulates the production of abscisic acid, phaseic acid, and to a lesser extent auxin (IAA) and jasmonates, resulting in increased expression of DHAR1 and multiple transcription factors associated with abiotic stress tolerance. *Int. J. Mol. Sci.* 22, 6743. doi: 10.3390/ijms22136743
- Buttrose, M. S., Hale, C. R., and Kliever, W. M. (1971). Effect of temperature on the composition of 'Cabernet sauvignon' Berries. *Am. J. Enol. Viticulture* 22, 71–75. doi: 10.5344/ajev.1971.22.2.71
- Cai, W.-J., Ye, T.-T., Wang, Q., Cai, B.-D., and Feng, Y.-Q. (2016). A rapid approach to investigate spatiotemporal distribution of phytohormones in rice. *Plant Methods* 12, 47. doi: 10.1186/s13007-016-0147-1
- Chappell, P. R. (2016). The climate and weather of Marlborough. *NIWA Sci. Technol. Ser.* (Wellington, New Zealand) 69, 40.
- Chorti, E., Guidoni, S., Ferrandino, A., and Novello, V. (2010). Effect of different cluster sunlight exposure levels on ripening and anthocyanin accumulation in nebbiolo grapes. *Am. J. Enol. Viticulture* 61, 23. doi: 10.5344/ajev.2010.61.1.23
- Cohen, S. D., Tarara, J. M., Gambetta, G. A., Matthews, M. A., and Kennedy, J. A. (2012a). Impact of diurnal temperature variation on grape berry development, proanthocyanidin accumulation, and the expression of flavonoid pathway genes. *J. Exp. Bot.* 63, 2655–2665. doi: 10.1093/jxb/err449
- Cohen, S. D., Tarara, J. M., and Kennedy, J. A. (2008). Assessing the impact of temperature on grape phenolic metabolism. *Analytica Chimica Acta* 621, 57–67. doi: 10.1016/j.aca.2007.11.029
- Cohen, S. D., Tarara, J. M., and Kennedy, J. A. (2012b). Diurnal temperature range compression hastens berry development and modifies flavonoid partitioning in grapes. *Am. J. Enol. Viticulture* 63, 112–120. doi: 10.5344/ajev.2011.11015
- Coombe, B. G. (1987). Influence of temperature on composition and quality of grapes. *Acta Hort.* 206, 23–35. doi: 10.17660/ActaHortic.1987.206.1
- Crippen, D., and Morrison, J. (1986). The effects of sun exposure on the compositional development of Cabernet Sauvignon berries. *Am. J. Enol. Viticulture* 37, 235–242. doi: 10.5344/ajev.1986.37.4.235
- Deis, L., Cavnagaro, B., Bottini, R., Wuilloud, R., and Fernanda Silva, M. (2011). Water deficit and exogenous ABA significantly affect grape and wine phenolic composition under in field and in-vitro conditions. *Plant Growth Regul.* 65, 11–21. doi: 10.1007/s10725-011-9570-5
- Deluc, L., Barrieu, F., Marchive, C., Lauvergeat, V., Decendit, A., Richard, T., et al. (2006). Characterization of a grapevine R2R3-MYB transcription factor that regulates the phenylpropanoid pathway. *Plant Physiol.* 140, 499–511. doi: 10.1104/pp.105.067231
- Deluc, L., Bogs, J., Walker, A. R., Ferrier, T., Decendit, A., Merillon, J.-M., et al. (2008). The transcription factor VvMYB5b contributes to the regulation of anthocyanin and

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphgy.2025.1701827/full#supplementary-material>

- proanthocyanidin biosynthesis in developing grape berries. *Plant Physiol.* 147, 2041–2053. doi: 10.1104/pp.108.118919
- de Rosas, I., Deis, L., Baldo, Y., Cavagnaro, J. B., and Cavagnaro, P. F. (2022). High temperature alters anthocyanin concentration and composition in grape berries of malbec, merlot, and pinot noir in a cultivar-dependent manner. *Plants* 11, 926. doi: 10.3390/plants11070926
- Düring, H., and Alleweldt, G. (1984). Zur möglichen Bedeutung der Abscisinsäure bei der Zuckereinlagerung in die Weinbeere. *Plant Biol.* 97, 101–113.
- Ferrara, G., Mazzeo, A., Matarrese, A. M. S., Pacucci, C., Punzi, R., Faccia, M., et al. (2015). Application of abscisic acid (S-ABA) and sucrose to improve colour, anthocyanin content and antioxidant activity of cv. Crimson Seedless grape berries. *Aust. J. Grape Wine Res.* 21, 18–29. doi: 10.1111/ajgw.12112
- Friedel, M., Stoll, M., Patz, C. D., Will, F., and Dietrich, H. (2015). Impact of light exposure on fruit composition of white 'Riesling' grape berries (*Vitis vinifera* L.). *Vitis* 54, 10.
- Gagné, S., Esteve, K., Deytieu-Belleau, C., Saucier, C., and Geny, L. (2006). Influence of abscisic acid in triggering "véraison"; in grape berry skins of *Vitis vinifera* L. cv. Cabernet-Sauvignon. *OENO One* 40, 7–14. doi: 10.20870/oeno-one.2006.40.1.882
- Gambetta, G. A., Matthews, M. A., Shaghisi, T. H., McElrone, A. J., and Castellarin, S. D. (2010). Sugar and abscisic acid signaling orthologs are activated at the onset of ripening in grape. *Planta* 232, 219–234. doi: 10.1007/s00425-010-1165-2
- Gladstones, J. (2011). *Wine, terroir and climate change* (Kent Town, South Australia: Wakefield Press).
- Greer, D. H., and Weston, C. (2010). Heat stress affects flowering, berry growth, sugar accumulation and photosynthesis of *Vitis vinifera* cv. Semillon grapevines grown in a controlled environment. *Funct. Plant Biol.* 37, 206–214. doi: 10.1071/FP09209
- Guidoni, S., Ferrandino, A., and Novello, V. (2008). Effects of seasonal and agronomical practices on skin anthocyanin profile of nebbiolo grapes. *Am. J. Enol. Viticulture* 59, 22–29. doi: 10.5344/ajev.2008.59.1.22
- Günther, C. S., Plunkett, B. J., Cooney, J. M., Jensen, D. J. J., Trower, T. M., Elborough, C., et al. (2022). Biotic stress-induced and ripening-related anthocyanin biosynthesis are regulated by alternate phytohormone signals in blueberries. *Environ. Exp. Bot.* 203, 105065. doi: 10.1016/j.envexpbot.2022.105065
- Guo, R., Lin, L., Huang, G., Shi, X., Wei, R., Han, J., et al. (2022). Transcriptome analysis of 'Kyoho' Grapevine leaves identifies heat response genes involved in the transcriptional regulation of photosynthesis and abscisic acid. *Agronomy* 12, 2591. doi: 10.3390/agronomy12102591
- He, F., Mu, L., Yan, G.-L., Liang, N.-N., Pan, Q.-H., Wang, J., et al. (2010). Biosynthesis of anthocyanins and their regulation in colored grapes. *Molecules* 15, 9057–9091. doi: 10.3390/molecules15129057
- Hiratsuka, S., Onodera, H., Kawai, Y., Kubo, T., Itoh, H., and Wada, R. (2001). ABA and sugar effects on anthocyanin formation in grape berry cultured *in vitro*. *Scientia Hort.* 90, 121–130. doi: 10.1016/S0304-4238(00)00264-8
- Jones, G. V., and Schultz, H. R. (2016). Climate change: Climate change and emerging cool climate wine regions. *Wine Viticulture J.* 31, 51–53.
- Jones, G. V., White, M. A., Cooper, O. R., and Storchmann, K. (2005). Climate change and global wine quality. *Climate Change* 73, 319–343. doi: 10.1007/s10584-005-4704-2
- Keller, M., Mills, L. J., Wample, R. L., and Spayd, S. E. (2005). Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. *Am. J. Enol. Viticulture* 56, 91–103. doi: 10.5344/ajev.2005.56.2.91
- Kliewer, W. M. (1977). Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. *Am. J. Enol. Viticulture* 28, 96–103. doi: 10.5344/ajev.1977.28.2.96
- Kliewer, W. M., and Torres, R. E. (1972). Effect of controlled day and night temperatures on grape coloration. *Am. J. Enol. Viticulture* 23, 71–77. doi: 10.5344/ajev.1972.23.2.71
- Kobayashi, S., Goto-Yamamoto, N., and Hirochika, H. (2005). Association of VvmybA1 gene expression with anthocyanin production in grape (*Vitis vinifera*) skin -color mutants. *J. Japanese Soc. Hortic. Sci.* 74, 196–203. doi: 10.2503/jjshs.74.196
- Koshita, Y., Mitani, N., Azuma, A., and Yakushiji, H. (2015). Effect of short-term temperature treatment to clusters on anthocyanin and abscisic acid content in the peel of 'Aki Queen' grape. *Vitis - J. Grapevine Res.* 54, 169–173. doi: 10.5073/vitis.2015.54.169-173
- Koyama, R., Roberto, S. R., de Souza, R. T., Borges, W. F. S., Anderson, M., Waterhouse, A. L., et al. (2018). Exogenous Abscisic Acid Promotes Anthocyanin Biosynthesis and Increased Expression of Flavonoid Synthesis Genes in *Vitis vinifera* × *Vitis labrusca* Table Grapes in a Subtropical Region. *Front. Plant Sci.* 9, Article 323. doi: 10.3389/fpls.2018.00323
- Lakso, A. N., and Kliewer, W. M. (1975). The influence of temperature on Malic acid metabolism in grape berries. I. Enzyme responses. *Plant Physiol.* 56, 370–372. doi: 10.1104/pp.56.3.370
- Lecourieux, F., Kappel, C., Pieri, P., Charon, J., Pillet, J., Hilbert, G., et al. (2017). Dissecting the biochemical and transcriptomic effects of a locally applied heat treatment on developing cabernet sauvignon grape berries. *Front. Plant Sci.* 8, 1–23. doi: 10.3389/fpls.2017.00053
- Liang, B., Zheng, Y., Wang, J., Zhang, W., Fu, Y., Kai, W., et al. (2020). Overexpression of the persimmon abscisic acid β -glucosidase gene (DkBG1) alters fruit ripening in transgenic tomato. *Plant J.* 102, 1220–1233. doi: 10.1111/tpj.14695
- Mazza, G. (1995). Anthocyanins in grapes and grape products. *Crit. Rev. Food Sci. Nutr.* 35, 341–371. doi: 10.1080/10408399509527704
- Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., and Ewert, B. (1999). Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *J. Agric. Food Chem.* 47, 4009–4017. doi: 10.1021/jf990449f
- Mori, K., Goto-Yamamoto, N., Kitayama, M., and Hashizume, K. (2007a). Effect of high temperature on anthocyanin composition and transcription of flavonoid hydroxylase genes in 'Pinot noir' grapes (*Vitis vinifera*). *J. Hortic. Sci. Biotechnol.* 82, 199–206. doi: 10.1080/14620316.2007.11512220
- Mori, K., Goto-Yamamoto, N., Kitayama, M., and Hashizume, K. (2007b). Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.* 58, 1935–1945. doi: 10.1093/jxb/erm055
- Mori, K., Saito, H., Goto-Yamamoto, N., Kitayama, M., Kobayashi, S., Sugaya, S., et al. (2005). Effects of abscisic acid treatment and night temperatures on anthocyanin composition in Pinot noir grapes. *Vitis* 44, 161.
- Mullins, M. G., Bouquet, A., and Williams, L. E. (1992). *Biology of the grapevine* (Cambridge, England: Cambridge University Press).
- Niculcea, M., López, J., Sánchez-Díaz, M., and Carmen Antolín, M. (2014). Involvement of berry hormonal content in the response to pre- and post-véraison water deficit in different grapevine (*Vitis vinifera* L.) cultivars. *Aust. J. Grape Wine Res.* 20, 281–291. doi: 10.1111/ajgw.12064
- Pereira, G. E., Gaudillere, J. P., Pieri, P., Hilbert, G., Maucourt, M., Deborde, C., et al. (2006). Microclimate influence on mineral and metabolic profiles of grape berries. *J. Agric. Food Chem.* 54, 6765–6775. doi: 10.1021/jf061013k
- Reynolds, A. G., Molek, T., and De Savigny, C. (2005). Timing of shoot thinning in vitis vinifera: impacts on yield and fruit composition variables. *Am. J. Enol. Viticulture* 56, 343–356. doi: 10.5344/ajev.2005.56.4.343
- Reynolds, A. G., Pool, R. M., and Matpick, L. R. (1986). Influence of cluster exposure on fruit composition and wine quality of Seyval blanc grapes. *Vitis* 25, 85–95.
- Rienth, M., Torregrosa, L., Sarah, G., Ardisson, M., Brillouet, J.-M., and Romieu, C. (2016). Temperature desynchronizes sugar and organic acid metabolism in ripening grapevine fruits and remodels their transcriptome. *BMC Plant Biol.* 16, 20. doi: 10.1186/s12870-016-0850-0
- Rinaldo, A. R., Cavallini, E., Jia, Y., Moss, S. M. A., McDavid, D. A. J., Hooper, L. C., et al. (2015). A grapevine anthocyanin acyltransferase, transcriptionally regulated by vMYBA, can produce most acylated anthocyanins present in grape skins. *Plant Physiol.* 169, 1897–1916. doi: 10.1104/pp.15.01255
- Rodriguez-Delgado, M. A., Malovaná, S., Pérez, J. P., Borges, T., and García Montelongo, F. J. (2001). Separation of phenolic compounds by high-performance liquid chromatography with absorbance and fluorimetric detection. *J. Chromatogr. A* 912, 249–257. doi: 10.1016/S0021-9673(01)00598-2
- Sadras, V. O., and Moran, M. A. (2012). Elevated temperature decouples anthocyanins and sugars in berries of Shiraz and Cabernet Franc. *Aust. J. Grape Wine Res.* 18, 115–122. doi: 10.1111/j.1755-0238.2012.00180.x
- Sepúlveda, G., and Kliewer, W. M. (1986).) effect of high temperature on grapevines (*Vitis vinifera*; L.). II. Distribution of soluble sugars. *Am. J. Enol. Viticulture* 37, 20.
- Shahab, M., Roberto, S. R., Adnan, M., Fahad, S., Koyama, R., Saleem, M. H., et al. (2023). Phenolic compounds as a quality determinant of grapes: A critical review. *J. Plant Growth Regul.* 42, 5325–5331. doi: 10.1007/s00344-023-10953-w
- Shi, S., Condron, L., Larsen, S., Richardson, A. E., Jones, E., Jiao, J., et al. (2011). *In situ* sampling of low molecular weight organic anions from rhizosphere of radiata pine (*Pinus radiata*) grown in a rhizotron system. *Environ. Exp. Bot.* 70, 131–142. doi: 10.1016/j.envexpbot.2010.08.010
- Soar, C. J., Collins, M. J., and Sadras, V. O. (2009). Irrigated Shiraz vines (*Vitis vinifera*) upregulate gas exchange and maintain berry growth in response to short spells of high maximum temperature in the field. *Funct. Plant Biol.* 36, 801–814. doi: 10.1071/FP09101
- Spayd, S. E., Tarara, J. M., Mee, D. L., and Ferguson, J. C. (2002). Separation of Sunlight and Temperature Effects on the Composition of *Vitis vinifera* cv. Merlot Berries. *Am. J. Enol. Viticulture* 53, 171–182. doi: 10.5344/ajev.2002.53.3.171
- Springob, K., Nakajima, J., Yamazaki, M., and Saito, K. (2003). Recent advances in the biosynthesis and accumulation of anthocyanins. *Natural Product Rep.* 20, 288–303. doi: 10.1039/b109542k
- Sweetman, C., Deluc, L. G., Cramer, G. R., Ford, C. M., and Soole, K. L. (2009). Regulation of malate metabolism in grape berry and other developing fruits. *Phytochemistry* 70, 1329–1344. doi: 10.1016/j.phytochem.2009.08.006
- Sweetman, C., Sadras, V. O., Hancock, R. D., Soole, K. L., and Ford, C. M. (2014). Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. *J. Exp. Bot.* 65, 5975–5988. doi: 10.1093/jxb/eru343
- Tarara, J. M., Ferguson, J. C., and Spayd, S. E. (2000). A chamber-free method of heating and cooling grape clusters in the vineyard. *Am. J. Enol. Viticulture* 51, 182–188. doi: 10.5344/ajev.2000.51.2.182

- Tarara, J. M., Lee, J., Spayd, S. E., and Scagel, C. F. (2008). Berry temperature and solar radiation alter acylation, proportion, and concentration of anthocyanin in merlot grapes. *Am. J. Enol. Viticulture* 59, 235–247. doi: 10.5344/ajev.2008.59.3.235
- Venios, X., Korkas, E., Nisiotou, A., and Banilas, G. (2020). Grapevine responses to heat stress and global warming. *Plants* 9, 1754. doi: 10.3390/plants9121754
- Wheeler, S., Loveys, B., Ford, C., and Davies, C. (2009). The relationship between the expression of abscisic acid biosynthesis genes, accumulation of abscisic acid and the promotion of *Vitis vinifera* L. berry ripening by abscisic acid. *Aust. J. Grape Wine Res.* 15, 195–204. doi: 10.1111/j.1755-0238.2008.00045.x
- Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., et al. (2021). ClusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation* 2, 100141. doi: 10.1016/j.xinn.2021.100141
- Wünsche, J. N., and Palmer, J. W. (1997). Portable through-flow cuvette system for measuring whole-canopy gas exchange of apple trees in the field. *HortScience* 32, 653–658. doi: 10.21273/HORTSCI.32.4.653
- Wurms, K. V., Reglinski, T., Buissink, P., Ah Chee, A., Fehlmann, C., McDonald, S., et al. (2023). Effects of drought and flooding on phytohormones and abscisic acid gene expression in kiwifruit. *Int. J. Mol. Sci.* 24, 7580. doi: 10.3390/ijms24087580
- Yamane, T., Jeong, S. T., Goto-Yamamoto, N., Koshita, Y., and Kobayashi, S. (2006). Effects of temperature on anthocyanin biosynthesis in grape berry skins. *Am. J. Enol. Viticulture* 57, 54–59. doi: 10.5344/ajev.2006.57.1.54
- Yamane, T., and Shibayama, K. (2006). Effects of changes in the sensitivity to temperature on skin coloration in 'Aki Queen' grape berries. *J. Japanese Soc. Hortic. Sci.* 75, 458–462. doi: 10.2503/jjshs.75.458