# Transcriptional survey of the light-induced anthocyanin pathway in non-GM purple tomatoes

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## Supplementary Material

**Table S1.** List of the oligonucleotide primers used for qPCR analyses.

| Gene Name | Primers_ID | Forward primer | Reverse primer |
|---|---|---|---|
| *Solyc06g005060* | *EF1a* | GCTGCTGTAACAAGATGGATGC | GGGGATTTTGTCAGGGTTGTAA |
| *Solyc04g071610* | *ASR1* | TTGCTGACCGTATGAGCAAG | GGACAATGGATGGACCAGAC |
| *Solyc09g007890* | *PAL5* | ATTGGGAAATGGCTGCTGATT | TCAACATTTGCAATGGATGCA |
| *Solyc10g086180* | *PAL10* | GGGACGGAATTGTTGACAGGA | CTAACAGACTGGTAGTGGAGCACCA |
| *Solyc06g068650* | *4CL1* | GCATTGGAGAATGGTGTGAA | CTCATCGGCCTGAATCAACT |
| *Solyc03g117870* | *4CL2* | TGGAGACGGTACCGAAATCTCC | GCAGCCAGTCTAGCTCTTAAGTC |
| *Solyc09g091510* | *CHS1* | TGGTCACCGTGGAGGAGTATC | GATCGTAGCTGGACCCTCTGC |
| *Solyc05g053550* | *CHS2* | GGCGACTCCTTCGAACTGTGTTG | ATCCAAAGAAGGAGCCATGTATTCAC |
| *Solyc05g010320* | *CHI* | CAGTTCCTTGTGTTGGGAATGCTG | TGGAATCCAGCACTGCTTCTGAC |
| *Solyc05g052240* | *CHI-like* | TGAGGCTATTGTGAATGCTCCAG | TAGCACTCTCTAGCTGCACACC |
| *Solyc02g083860* | *F3H* | CACACCGATCCAGGAACCAT | GCCCACCAACTTGGTCTTGTA |
| *Solyc03g115220* | *F3'H* | GCACCACGAATGCACTTGC | CGTTAGTACCGTCGGCGAAT |
| *Solyc11g066580* | *F3'5'H* | GGCAATTGGACGAGATCCTG | AAGGAACCTCTCGGGAGTGAA |
| *Solyc11g013110* | *FLS* | GAGCATGAAGTTGGGCCAAT | TGGTGGGTTGGCCTCATTAA |
| *Solyc02g085020* | *DFR* | TCCGAAGACGACAACGGTTT | TGACAAGCCAAGAGCCGATAA |
| *Solyc08g080040* | *ANS* | GAACTAGCACTTGGCGTCGAA | TTGCAAGCCAGGCACCATA |
| *Solyc10g083440* | *UFGT* | CGAACGACGAAACACTGTTGA | TGCAGCATAGATGGCATTGG |
| *Solyc01g079620* | *MYB12* | ATGCCGGATTATTGAGATGCG | GCTATCAACTTTTCGACTTAGATGAGAG |
| *Solyc10g086290* | *AN2-like* | ATATTGCCAAGACATTGGGAGTG | CCATACTTGTCAATACATTTCCTCA |
| *Solyc08g081140* | *JAF13* | TCAGGGGATCACTACCGAAC | TCCCATCAAGGTTGGAAGAC |
| *Solyc09g065100* | *AN1* | CTAAGAGTGCCCGCATACAGAC | ATCCGAAGTGGAGTGCTCAGATA |
| *Solyc03g097340* | *AN11* | ATGAAGTGGAGCCGAGAAGA | TCCATCAGCAGAAACAGA |
| *Solyc07g052490* | *MYB-ATV* | GATTGGAATAGATCAAGCACATCA | TTCGTTGGTAGTCTCTAATGCAAC |
| *Solyc12g005800* | *MYB-ATV-like* | CCTTCATAGACTCCCCTCCATTTG | GACCACCTCTCACCAACCAAGTT |
| *Solyc10g055410* | *THM27* | ACAAGTGGTCGCTTATAGCAGGAA | TCATGAGCAGCAGCAAAAGTAATC |
| *Solyc05g008250* | *MYB76* | CTGCTGGCCTGCTTCGTTGT | CCGTTCGTCCTGGCAATCTTC |
| *Solyc10g084380* | *WRKY44* | TGGAGAGAGCTCTTCACAGGTTGG | CCTTGGTGCAAGTTTAGCTATGGG |
| *Solyc03g120620* | *GL2* | AGGAAATGCAGTCTCAATCCAAGC | TGCATGCCTGCAATGTCCAC |
| *Solyc02g077790* | *ERF.G3-like* | TAAGGATGTCTGGCGCGGAATG | AGGAAAATTTACAGTCCGAGGGT |
| *Solyc05g018620* | *UVR8* | GGGAAGAGGTACAAATGGTCAGC | ATCCTTGGGACAGACCTATCAACG |
| *Solyc04g074180* | *CRY1a* | TCCACAGTTTGTGGGATACAAGTG | CCATGGATGGTGTATCCATTCGG |
| *Solyc12g057040* | *CRY1b* | TTCGACGATGGCTTCCTGAACTTG | CCCATGGGTGGTGTATCCATTCTG |
| *Solyc09g090100* | *CRY2* | GGGATCGTTTAATGCAAGCTATAATT | CGAGTTATCAAACACAACTTCAACAG |
| *Solyc08g074270* | *CRY-DASH* | TACTTCAGCATTCCGAAGCAAGC | TCCATGTTTGAGAGCTACGATTGG |
| *Solyc10g044670* | *PhyA* | ATCTGTGAACGCTACACCAAGTG | TCCTGAATTCCAAGAGAGCAAGC |
| *Solyc01g059870* | *PhyB1* | TGCCAATATCTGATGGAGCAACTG | TTCGCCAGGGCATATAATCCTGAG |
| *Solyc05g053410* | *PhyB2* | GCTTGTGAGAGGCAGATGTCCAAG | ACCTTTCTCAAGCTCCAGGGAACC |
| *Solyc08g061130* | *HY5* | GGACAACGTCAGCTTCTGGAAGAG | TGAGTCCCAGCTGATGGTTGAG |
| *Solyc12g005950* | *COP1 homolog* | AGAGCATGAGAAACGGGCTTGG | TCGTGCACCAAACTTTGACCTTAC |
| *Solyc11g011980* | *COP1like isoform X1* | TGAGCTAGCTTCTGCATCAACCG | TGTCCTTTAAACGTACGCAGTGG |
| *Solyc11g005190* | *RUP* | CCGATGACGGAACCATGCAAATG | AGCTAAACACTTGCCACTGTCACC |
| *Solyc12g089240* | *BBX20* | GTTTACTCCAAGAATATTGGTGATGA | TGGTGGAAATGAACTAAATTGACTC |
| *Solyc04g081020* | *BBX21* | AGCAGCTTCATGTAGTGCGAATC | TGCTACTGACACTTGTTGTTGAGG |
| *Solyc07g062160* | *BBX22* | TGGATCATCTAAGGCGGATAATG | AGCACTCATCACCATCTAGTTCTTC |
| *Solyc06g073180* | *BBX24* | GATACAGTGTGATGTGTGTGAGAAAG | GTTAGATAGGCACTGAAGATGAAGC |

**Table S2.** List of the genes analyzed in the study containing relative category and function.

| Name and abbreviation | Category | Function |
|---|---|---|
| *Phenylalanine ammonia lyase 5 (PAL5)* | Structural gene | Enzyme of the phenylpropanoid biosynthetic pathway |
| *Phenylalanine ammonia lyase 10 (PAL10)* | Structural gene | Enzyme of the phenylpropanoid biosynthetic pathway |
| *4-coumaryol CoA ligase 1 (4CL1)* | Structural gene | Enzyme of the phenylpropanoid biosynthetic pathway |
| *4-coumaryol CoA ligase 2 (4CL2)* | Structural gene | Enzyme of the phenylpropanoid biosynthetic pathway |
| *Chalcone synthase 1 (CHS1)* | Structural gene | Early enzyme of the flavonoid biosynthetic pathway |
| *Chalcone synthase 2 (CHS2)* | Structural gene | Early enzyme of the flavonoid biosynthetic pathway |
| *Chalcone isomerase (CHI)* | Structural gene | Early enzyme of the flavonoid biosynthetic pathway |
| *Chalcone isomerase-like (CHI-like)* | Structural gene | Early enzyme of the flavonoid biosynthetic pathway |
| *Flavanone-3-hydroxylase (F3H)* | Structural gene | Early enzyme of the flavonoid biosynthetic pathway |
| *Flavanone 3'-hydroxylase (F3'H)* | Structural gene | Early enzyme of the flavonoid biosynthetic pathway |
| *Flavanone 3'5'-hydroxylase (F3'5'H)* | Structural gene | Early enzyme of the flavonoid biosynthetic pathway |
| *Flavonol synthase (FLS)* | Structural gene | Late enzyme of the flavonoid biosynthetic pathway |
| *Dihydroflavonol 4-reductase (DFR)* | Structural gene | Late enzyme of the flavonoid biosynthetic pathway |
| *Anthocyanidin synthase (ANS)* | Structural gene | Late enzyme of the flavonoid biosynthetic pathway |
| *UDP glucose flavonoid 3-O-glucosyltransferase (UFGT)* | Structural gene | Late enzyme of the flavonoid biosynthetic pathway |
| *MYB12* | Regulatory gene | Activator of early biosynthetic genes |
| *Anthocyanin 2-like (AN2-like)* | Regulatory gene | Activator of late biosynthetic genes and component of the MBW complex |
| *John and Francesca 13 (JAF13)* | Regulatory gene | Activator of late biosynthetic genes and component of the MBW complex |
| *Anthocyanin 1 (AN1)* | Regulatory gene | Activator of late biosynthetic genes and component of the MBW complex |
| *Anthocyanin 11 (AN11)* | Regulatory gene | Activator of late biosynthetic genes and component of the MBW complex |
| *MYB-atroviolacea (MYB-ATV)* | Regulatory gene | Repressor of late biosynthetic genes |
| *MYB-atroviolacea –like (MYB-ATV-like)* | Regulatory gene | Repressor of late biosynthetic genes |
| *THM27* | Regulatory gene | Repressor of early and late biosynthetic genes |
| *MYB76* | Regulatory gene | Repressor of early and late biosynthetic genes |
| *WRKY44* | Regulatory gene | Putative regulator of anthocyanin synthesis |
| *Glabra 2 (GL2)* | Regulatory gene | Putative regulator of anthocyanin synthesis |
| *Ethylene response factor G3-like (ERF.G3-like)* | Regulatory gene | Putative regulator of anthocyanin synthesis |
| *UV-B Resistance 8 (UVR8)* | Photoreceptor gene | Photoreceptor of UV light and activator of *MYB12* and early biosynthetic genes in tomato |
| *Cryptochrome 1a (CRY1a)* | Photoreceptor gene | Apoprotein of UVA-blue light photoreceptor and putative regulator of tomato fruit pigmentation |
| *Cryptochrome 1b (CRY1b)* | Photoreceptor gene | Apoprotein of UVA-blue light photoreceptor and activator of flavonoid production in tomato fruit |
| *Cryptochrome 2 (CRY2)* | Photoreceptor gene | Apoprotein of UVA-blue light photoreceptor and activator of flavonoid production in tomato fruit |
| *Cryptochrome DASH (CRY-DASH)* | Photoreceptor gene | Apoprotein of UVA-blue light photoreceptor and putative regulator of tomato fruit pigmentation |
| *Phytochrome A (PhyA)* | Photoreceptor gene | Apoprotein of red/far-red light photoreceptor and putative regulator of tomato fruit pigmentation |

| | | |
|---|---|---|
| *Phytochrome B1 (PhyB1)* | Photoreceptor gene | Apoprotein of red/far-red light photoreceptor and putative regulator of tomato fruit pigmentation |
| *Phytochrome B2 (PhyB2)* | Photoreceptor gene | Apoprotein of red/far-red light photoreceptor and putative regulator of tomato fruit pigmentation |
| *ELONGATED HYPOCOTYL 5 (HY5)* | Light signalling gene | Activator of light-responsive genes |
| *CONSTITUTIVE PHOTOMORPHOGENESIS 1 homolog (COP1 homolog)* | Light signalling gene | Putative component of the ubiquitin ligase complex destabilizing light responsive factors |
| *CONSTITUTIVE PHOTOMORPHOGENESIS 1 like isoform X1 (COP1like isoform X1)* | Light signalling gene | Putative component of the ubiquitin ligase complex destabilizing light responsive factors |
| *REPRESSOR OF UV-B PHOTOMORPHOGENESIS (RUP)* | Light signalling gene | Light signalling factor putatively involved in anthocyanin repression |
| *B-BOX 20 (BBX20)* | Light signalling gene | Light signalling factor involved in photomorphogenesis and putative activator of anthocyanin synthesis |
| *B-BOX 21 (BBX21)* | Light signalling gene | Light signalling factor involved in photomorphogenesis and putative activator of anthocyanin synthesis |
| *B-BOX 22 (BBX22)* | Light signalling gene | Light signalling factor involved in photomorphogenesis and putative activator of anthocyanin synthesis |
| *B-BOX (BBX24)* | Light signalling gene | Light signalling factor involved in photomorphogenesis and putative repressor of anthocyanin synthesis |

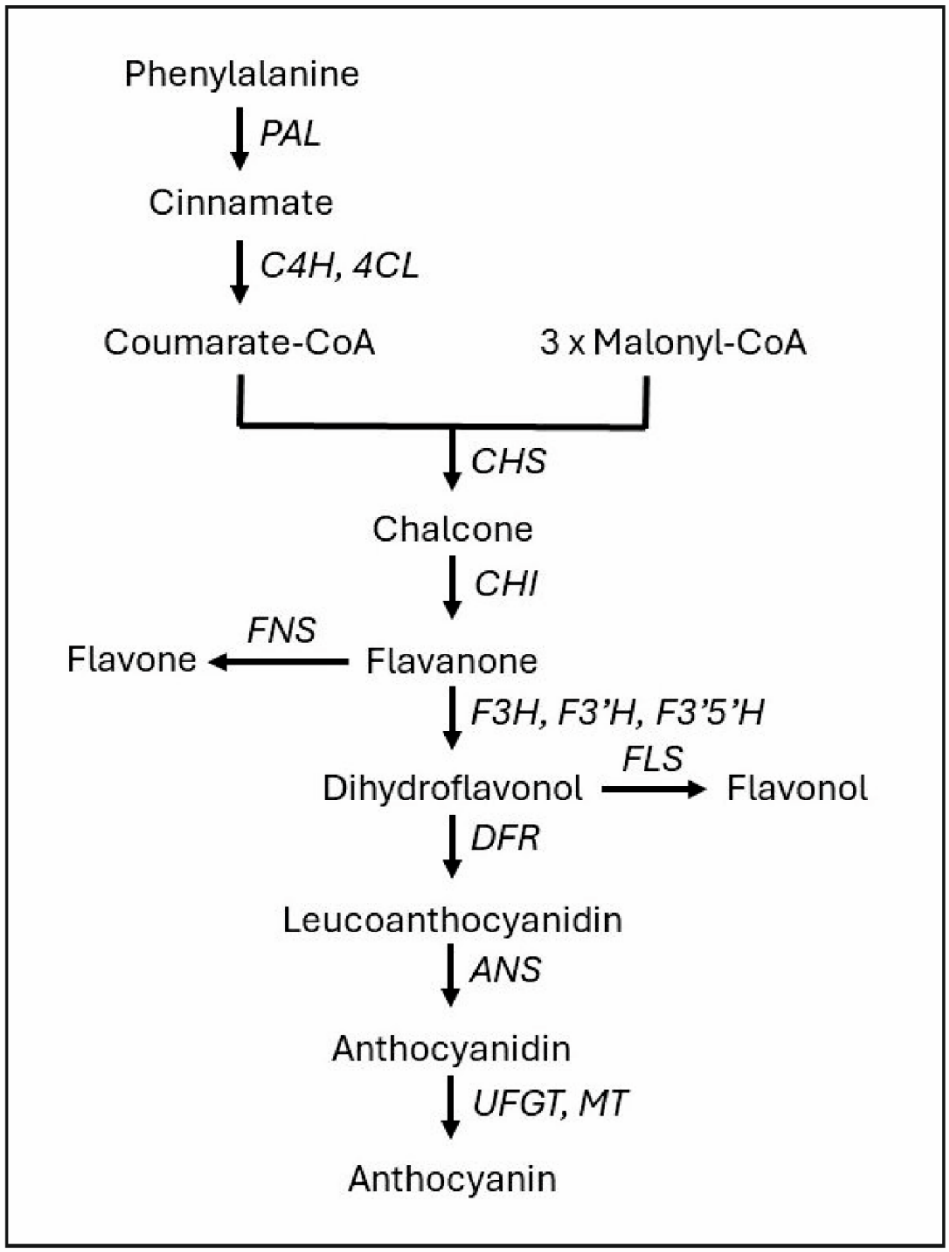



**Figure S1.** Anthocyanin biosynthetic pathway. Phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumaryol CoA ligase (4CL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone-3-hydroxylase (F3H), flavanone 3'-hydroxylase (F3'H), flavanone 3'5'-hydroxylase (F3'5'H), flavonol synthase (FLS), flavone synthase (FNS), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), UDP glucose flavonoid 3-O-glucosyltransferase (UFGT), methyltransferase (MT).

*Aft/atv*

*Aft/atv/hp2*

**Figure S2.** Pictures of *Aft*/*atv* and *Aft*/*atv*/*hp2* anthocyanin-enriched tomato fruits at the immature green stage.

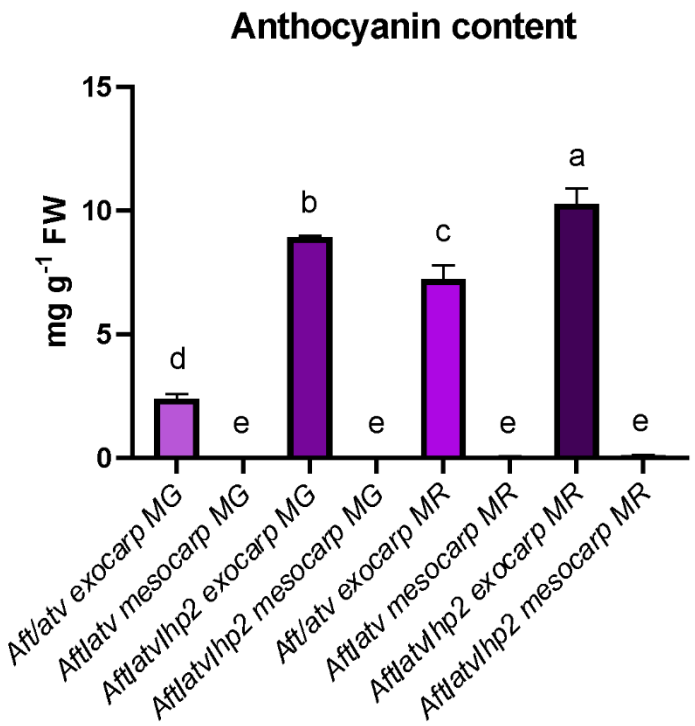



**Figure S3.** Anthocyanins measured in exocarp and mesocarp of *Aft*/*atv* and *Aft*/*atv*/*hp2* fruits at both mature green (MG) and mature red (MR), expressed as petunidin-3-(p-coumaroyl rutinoside)-5-glucoside per gram exocarp fresh weight (FW). Data are means (±SEM) of three biological replicates. One-way ANOVA with Tukey's HSD post hoc test was performed. Different letters indicate significant differences at $P \leq 0.05$.

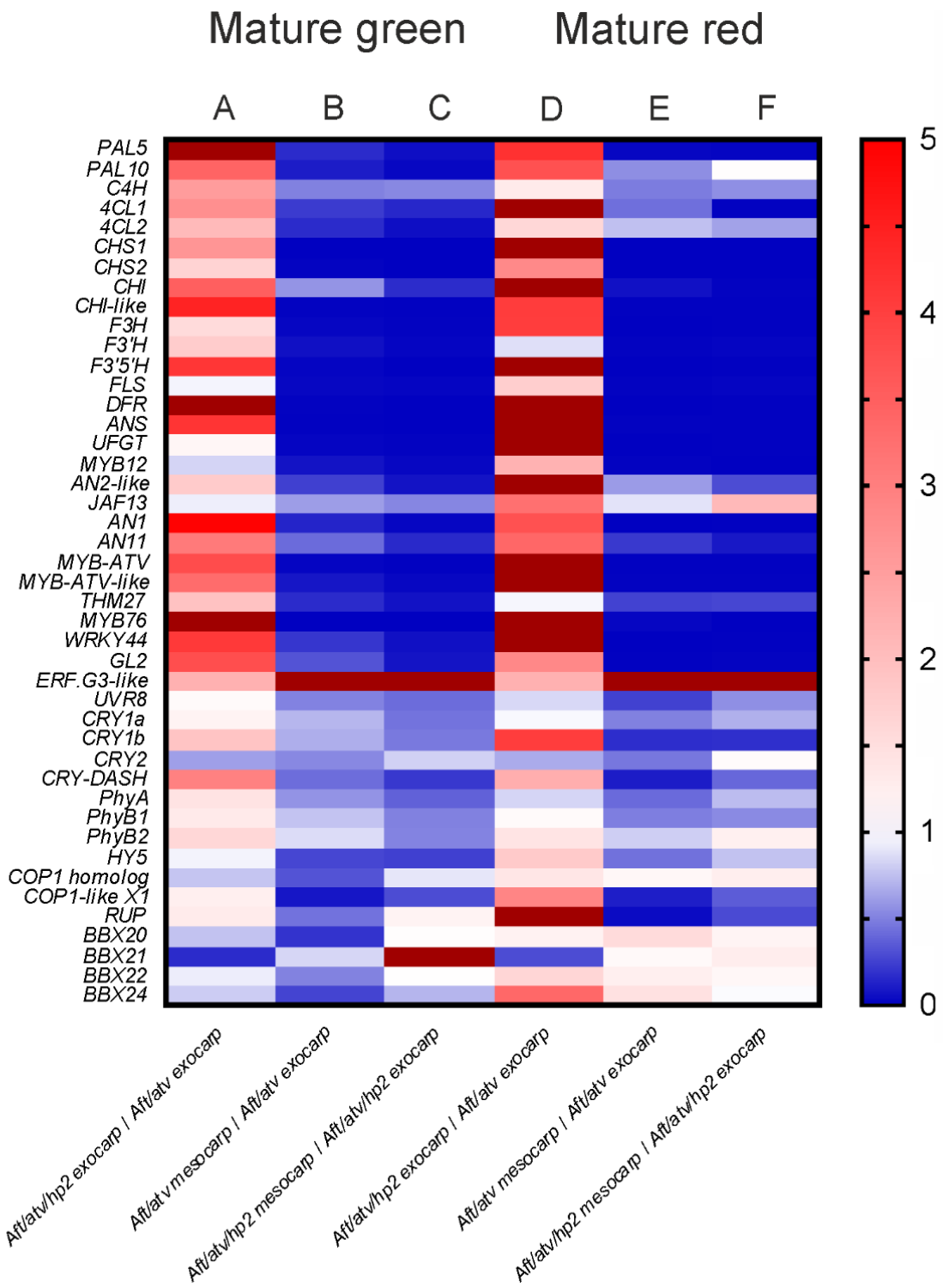



**Figure S4.** Heatmap showing the ratio between gene expressions measured in *Aft/atv/hp2* exocarp and *Aft/atv* exocarp [column A (mature green), column D (mature red)], the ratio between gene expressions measured in *Aft/atv* mesocarp and exocarp [column B (mature green), column E (mature red)], and the ratio between gene expressions measured in *Aft/atv/hp2* mesocarp and exocarp [column C (mature green), column F (mature red)] of the structural, regulatory, photoreceptors and light signalling genes of the anthocyanin biosynthetic pathway. The colour bar on the right shows the ratio scale, from 0 to 5. Some scattered ratios exceeding the maximum value represented in the colour bar are represented by magenta colour.

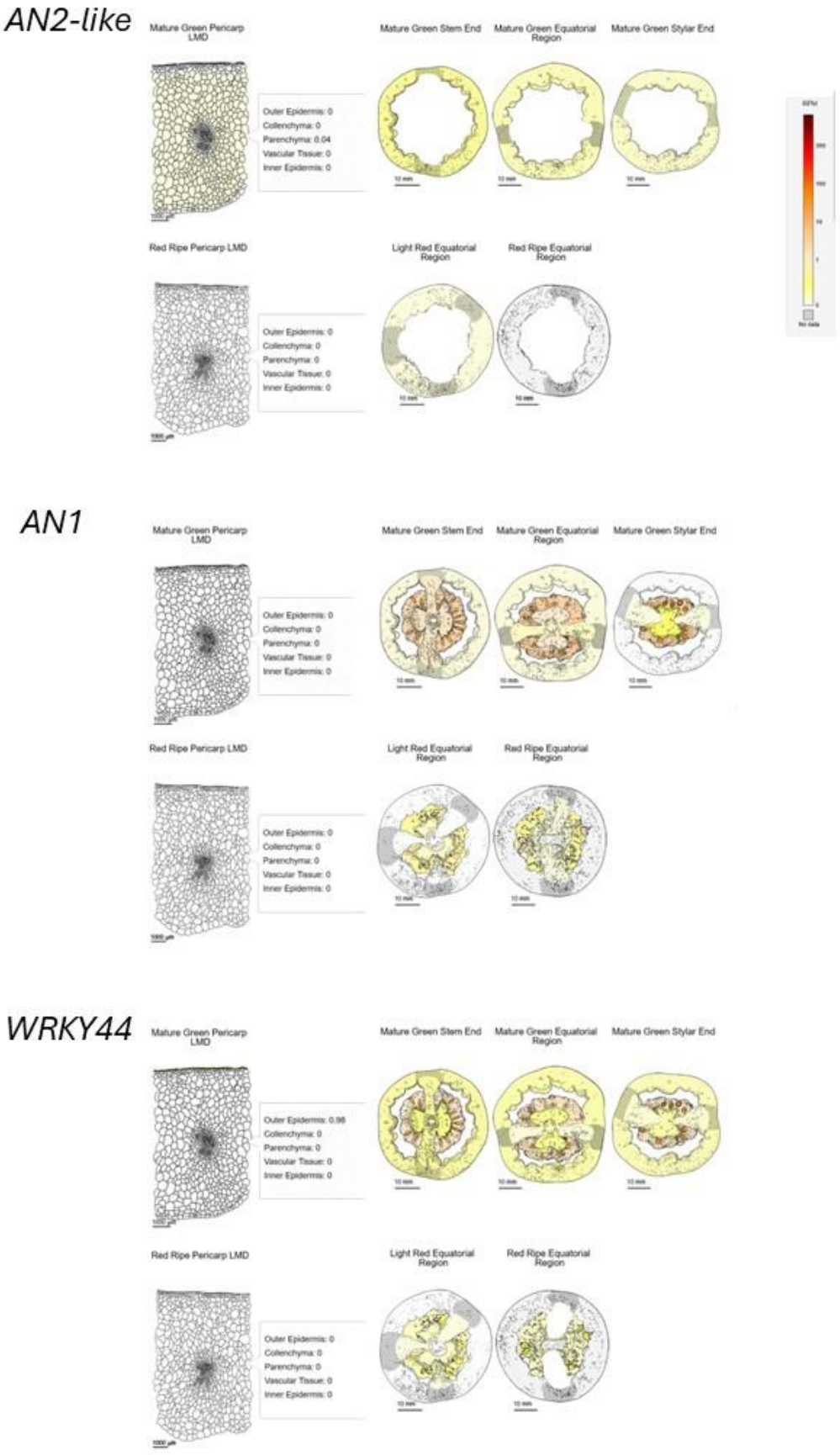
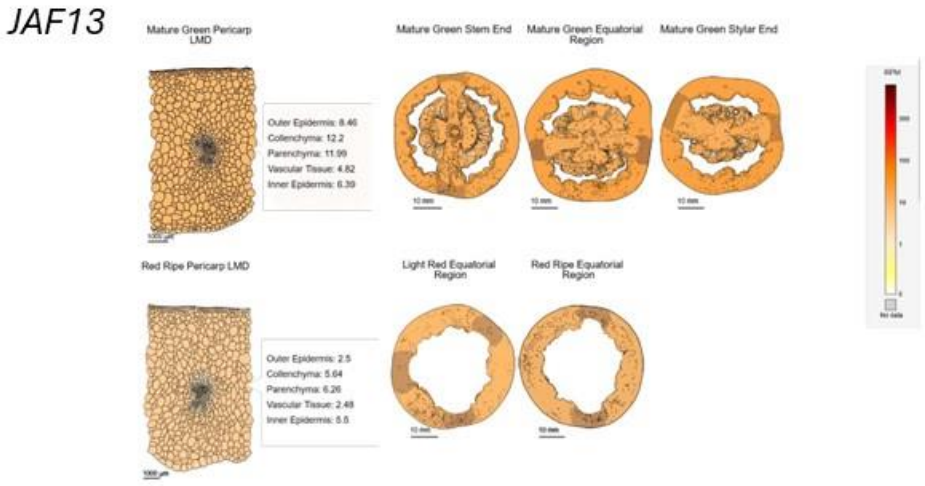



**Figure S5.** Expression of *AN2-like*, *AN1* and *WRKY44* in *S. lycopersicum* fruits in mature green and red ripe pericarp and other tissues shown in different fruit cross-sections. Images are from Tomato Expression Atlas (https://tea.solgenomics.net).



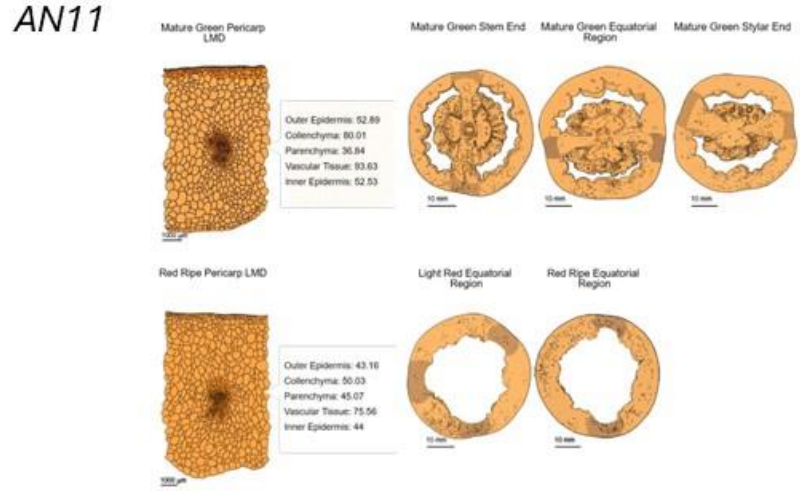




**Figure S6.** Expression of *JAF13* and *AN11* in *S. lycopersicum* fruits in mature green and red ripe pericarp and other tissues shown in different fruit cross-sections. Images are from Tomato Expression Atlas (https://tea.solgenomics.net).

A

*Table below expresses data for NS1 (upper row) / NS12 (lower row).*

| UV | B | G | R | FR |
|---|---|---|---|---|
| 1% | 20% | 39% | 35% | 5% |
| 0,5% | 21% | 38% | 35% | 6% |

| PAR | CCT | CRI | B:G | R:FR |
|---|---|---|---|---|
| 94% | 4800 | 90 | 0,7 | 10,4 |
| 94% | 5000 | 91 | 0,6 | 4,6 |

B

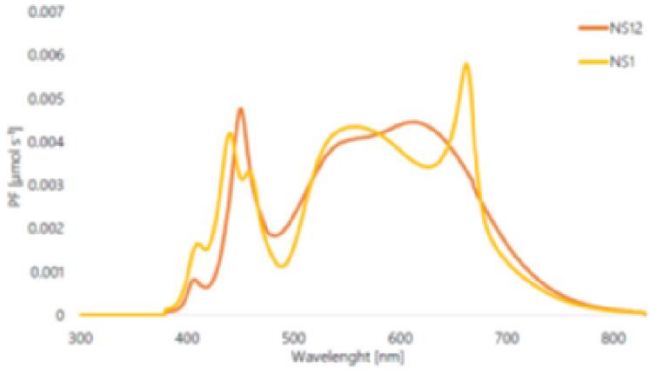



*Figure 1: Spectra wavelength. The integral area of each curve is equivalent to 1 µmol.s⁻¹.*

**Figure S7.** Light wavelength spectra of the NS1 LED lamps used in the experimental set-up. Pictures A and B are from Valoya (www.valoya.com).