

# Supplements

## **Early metabolic and transcriptional variations in fruit of natural white-fruited *Fragaria vesca* genotypes**

**Katja Härtl<sup>1</sup>, Alisandra Denton<sup>2</sup>, Katrin Franz-Oberdorf<sup>1</sup>, Thomas Hoffmann<sup>1</sup>, Melanie Spornraft<sup>3</sup>, Björn Usadel<sup>2</sup>, Wilfried Schwab<sup>1\*</sup>**

<sup>1</sup> Biotechnology of Natural Products, Technische Universität München, Liesel-Beckmann-Str. 1, 85354 Freising, Germany

<sup>2</sup> Institute for Biology I, RWTH Aachen University, Worringer Weg 3, 52074 Aachen, Germany

<sup>3</sup> Physiology Weihenstephan, TU München, Weihenstephaner Berg 3, 85354 Freising, Germany

# Tables

| <b>Table S1: RIN – values</b> |                     |                |            |     |
|-------------------------------|---------------------|----------------|------------|-----|
| ID                            | Strawberry Variety  | Ripening Stage | Tissue     | RIN |
| 1                             | “Reine des Vallees” | ripe           | Receptacle | 8.5 |
| 2                             |                     |                | Achenes    | 6.2 |
| 3                             |                     | intermediate   | Receptacle | 8.5 |
| 4                             |                     |                | Achenes    | 7.0 |
| 5                             |                     | green          | Receptacle | 8.7 |
| 6                             |                     |                | Achenes    | 7.1 |
| 7                             | “Yellow Wonder”     | ripe           | Receptacle | 8.7 |
| 8                             |                     |                | Achenes    | 8.3 |
| 9                             |                     | intermediate   | Receptacle | 8.3 |
| 10                            |                     |                | Achenes    | 7.2 |
| 11                            |                     | green          | Receptacle | 7.0 |
| 12                            |                     |                | Achenes    | 8.3 |
| 13                            | “Hawaii 4”          | ripe           | Receptacle | 9.3 |
| 14                            |                     |                | Achenes    | 7.9 |
| 15                            |                     | intermediate   | Receptacle | 8.6 |
| 16                            |                     |                | Achenes    | 7.5 |
| 17                            |                     | green          | Receptacle | 8.3 |
| 18                            |                     |                | Achenes    | 7.7 |

**Table S2: Sequencing Results;** Sequences are demultiplexed according to the 6 bp index code with 0 mismatch allowed. “Yield (Mbp)” specifies the number of bases called in mega bases. All reads have passed the Illumina chastity filter. “%Q30” represents the percentage of bases with a quality score of at least 30 (inferred base call accuracy of 99.9%). The Q-score is a prediction of the probability of a wrong base call.

| Lane | ID | Index  | Yield (Mbp)     | #Cluster             | %Q30  | Mean Q |
|------|----|--------|-----------------|----------------------|-------|--------|
| 2    | 1  | ATCACG | 1,921           | 19,205,021           | 95.49 | 36.97  |
| 2    | 2  | CGATGT | 1,181           | 11,811,431           | 96.66 | 37.32  |
| 2    | 3  | TTAGGC | 1,264           | 12,635,961           | 96.18 | 37.20  |
| 2    | 4  | TGACCA | 1,454           | 14,539,863           | 95.61 | 36.97  |
| 2    | 5  | ACAGTG | 2,058           | 20,584,618           | 96.66 | 37.38  |
| 2    | 6  | GCCAAT | 1,156           | 11,556,879           | 96.58 | 37.34  |
| 2    | 7  | CAGATC | 1,350           | 13,500,409           | 96.56 | 37.31  |
| 2    | 8  | ACTTGA | 1,932           | 19,322,039           | 95.86 | 37.04  |
| 2    | 9  | GATCAG | 1,568           | 15,681,771           | 96.39 | 37.28  |
| 2    |    |        | $\Sigma$ 13,884 | $\Sigma$ 138,837,992 |       |        |
| 3    | 10 | ATCACG | 1,200           | 11,997,650           | 97.23 | 37.53  |
| 3    | 11 | CGATGT | 1,086           | 10,863,231           | 97.41 | 37.59  |
| 3    | 12 | TTAGGC | 1,180           | 11,798,844           | 97.24 | 37.53  |
| 3    | 13 | TGACCA | 1,307           | 13,072,075           | 97.43 | 37.67  |
| 3    | 14 | ACAGTG | 1,217           | 12,167,220           | 97.14 | 37.49  |
| 3    | 15 | GCCAAT | 1,237           | 12,366,260           | 97.02 | 37.49  |
| 3    | 16 | CAGATC | 1,255           | 12,545,408           | 97.13 | 37.45  |
| 3    | 17 | ACTTGA | 1,321           | 13,210,822           | 95.82 | 36.98  |
| 3    | 18 | GATCAG | 1,272           | 12,722,858           | 97.35 | 37.58  |
| 3    |    |        | $\Sigma$ 11,075 | $\Sigma$ 110,744,368 |       |        |
|      |    |        | $\Sigma$ 24,959 | $\Sigma$ 249,582,360 |       |        |

**Table S3: Read Mapping Results**

| ID                   | Input      | Mapped               | Rate [%]      |
|----------------------|------------|----------------------|---------------|
| 1                    | 19,127,187 | 14,995,534           | 78.4          |
| 2                    | 11,765,186 | 10,191,209           | 86.6          |
| 3                    | 12,584,685 | 10,251,152           | 81.5          |
| 4                    | 14,481,463 | 10,963,855           | 75.7          |
| 5                    | 20,505,771 | 17,115,834           | 83.5          |
| 6                    | 11,512,157 | 9,757,056            | 84.8          |
| 7                    | 13,450,559 | 10,700,255           | 79.6          |
| 8                    | 19,244,492 | 15,093,497           | 78.4          |
| 9                    | 15,620,144 | 12,971,680           | 83.0          |
| 10                   | 11,964,382 | 10,300,023           | 86.1          |
| 11                   | 10,831,642 | 9,786,653            | 90.4          |
| 12                   | 11,763,556 | 10,621,766           | 90.3          |
| 13                   | 13,032,881 | 11,963,684           | 91.8          |
| 14                   | 12,131,564 | 10,711,818           | 88.3          |
| 15                   | 12,328,783 | 10,643,386           | 86.3          |
| 16                   | 9,402,323  | 8,214,526            | 87.4          |
| 17                   | 13,170,437 | 8,933,261            | 67.8          |
| 18                   | 12,686,615 | 11,673,334           | 92.0          |
| $\Sigma$ 245,603,827 |            | $\Sigma$ 204,888,523 | $\Sigma$ 83.4 |

**Table S4:** Levels of secondary metabolites in per mil equivalents of the dry weight (% equ. dw.) measured in **achene** tissues of strawberry fruits in green, intermediate and ripe developmental stage.

| Metabolite                      | RdV<br>green<br>Ac | RdV<br>inter<br>Ac | RdV<br>ripe<br>Ac | YW<br>green<br>Ac | YW<br>inter<br>Ac | YW<br>ripe<br>Ac | HW4<br>green<br>Ac | HW4<br>inter<br>Ac | HW4<br>ripe<br>Ac |
|---------------------------------|--------------------|--------------------|-------------------|-------------------|-------------------|------------------|--------------------|--------------------|-------------------|
| Gallic acid                     | 0.08±0.01          | 0.04±0.01          | 0.04±0.02         | 0.07±0.03         | 0.02±0.02         | 0.02±0.01        | 0.06±0.02          | 0.05±0.01          | 0.04±0.01         |
| Gallic acid glucose ester       | 0.17±0.03          | 0.14±0.03          | 0.16±0.08         | 0.19±0.08         | 0.06±0.04         | 0.09±0.02        | 0.21±0.06          | 0.16±0.03          | 0.12±0.03         |
| Ellagic acid                    | 1.80±0.69          | 1.28±0.30          | 1.38±0.93         | 1.83±0.93         | 1.56±0.44         | 1.77±0.61        | 1.34±0.59          | 1.92±0.35          | 1.70±0.44         |
| Cinnamic acid glucose ester     | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         | 0.00±0.00         | 0.00±0.00         | 0.00±0.00        | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         |
| 4-Coumaric acid glucose ester   | 0.00±0.00          | 0.00±0.00          | 0.07±0.02         | 0.00±0.00         | 0.00±0.00         | 0.00±0.00        | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         |
| Caffeic acid glucose ester      | 0.00±0.00          | 0.00±0.00          | 0.05±0.01         | 0.00±0.00         | 0.02±0.00         | 0.04±0.00        | 0.00±0.00          | 0.00±0.00          | 0.03±0.01         |
| Quercetin glucuronide           | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         | 0.00±0.00         | 0.00±0.00         | 0.00±0.00        | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         |
| Kaempferol glucuronide          | 1.37±0.15          | 1.73±0.34          | 1.85±0.28         | 1.17±0.33         | 1.77±0.53         | 1.82±0.31        | 1.05±0.25          | 1.62±0.21          | 1.66±0.38         |
| Kaempferol glucoside            | 0.24±0.01          | 0.38±0.12          | 0.37±0.09         | 0.12±0.05         | 0.11±0.12         | 0.20±0.16        | 0.14±0.05          | 0.22±0.03          | 0.21±0.08         |
| Catechin                        | 0.25±0.08          | 0.37±0.15          | 0.28±0.15         | 0.14±0.05         | 0.14±0.07         | 0.30±0.06        | 0.25±0.04          | 0.19±0.07          | 0.18±0.04         |
| Epicatechin catechin dimers     | 0.70±0.01          | 0.88±0.22          | 0.71±0.22         | 0.50±0.12         | 0.44±0.06         | 0.99±0.19        | 0.55±0.13          | 0.46±0.11          | 0.52±0.17         |
| Epiafzelechin catechin dimers   | 0.42±0.04          | 0.51±0.15          | 0.48±0.16         | 0.27±0.09         | 0.25±0.04         | 0.59±0.14        | 0.37±0.14          | 0.34±0.07          | 0.40±0.16         |
| Pelargonidin glucoside          | 0.00±0.00          | 0.21±0.06          | 4.50±1.78         | 0.00±0.00         | 0.00±0.00         | 0.00±0.00        | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         |
| Pelargonidin glucoside malonate | 0.00±0.00          | 0.33±0.11          | 3.85±1.57         | 0.00±0.00         | 0.00±0.00         | 0.00±0.00        | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         |
| Cyanidin glucoside              | 0.00±0.00          | 0.38±0.10          | 3.26±1.35         | 0.00±0.00         | 0.00±0.00         | 0.00±0.00        | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         |

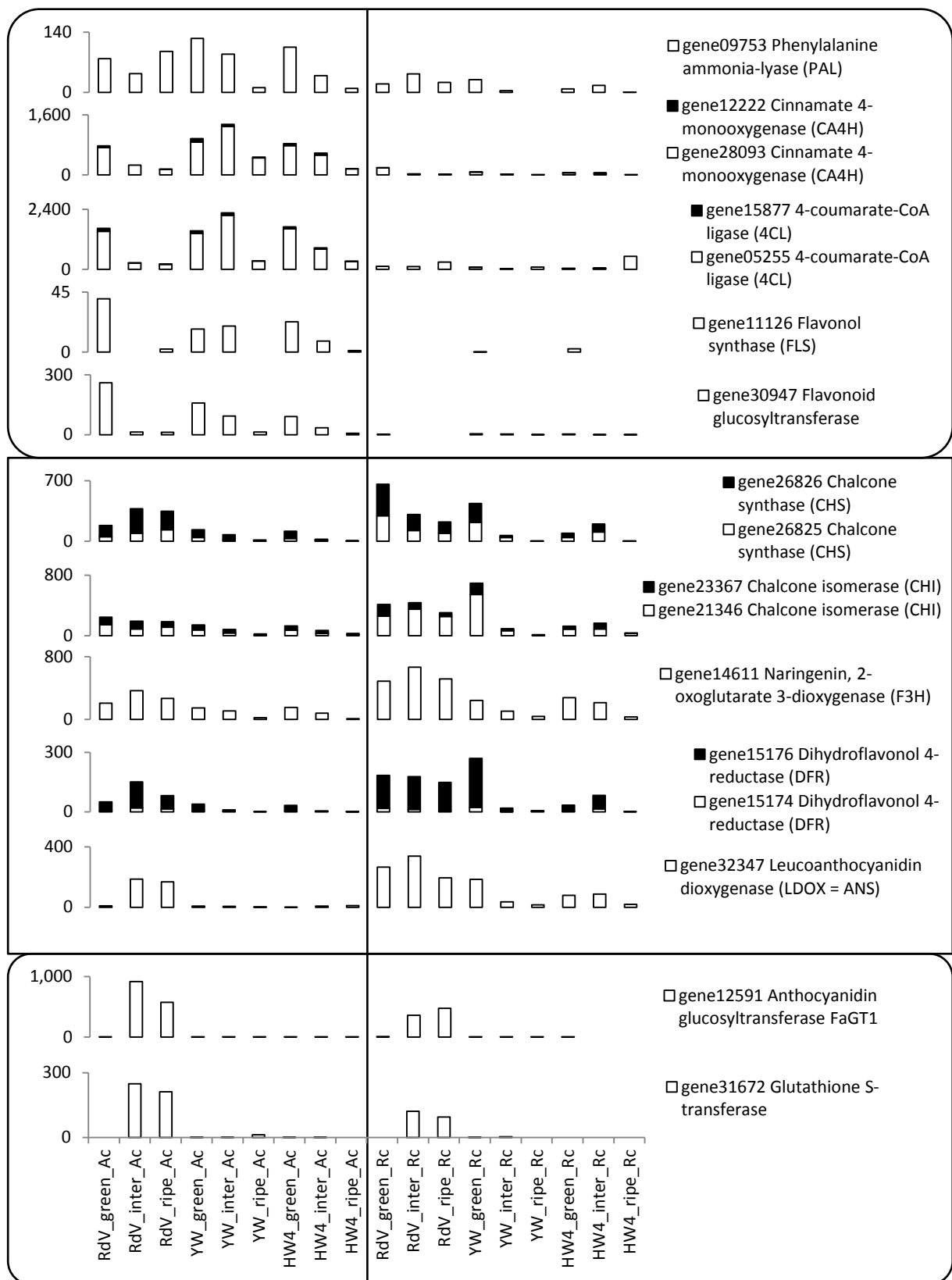
**Table S5:** Levels of secondary metabolites in per mil equivalents of the dry weight (% equ. dw.) measured **in receptacle** tissues of strawberry fruits in green, intermediate and ripe developmental stage.

| Metabolite                      | RdV<br>green<br>Rc | RdV<br>inter<br>Rc | RdV<br>ripe<br>Rc | YW<br>green<br>Rc | YW<br>inter<br>Rc | YW<br>ripe<br>Rc | HW4<br>green<br>Rc | HW4<br>inter<br>Rc | HW4<br>ripe<br>Rc |
|---------------------------------|--------------------|--------------------|-------------------|-------------------|-------------------|------------------|--------------------|--------------------|-------------------|
| Gallic acid                     | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         | 0.00±0.00         | 0.00±0.00         | 0.00±0.00        | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         |
| Gallic acid glucose ester       | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         | 0.00±0.00         | 0.00±0.00         | 0.00±0.00        | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         |
| Ellagic acid                    | 0.13±0.02          | 0.09±0.02          | 0.28±0.05         | 0.30±0.03         | 0.18±0.02         | 0.24±0.07        | 0.27±0.04          | 0.17±0.03          | 0.24±0.05         |
| Cinnamic acid glucose ester     | 0.00±0.00          | 0.01±0.00          | 0.15±0.02         | 0.00±0.00         | 0.00±0.00         | 0.04±0.00        | 0.00±0.00          | 0.00±0.00          | 0.05±0.01         |
| 4-Coumaric acid glucose ester   | 0.00±0.00          | 0.04±0.01          | 0.59±0.13         | 0.00±0.00         | 0.00±0.00         | 0.00±0.00        | 0.00±0.00          | 0.00±0.00          | 0.05±0.01         |
| Caffeic acid glucose ester      | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         | 0.00±0.00         | 0.00±0.00         | 0.00±0.00        | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         |
| Quercetin glucuronide           | 0.15±0.03          | 0.10±0.02          | 0.25±0.02         | 0.24±0.03         | 0.15±0.01         | 0.10±0.01        | 0.18±0.04          | 0.13±0.03          | 0.11±0.02         |
| Kaempferol glucuronide          | 0.48±0.11          | 0.45±0.03          | 0.59±0.15         | 1.12±0.15         | 0.69±0.02         | 0.63±0.09        | 0.80±0.13          | 0.55±0.05          | 0.58±0.05         |
| Kaempferol glucoside            | 0.03±0.01          | 0.03±0.00          | 0.08±0.02         | 0.09±0.04         | 0.16±0.20         | 0.22±0.16        | 0.05±0.01          | 0.04±0.01          | 0.04±0.01         |
| Catechin                        | 1.83±0.24          | 1.71±0.33          | 1.59±0.31         | 2.75±0.45         | 1.72±0.09         | 1.49±0.27        | 2.78±0.44          | 1.92±0.11          | 1.58±0.20         |
| Epicatechin catechin dimers     | 4.60±0.42          | 6.30±0.93          | 5.13±0.67         | 8.03±1.05         | 5.75±0.17         | 4.88±0.83        | 6.11±0.60          | 6.71±0.53          | 4.77±0.30         |
| Epiafzelechin catechin dimers   | 0.64±0.08          | 1.00±0.14          | 0.68±0.10         | 1.09±0.23         | 0.81±0.03         | 0.34±0.08        | 0.73±0.05          | 0.96±0.09          | 0.55±0.08         |
| Pelargonidin glucoside          | 0.00±0.00          | 0.04±0.01          | 4.68±0.51         | 0.00±0.00         | 0.00±0.00         | 0.00±0.00        | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         |
| Pelargonidin glucoside malonate | 0.00±0.00          | 0.00±0.00          | 0.14±0.03         | 0.00±0.00         | 0.00±0.00         | 0.00±0.00        | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         |
| Cyanidin glucoside              | 0.00±0.00          | 0.08±0.03          | 3.16±0.35         | 0.00±0.00         | 0.00±0.00         | 0.00±0.00        | 0.00±0.00          | 0.00±0.00          | 0.00±0.00         |

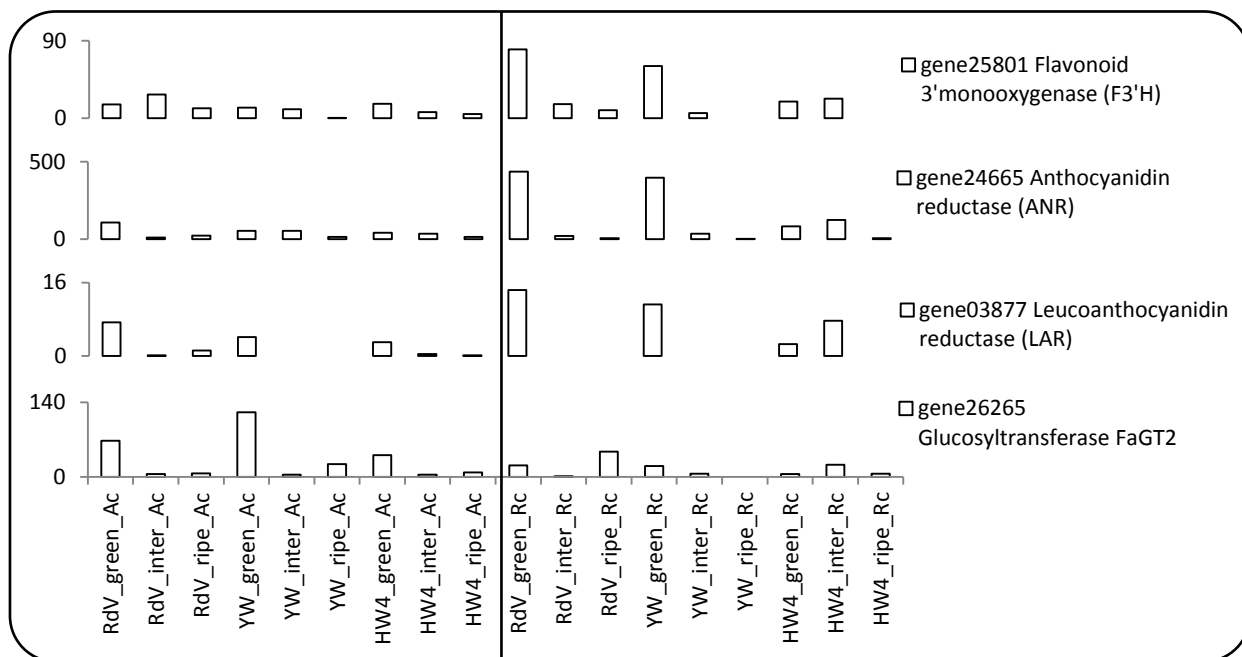
**Table S6:** RT-PCR primers

| Gene           | Nomenclature | Sequence               | Reference |
|----------------|--------------|------------------------|-----------|
| <i>FvGT1</i>   | fw           | CACTCACGCCGCCCCACTG    | 27        |
|                | rev          | CACACCATCCGCCACCTCACAC |           |
| <i>FvMYB10</i> | fw           | TCAAATCAGGCTTAAACAGA   | 54        |
|                | rev          | TTAAAGACCACCTGTTTCCT   |           |
| <i>FvUBC9</i>  | fw           | ATCTGCTCACTGTTGACGGA   | 54        |
|                | rev          | AGCTCCTTGCTGTTGTCTCA   |           |

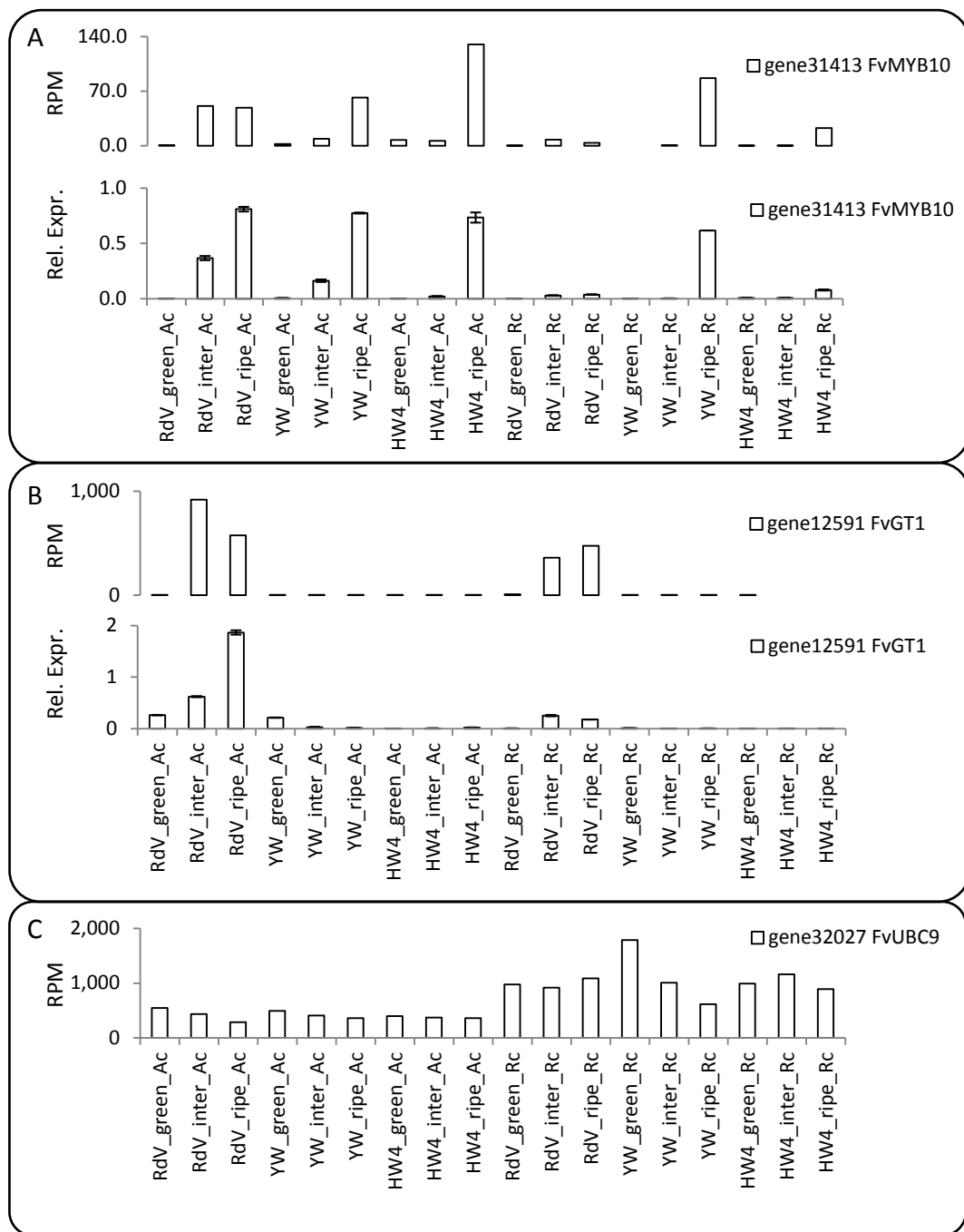
# Figures



**Figure S1:** Transcript levels (normalized RPM) of genes encoding enzymes involved in flavonoid/anthocyanin formation in receptacle (Rc) and achenes (Ac) of *F. vesca* cv. RdV, YW, and HW4 at the green, intermediate and ripe developmental stage.



**Figure S2:** Transcript levels (normalized RPM) of genes encoding enzymes involved in proanthocyanidin, and  $\beta$ -glucogallin formation in receptacle (Rc) and achenes (Ac) of *F. vesca* cv. RdV, YW, and HW4 at the green, intermediate and ripe developmental stage.



**Figure S3:** Comparison of gene transcript levels determined by RNA sequencing (RPM) and RT-PCR (relative Expression, Rel. Expr.) for *MYB10* (A) and *GT1* (B), and RPM of the reference gene *UBC9* (C) in receptacle (Rc) and achenes (Ac) of *F. vesca* RdV, YW, and HW4 at the green, intermediate, and ripe developmental stage.