A comparative study of ripening among berries of the grape cluster reveals altered transcriptional program and enhanced ripening rate in delayed berries

Satyanarayana Gouthu, Shawn T. O’Neil, Yanming Di, Mitra Ansarolia, Molly Megraw and Laurent G. Deluc

Supplementary text

Ripening progress in each class in relation to RS berries during the first 3-week period

To assess the progress of ripening during early-véraison in GH, GS, and PS berries relative to that of RS berries, principle component analyses were performed using TSS, color index, and elasticity data of individual berries from each under-ripe berry class along with those of RS berry class during the first 3-week period following mid-véraison (Supplementary Fig. S3A-C). No ripening progress was observed in GH berries between V and Week 1 (Supplementary Fig. S3A). In contrast, the ripening progress of GS berries was apparent at V but still lagged behind RS berries at Week 3 (Supplementary Fig. S3B). PS berries matched the RS berry ripening state by Week 1 and further progress was concomitant in both classes (Supplementary Fig. S3C). Discriminant analyses to examine the advancement of individual berries of a class (Supplementary Fig. S3D-G) showed that the majority of berries in a class advanced together during the first 3-week period, after which each berry class ceases to maintain its unique identity.

Rates of accumulation of sugars and pigments in berry classes

Increases in total soluble solids (TSS, °Brix) and color index [(180-h)/(C+L)] (Carreno and Martinez, 1995) were monitored in five clusters each of six plants to assess ripening progress during their equivalent ripening periods. GH, GS, PS, and RS berries were identified and tagged with color-coded strings. One berry from each ripening class per cluster was sampled every week and sugar and color data was obtained for each berry. At V, TSS and color index in RS berries were 12.6 and 3.4 respectively, levels that were observed one or two weeks later in GH, GS, and PS berries. Weekly increases in TSS and color index over a two-week period were plotted from these physiologically similar stages in each class. Equations to calculate the days required to reach a given TSS and
color stage at the ripening rate characteristic of each berry class were obtained by fitting second-order polynomial curves. Using the respective equations we could predict when GH, GS, and PS berries would reach the TSS and color levels observed in RS berries (Supplementary worksheet 2B). The TSS level of RS berries at V, and at 1 and 2 weeks past-V were designated as Brix/color stages of R1, R2, and R3 respectively. Although RS berries took seven days to traverse the increments between the stages, the times calculated for under-ripe berries to traverse the same increments were lower (Table 1).

RNA isolation and NimbleGen Vitis vinifera GeneChip Array Hybridization and data analysis
Total RNA was isolated from the skin, pulp and seed tissues of the pooled berries using the RNeasy Midi Kit (Qiagen Inc., Valencia, CA). Because of the high sugar and phenolic content of the tissues, Qiagen RLC buffer (2% polyethylene glycol (MW 20,000), 0.2 M sodium acetate (pH 5.2), and 1% β-mercaptoethanol) was substituted for lysis buffer. For the reminder of the procedure, the manufacturer’s protocol was followed, including on-column DNase digestion (RNase-free DNase, Qiagen, Valencia, CA). First-strand cDNA was prepared from 10 µg total RNA using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA) and oligo dT primers. The second-strand cDNA synthesis reaction contained 3 µg random primer, 5U Klenow fragment (New England Biolabs, Ipswich, MA), Klenow buffer, and 2.5 mM dNTP mixture. Following incubation at 37 °C for 1.5 h, the double-stranded cDNA was purified using MiniElute PCR Purification Spin Columns (Qiagen Inc., Valencia, CA). The double-stranded cDNA was labeled using the NimbleGen One-Color DNA Labeling Kit (NimbleGen, Madison, WI) following the NimbleGen Array user guide. A 2 µg sample of Cy3-labeled cDNA was hybridized to the microarray according to the manufacturer’s instructions. NimbleGen Microarray 090818 Vitis exp HX12 (Roche, NimbleGen) contains 29,971 non-redundant probes in quadruplicate, based on annotations of the Grape 12X Assembly performed by The Institute of Applied Genomics (Italy). Hybridization and washes were performed using NimbleGen reagents and hardware, and microarrays were scanned with an Axon GenePix 4200A Pro Scanner, according to the protocols provided by NimbleGen. Quality control, normalization, and the signal-to-noise
ratio for hybridization signals for samples in V and PostV were performed using the web-based ANAIS tools (Simon and Biot, 2010). The Robust Multichip Average (RMA) normalization process involved background adjustment and quantile normalization (Irizarry et al., 2003).

Quantitative Real-time RT-PCR analysis
RT-PCR was performed using ABI 7500 Fast Real-Time PCR system (Applied Biosystems). Briefly, 5-10 ng of cDNA was used as template for analysis using QuantiFast SYBR Green (Qiagen). Specific oligonucleotide primer pairs were designed with Primer 3 software (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). Amplification of the single amplicon per PCR product was verified by analyzing dissociation curves. In this study, six V. vinifera reference genes were evaluated: gamma tonoplast aquaporin, 3 sucrose transporter 11, ERD6-like 16, vacuolar invertase 1, UDP-Glucose glycosyltransferase, and trehalose-6P phosphatase. Peptidyl-prolyl cis-trans isomerase (VIT_06s0004g06610) was used as a reference gene for normalization based on its low M value in our expression data (Vandesompele et al., 2002). All PCR reactions were done with three biological and two technical replicates. The reaction conditions were: heat activation at 95 °C for 5 min (one cycle); 95 °C for 10 s, 60 °C for 30 s (40 cycles). Data were acquired and exported with the 7500 Fast Software version 2.0.6 (Applied Biosystems) and relative gene expression was calculated using the ΔΔCt method. Relative fold-expression for each gene was calculated, in which the level of expression in GS berries at V was set at 1.

Oligonucleotide sequences

| Name                     | Vitis ID         | Forward                     | Reverse                     |
|--------------------------|------------------|-----------------------------|-----------------------------|
| Gamma tonoplast aquaporin 3 | VIT_06s0061g00730 | ATCAGGGCCGGTCCATTTAT        |AACGCCAATTACATGCAATCA        |
| Sucrose transporter 11   | VIT_18s0001g08210 | GGACCATGGGGATCAACTTTTT      |AGATCGAGGAAATAGCCAAGATG      |
| ERD6-like 16             | VIT_05s0020g02170 | TTTTTGGCCAAAGATCGTG         |TCCGAGTACCATCTGCAATG         |
| Vacuolar invertase 1     | VIT_16s0022g00670 | CCTCTACATTAGCTCCGGTGG       |CAGGACATGACAAAGGATTGAA       |
| UDP-Glucose glycosyltransferase | VIT_00s0324g00050 | GCTGTGGTATGGTCACCTGG       |GGCAAAGATGGCTGTGACTT         |
| Trehalose – 6P phosphatase | VIT_00s0233g00030 | AATAAAGGTGGGTTGCTG         |AGGCATTACAAAGTGCCAGG         |
Identification of transcriptional modules during the ripening transitions

The data integration framework, ‘DISTILLER’ was used to identify transcriptional modules (co-expressed genes sharing overrepresented \textit{cis}-regulatory elements or motifs) in the data of differentially expressed genes between the berry ripening stages (Lemmens \textit{et al.}, 2009). DISTILLER identifies condition-dependent expression modularity by integrating the overrepresentation score of motifs identified in the promoter region of analyzed genes and the expression dynamics of the same genes. Overrepresented motifs in the data were mapped and the Positional Weight Matrices (PWMs) representing these regulatory motif sites were extracted using DREME algorithm (Bailey, 2011). Nature of the stimuli that elicit responses from known regulatory motifs was extracted from PLACE database (Higo \textit{et al.}, 1999). The PWMs are used to scan the 2 kb upstream promoter region of each gene in order to discover the putative binding sites of each regulatory element represented by a PWM. A log-likelihood-based score compares the probability of observing a particular subsequence in promoter region according to the PWM model to the probability of observing that subsequence according to a background model. The scanning is performed with the Scanner Toolset for Transcription Factor Binding Site Discovery (\url{http://megraw.cgrb.oregonstate.edu/software/TFBS-Scan/}) (Megraw \textit{et al.}, 2013). A high score is indicative of a good match to the TF binding motif. The motifs form a binary matrix in which the columns correspond to each motif and rows indicate each gene; motifs found to have at least one putative binding site in the promoter region of a gene receive value 1, otherwise 0. This binary matrix is used as an input to DISTILLER for identifying transcriptional modules. Along with the GS, PS, and RS datasets of mid-\textit{véraison} and maturity cluster stages, we included gene expression data of berries of pre-\textit{véraison} cluster that comes before GS stage, forming a linear sequence of seven developmental stages (PV, GS, PS, RS, GSH, PSH, RSH), to increase the statistical strength of the DISTILLER analysis. Expression differences of each gene between two successive ripening stages was assigned to conditions A, B, C, D, E, F. For example, condition A has the expression difference of a gene from pre-\textit{véraison} (PV) to
green soft stage (GS), which contained the information on the direction of gene expression (up or down-regulation), and the extent of expression differences between the stages. To identify the relative functional activity of motifs across the conditions, overall activity scores were assigned for each motif based on the total number of modules it belongs to and the number of genes in those modules. Similar scores for motifs in each condition were assigned, and integrated with the overall activity score, which was normalized across the motifs. So the final activity scores reflect the relative activity of each motif between tissues, conditions, and across the motifs.

REFERENCES

Bailey TL. 2011. DREME: motif discovery in transcription factor ChIP-seq data. Bioinformatics (Oxford, England) 27, 1653–1659.

Carreno J, Martinez A. 1995. Proposal of an index for the objective evaluation of the color of red table grapes. Food Research International 28, 373–377.

Higo K, Ugawa Y, Iwamoto M, Korenaga T. 1999. Plant cis-acting regulatory DNA elements (PLACE) database: 1999. Nucleic acids research 27, 297–300.

Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics (Oxford, England) 4, 249–264.

Lemmens K, De Bie T, Dhollander T, et al. 2009. DISTILLER: a data integration framework to reveal condition dependency of complex regulons in Escherichia coli. Genome biology 10, R27.

Megraw M, Mukherjee S, Ohler U. 2013. Sustained-input switches for transcription factors and microRNAs are central building blocks of eukaryotic gene circuits. Genome biology 14, R85.

Simon A, Biot E. 2010. ANAIS: analysis of NimbleGen arrays interface. Bioinformatics (Oxford, England) 26, 2468–2469.

Vandesompele J, De Preter K, Pattyn F, 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, 0034.1–0034.11.
**Supplementary Table S1.** Evaluation of variances in ripening parameters of berries from different clusters and experimental plants.

| Comparison                  | Brix   | Color index   |
|-----------------------------|--------|---------------|
| ripening class              | <0.0001| <0.0001       |
| ripening class X cluster    | 0.8242 | 0.1748        |
| ripening class X plant      | 0.0379 | 0.4959        |
| ripening class X plant X cluster | 0.4253 | 0.7181        |

The table displays the p values of ANOVA tests performed between different comparisons. Total soluble solids and color index of individual berry were used in the tests. Seventy-five berries of each ripening class that were used in the study were sampled from five different clusters of three experimental plants.
**Supplementary Table S2.** Discriminant analyses showing the percentage of berries from each berry class sampled as GH, GS, PS, or RS berries (rows) assigned to ripening classes (columns).

|          | **Mid-véraison** |          | **5-Week Post- véraison** |
|----------|------------------|----------|--------------------------|
|          | GH   | GS   | PS   | RS   | GH   | GS   | PS   | RS   |
| GH       | 69   | 31   | 0    | 0    | 21   | 32   | 21   | 26   |
| GS       | 20   | 79   | 1    | 0    | 15   | 36   | 21   | 28   |
| PS       | 0    | 4    | 93   | 3    | 14   | 20   | 34   | 31   |
| RS       | 0    | 0    | 4    | 96   | 17   | 13   | 22   | 48   |

Color (L [lightness], h [hue angle], and C [chroma]), and total soluble solid content (°Brix) of individual berries were used as discriminant factors. Number of berries (n) is 75 for each class in the V plot; and 67, 61, 64, and 47 berries for GH, GS, PS, and RS respectively for PostV.
**Supplementary Table S3.** Percentages of genes with specific trends of expression from V to PostV ripening stages in high- and low-RV gene sets.

| V to PostV expression* | Low RV set | High RV set |
|------------------------|------------|-------------|
| Up-regulated           | 22         | 40          |
| Down-regulated         | 37         | 22          |
| Plateau                | 41         | 38          |

*: Significance of the V to PostV expression was calculated using two-sided contrast analysis (See Material and Methods; Supplementary worksheet 1B)
**Supplementary Table S4.** Ripening associated genes identified using reduction in variance as the selection criterion

| Gene ID            | Gene Name                                                                 | Tissue | $RV^a$  | $Var^b$ | Synch stage$^c$ |
|--------------------|---------------------------------------------------------------------------|--------|---------|---------|-----------------|
| VIT_12s0034g01920  | No hit                                                                    | Pulp   | 30397   | 4.69    | ES              |
| VIT_01s0011g05930  | S-adenosyl-L-methionine:carboxyl methyltransferase                         | Seed   | 14429   | 11.20   | LS              |
| VIT_12s0057g00940  | Glucose-6-phosphate 1-dehydrogenase 2                                   | Pulp   | 7875    | 0.44    | ES              |
| VIT_10s0003g01830  | Aquaporin NIP1;2                                                         | Skin   | 7467    | 0.78    | ES              |
| VIT_18s0001g14350  | No hit                                                                    | Pulp   | 6608    | 10.81   | LS              |
| VIT_00s0394g00040  | Alliinase EGF                                                             | Pulp   | 6606    | 6.99    | ES              |
| VIT_02s0025g04300  | Thaumatin                                                                 | Seed   | 5844    | 1.56    | LS              |
| VIT_13s0067g00660  | Steroid 23-alpha-hydroxylase                                             | Skin   | 5455    | 0.34    | ES              |
| VIT_07s0104g01710  | HAK5 (High affinity K+ transporter 5)                                     | Pulp   | 5194    | 2.50    | LS              |
| VIT_06s0004g06770  | No hit                                                                    | Skin   | 5085    | 0.38    | LS              |
| VIT_05s0051g00640  | Purple acid phosphatase 23-ATPAP23/PAP23                                 | Skin   | 4698    | 0.13    | LS              |
| VIT_00s0324g00050  | UDP-glucose glucosyltransferase                                           | Skin   | 3570    | 1.16    | ES              |
| VIT_07s0031g02560  | UVB-resistance protein UVR8                                               | Pulp   | 3431    | 1.62    | LS              |
| VIT_18s0001g14340  | No hit                                                                    | Pulp   | 3288    | 5.01    | LS              |
| VIT_13s0067g03820  | Chalcone isomerase                                                        | Pulp   | 3192    | 0.75    | ES              |
| VIT_11s0016g04820  | Agenet domain-containing protein                                         | Skin   | 3189    | 1.01    | ES              |
| VIT_14s0128g00520  | Alpha-L-fucosidase 2 precursor                                           | Pulp   | 2377    | 0.72    | ES              |
| VIT_00s1286g00020  | Elongation factor EF-G                                                   | Pulp   | 2310    | 0.06    | LS              |
| VIT_18s0001g11730  | No hit                                                                    | Skin   | 2133    | 0.66    | LS              |
| VIT_02s0154g00110  | Trehalose-6-phosphate phosphatase                                        | Pulp   | 2061    | 2.57    | ES              |
| VIT_12s0005g00320  | EF hand                                                                   | Pulp   | 1979    | 1.64    | ES              |
| VIT_18s0001g01130  | Alpha-expansin 1 precursor                                               | Seed   | 1943    | 0.69    | LS              |
| VIT_18s0001g00990  | Calmodulin-domain protein kinase 9 CPK9                                   | Pulp   | 1929    | 0.38    | ES              |
| VIT_18s0001g06390  | Auxin-independent growth promoter                                        | Pulp   | 1923    | 0.24    | LS              |
| VIT_15s0046g02400  | Glycerol-3-phosphate acyltransferase 8                                   | Pulp   | 1888    | 1.61    | ES              |
| VIT_07s0005g02370  | Germin-like protein 2 [Vitis vinifera]                                    | Seed   | 1765    | 6.49    | LS              |
| VIT_10s0003g00410  | MLO6 (mildew resistance locus O 6)                                        | Seed   | 1701    | 1.99    | LS              |
| VIT_03s0180g00110  | Stress enhanced protein 1 (SEP1)                                         | Pulp   | 1658    | 0.09    | ES              |
| VIT_03s0063g01290  | Gibberellin 20 oxidase 2                                                  | Pulp   | 1637    | 1.03    | ES              |
| VIT_18s0001g15140  | Unknown                                                                   | Skin   | 1580    | 1.14    | LS              |
| VIT_04s0023g03790  | Jasmonate methyltransferase                                               | Skin   | 1555    | 4.65    | LS              |
| VIT_12s0059g00490  | Unknown                                                                   | Skin   | 1552    | 0.34    | LS              |
| VIT_08s0040g01840  | No hit                                                                    | Pulp   | 1552    | 1.43    | LS              |
| VIT_00s0015g00010  | Cinnamyl alcohol dehydrogenase                                           | Seed   | 1547    | 0.25    | LS              |
| VIT_18s0001g07610  | EMB1674 (embryo defective 1674) kinase interacting family protein         | Pulp   | 1365    | 0.82    | ES              |
| VIT_00s0260g00080  | Ribosomal protein L32 60S                                                 | Skin   | 1292    | 0.05    | ES              |
| VIT_18s0001g11430  | flavonoid 3-monooxygenase                                                 | Seed   | 1291    | 7.37    | LS              |
| VIT_13s0067g02930  | Expansin [Vitis labrusca x Vitis vinifera] EXPA8                          | Seed   | 1277    | 0.67    | LS              |
| VIT_00s0865g00020  | Unknown protein                                                           | Pulp   | 1253    | 3.25    | LS              |
| VIT_07s0031g01930  | Myb TK1 (TSL-kinase 1)                                                    | Skin   | 1185    | 1.22    | LS              |
| VIT_00s0225g00230  | Alliin lyase precursor                                                    | Skin   | 1178    | 5.25    | LS              |
| VIT_13s0106g00280  | CYP79A2                                                                   | Pulp   | 1164    | 8.06    | LS              |
| VIT_12s0134g00160  | Xyloglucan endotransglycosylase/hydrolase                                 | Seed   | 1042    | 0.58    | ES              |
| Gene ID                | Description                                      | Tissue   | LS  | ES  |
|-----------------------|--------------------------------------------------|----------|-----|-----|
| VIT_08s0040g01600     | TCP family transcription factor TCP11            | Pulp     | 1025| 0.92|
| VIT_03s0017g00390     | MADS-box protein SVP                             | Skin     | 960 | 1.35|
| VIT_07s0031g00480     | Protein kinase family                             | Pulp     | 953 | 0.60|
| VIT_04s0023g01750     | TVLP1                                            | Skin     | 942 | 0.07|
| VIT_13s0067g02300     | Hypoxia-responsive                                | Pulp     | 936 | 2.17|
| VIT_06s0004g02560     | Kiwelin Ripening-related protein grip22          | Seed     | 911 | 0.58|
| VIT_11s0016g02530     | Protein arginine N-methyltransferase             | Pulp     | 911 | 0.23|
| VIT_02s0025g04340     | Osmotin                                          | Seed     | 897 | 0.51|
| VIT_18s0089g00160     | 1,4-beta-mannan endohydrolase                    | Skin     | 876 | 3.43|
| VIT_17s0000g06880     | Heparanase protein 2 precursor                   | Pulp     | 873 | 0.30|
| VIT_05s0077g01880     | DAG protein                                      | Pulp     | 869 | 0.10|
| VIT_03s0063g01310     | Oxidoreductase, 2OG-Fe(II) oxygenase             | Pulp     | 861 | 1.99|
| VIT_12s0057g00700     | Glucan endo-1,3-beta-glucosidase 3               | Seed     | 849 | 0.28|
| VIT_05s0051g00830     | Dihydroxy-acid dehydratase                       | Pulp     | 819 | 0.04|
| VIT_00s0684g00030     | SEN1 (dark inducible 1)                          | Pulp     | 815 | 0.66|
| VIT_01s0010g03390     | Gag-pol polyprotein                              | Pulp     | 780 | 0.16|
| VIT_03s0038g04330     | Unknown                                          | Seed     | 760 | 9.28|
| VIT_18s0001g06390     | Auxin-independent growth promoter                | Skin     | 741 | 0.17|
| VIT_03s0038g03410     | NAC domain containing protein 36                 | Pulp     | 737 | 2.05|
| VIT_15s0048g00500     | Pectinesterase family                            | Pulp     | 733 | 3.53|
| VIT_18s0001g06790     | Ripening regulated protein DDTFR18               | Seed     | 726 | 3.90|
| VIT_12s0034g01910     | Cupin family protein                             | Pulp     | 719 | 4.97|
| VIT_01s0011g03670     | Bifunctional nuclease                            | Skin     | 718 | 3.10|
| VIT_19s0015g00140     | Ribosomal protein S10 30S                        | Pulp     | 716 | 0.03|
| VIT_18s0001g11490     | CYP82C1p                                         | Seed     | 706 | 3.67|
| VIT_12s0034g01890     | Cupin region                                     | Pulp     | 702 | 4.90|
| VIT_00s0394g00040     | Alliinase EGF                                     | Skin     | 699 | 6.11|
| VIT_19s0015g02090     | CYP72A59                                         | Seed     | 682 | 0.50|
| VIT_18s0164g00170     | Diphenol oxidase                                  | Seed     | 676 | 2.98|
| VIT_10s0071g00320     | GRAM domain-containing protein / ABA-responsive    | Pulp     | 668 | 0.51|
| VIT_18s0001g00950     | Prolyl 4-hydroxylase alpha-2 subunit             | Pulp     | 660 | 0.16|
| VIT_18s0001g11520     | Flavonoid 3-monoxygenase                         | Seed     | 645 | 4.14|
| VIT_18s0001g15640     | Pathogenesis-related                             | Seed     | 639 | 0.41|
| VIT_06s0004g06030     | Ca2+/calmodulin-regulated receptor kinase        | Pulp     | 638 | 0.15|
| VIT_01s0137g00520     | CYP71B35                                         | Skin     | 635 | 6.48|
| VIT_16s0050g02400     | Ethylene-responsive transcription factor          | Pulp     | 622 | 0.23|
| VIT_16s0115g00120     | Transformation/transcription domain-associated protein | Skin   | 617 | 1.63|
| VIT_14s0066g01710     | Leaf senescence protein                          | Pulp     | 615 | 2.40|
| VIT_12s0142g00110     | Splicing factor, arginine/serine-rich 2          | Skin     | 596 | 0.59|
| VIT_08s0007g04220     | SER/ARG-rich protein kinase 4 SRPK4              | Skin     | 595 | 0.06|
| VIT_00s0276g00030     | No hit                                           | Skin     | 592 | 0.84|
| VIT_02s0025g04340     | Osmotin                                          | Pulp     | 584 | 2.80|
| VIT_05s0020g03200     | Spermine synthase                                | Pulp     | 582 | 4.63|
| VIT_18s0001g14340     | No hit                                           | Skin     | 572 | 5.17|
| VIT_06s0080g00920     | Photosystem I subunit O (PSAO)                   | Pulp     | 570 | 3.51|
| VIT_12s0034g01900     | Globulin-like protein                            | Pulp     | 566 | 5.27|
| VIT_02s0025g03590     | Phospholipid hydroperoxide glutathione peroxidase| Pulp     | 561 | 0.37|
| VIT_18s0086g00680     | No hit                                           | Pulp     | 559 | 6.13|
| VIT_18s0086g00320     | No hit                                           | Pulp     | 549 | 4.30|
| VIT_14s0068g01400     | UPF0497 family                                   | Pulp     | 545 | 6.66|
Top 100 genes ranked in the order of their reduction in expression variance$^a$ (RV) from *véraison* to maturity among the berry classes. Marked in red are the 15% of the genes that were identified in the top 100 based on their *véraison to maturity*-expression differences$^b$ (Var) alone. Synch stage$^c$ denotes the timing of synchronization during the ripening progress assessed from *véraison* to maturity expression trend as early *véraison* stage (ES) and late maturity stage (LS) synchronizing genes (Supplementary worksheet 1B). RV is calculated as the ratio between *véraison*- and maturity-stage expression variances. Very high numbers for RV numbers are due to very low to zero variance at maturity stage.
**Supplementary Table S5.** Ranges of TSS and color index values in GS and RS berry classes at equivalent ripening stages

| Ripening stage | TSS range  | Color index range |
|----------------|------------|-------------------|
| R1             | 11.5-12.5  | 3.2-3.3           |
| R2             | 13.9-14.1  | 4.3-4.4           |
| R3             | 16.0-17.2  | 5.0-5.5           |
| R4             | 18.1-18.5  | 5.8-6.0           |

R1 to R4 are the physiological stages of similar ripening levels across all the berry classes. Total soluble solids and color index levels of mid-véraison-RS, and 1, 2, 3 weeks past mid-véraison, act as reference. Table shows the range of differences in the total soluble solid (brix) and color index for green and red soft berries at each considered stage. Under-ripe green berries were selected when they reach the closest reference levels for the purpose of comparing sugar and pigment accumulation rates, hormone levels, and gene expression dynamics between both the berry classes.
**Supplementary Table S6.** Component loadings of the principle component analyses.

A.

Eigen vector contributions to component scores of Fig. 1

**Mid-véraison PCA**

| Vector | Component 1 | Component 2 | Component 3 | Component 4 |
|--------|-------------|-------------|-------------|-------------|
| Brix   | -0.50593    | 0.21194     | 0.82006     | 0.16316     |
| L      | 0.50285     | -0.44850    | 0.52883     | -0.51607    |
| C      | 0.46948     | 0.85782     | 0.10404     | -0.18142    |
| h      | 0.52035     | -0.13448    | 0.19242     | 0.82106     |
| Eigen value | 86.1%    | 7.79%       | 3.47%       | 1.75%       |
|        | (<0.0001*) | (<0.0001*)  | (<0.0001*)  | (NS)        |

**Five week post-véraison PCA**

| Vector | Component 1 | Component 2 | Component 3 | Component 4 |
|--------|-------------|-------------|-------------|-------------|
| Brix   | -0.58008    | 0.25479     | 0.34371     | 0.69315     |
| L      | 0.31605     | 0.87731     | 0.29713     | -0.20532    |
| C      | 0.58489     | 0.09655     | -0.44204    | 0.67319     |
| h      | 0.47065     | -0.39509    | 0.77342     | 0.15560     |
| Eigen value | 44.1%       | 23.9%       | 18.7%       | 13.3%       |
|        | (<0.0001*)  | (<0.001*)   | (<0.0308*)  | (NS)        |
### B. Eigen vector contributions to component scores of Fig. 4 PCAs

#### Mid-véraison, Day 0 PCA

| Vector     | Component 1 | Component 2 | Component 3 |
|------------|-------------|-------------|-------------|
| Elasticity | -0.30653    | 0.95175     | 0.01455     |
| Brix       | 0.67167     | 0.22710     | -0.70518    |
| Color Index| 0.67446     | 0.20638     | 0.70888     |
| Eigen value| 60.5% (<0.0001*) | 27.2% (<0.0001*) | 6.4% (NS) |

#### Week 1 PCA

| Vector     | Component 1 | Component 2 | Component 3 |
|------------|-------------|-------------|-------------|
| Brix       | 0.59788     | 0.47081     | -0.64875    |
| Elasticity | -0.49845    | 0.85220     | 0.15910     |
| Color Index| 0.62777     | 0.22825     | 0.74418     |
| Eigen value| 69.6% (<0.0001*) | 21.8% (<0.0001*) | 8.6% (NS) |

#### Week 2 PCA

| Vector     | Component 1 | Component 2 | Component 3 |
|------------|-------------|-------------|-------------|
| Brix       | 0.70531     | 0.03165     | 0.70819     |
| Elasticity | -0.11115    | 0.99158     | 0.06638     |
| Color Index| 0.70013     | 0.12553     | -0.70290    |
| Eigen value| 50.8% (<0.0001*) | 33.2% (<0.0005*) | 16.0% (NS) |

#### Week 3 PCA

| Vector     | Component 1 | Component 2 | Component 3 |
|------------|-------------|-------------|-------------|
| Brix       | 0.65973     | -0.08929    | 0.74618     |
| Elasticity | -0.57865    | 0.57318     | 0.58020     |
| Color Index| 0.47950     | 0.81455     | -0.32648    |
| Eigen value| 53.8% (<0.0001*) | 28.8% (<0.0267*) | 17.4% (NS) |
| Week 5 PCA | Vector | Component 1 | Component 2 | Component 3 |
|-----------|--------|-------------|-------------|-------------|
| Elasticity | -0.70732 | -0.00252   | 0.70689     |
| Brix      | 0.68926  | -0.22443   | 0.68888     |
| Color Index | 0.15691 | 0.97449    | 0.16048     |
| Eigen value | 46.8% (<0.0049*) | 33.4% (<0.0436*) | 19.8% (NS) |
| Week 6 PCA | Vector | Component 1 | Component 2 | Component 3 |
| Brix      | 0.66821  | -0.24894   | 0.70109     |
| Elasticity | 0.31002  | 0.94981    | 0.04178     |
| Color Index | 0.67630 | -0.18944   | -0.71185    |
| Eigen value | 36.8% (NS) | 32.1% (NS) | 31.1% (NS) |

Minimum weight of the vector scores to each component is zero. In the last row of each table, Eigen values for each component are given in percentage. p-values in parenthesis in the last row indicate the significance for variance between eigen values.
Supplementary Fig. 1. PCA plot of skin, pulp, and seed tissues of different berry classes from mid-véraison (V) and post-véraison (H) maturity stages according to their normalized expression. The assigned are Green for green soft; red for pink soft; and purple for red soft berries in each tissue and sampling times V and H.
**Supplemental Figure S2.** Relationship between ‘level of expression’ and ‘expression variance among berry classes’ at mid-\textit{véraison} (V) and 5-weeks post-\textit{véraison} (PostV). Genes with expression value below and above 11 were grouped into (A and C) low (1505 genes) and (B and D) high (1694 genes) expression sets, respectively, and distribution of their expression variance is plotted in each set. Whiskers indicate the range of expression variance and the boxes indicate the 25, 50 and 75 percentile distribution with outliers shown as dots.
Supplementary Figure S3. Increases in sugar (A) and pigment (B) accumulation monitored at seven-day intervals from mid-véraison (0) until maturity (day 42) in GH, GS, PS, and RS berries. Data are mean of six plants where five berries per ripening class were sampled from five clusters of each plant. Errors bars represent ± SEM. R1, R2, and R3 represent reference stages at which total soluble solids (Brix)/color index across the four berry classes were similar and berries of all classes were at common physiological ripening stages. The width of the color blocks at the bottom part of the plots shows the length of time for each berry class to progress from R1 to R3 ripening stages.
Supplementary Figure S4. Ripening progress in GH, GS, and PS berry classes in relation to that of the RS berry class. A to C, Principle component analyses of the total soluble solid content, color parameters, and elasticity of individual GH (A), GS (B), or PS (C) berries (n = 30) at mid-véraison (V) and at one, two, or three weeks past-V compared to the corresponding data for RS berries (n = 30). Purple color denotes RS berries, and dark green, light green, and orange colors represent GH, GS, and PS berries, respectively. The stages V and 1, 2, and 3-weeks past-V are indicated with plus mark, closed circles, open circles, and closed squares, respectively. D to G, Discriminant analyses showing the ripening advancement of individual berries within a class. Numbers in the columns are the percentage of berries assigned to the corresponding times based on berry ripening parameters, Color Index, total soluble solid content, and Elasticity.