

# Light Microclimate-Driven Changes at Transcriptional Level in Photosynthetic Grape Berry Tissues

**Andreia Garrido** <sup>1,2,\*</sup>, **Ric C. H. De Vos** <sup>3</sup>, **Artur Conde** <sup>1,2,†</sup> and **Ana Cunha** <sup>1,2,4,\*†</sup>

<sup>1</sup> Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; arturconde@bio.uminho.pt

<sup>2</sup> Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal

<sup>3</sup> Business Unit Bioscience, Wageningen Plant Research, Wageningen University and Research (Wageningen-UR), PO Box 16, 6700 AA Wageningen, The Netherlands; ric.devos@wur.nl

<sup>4</sup> Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

\* Correspondence: andreia Garrido@sapo.pt (A.G.); accunha@bio.uminho.pt (A.C.)

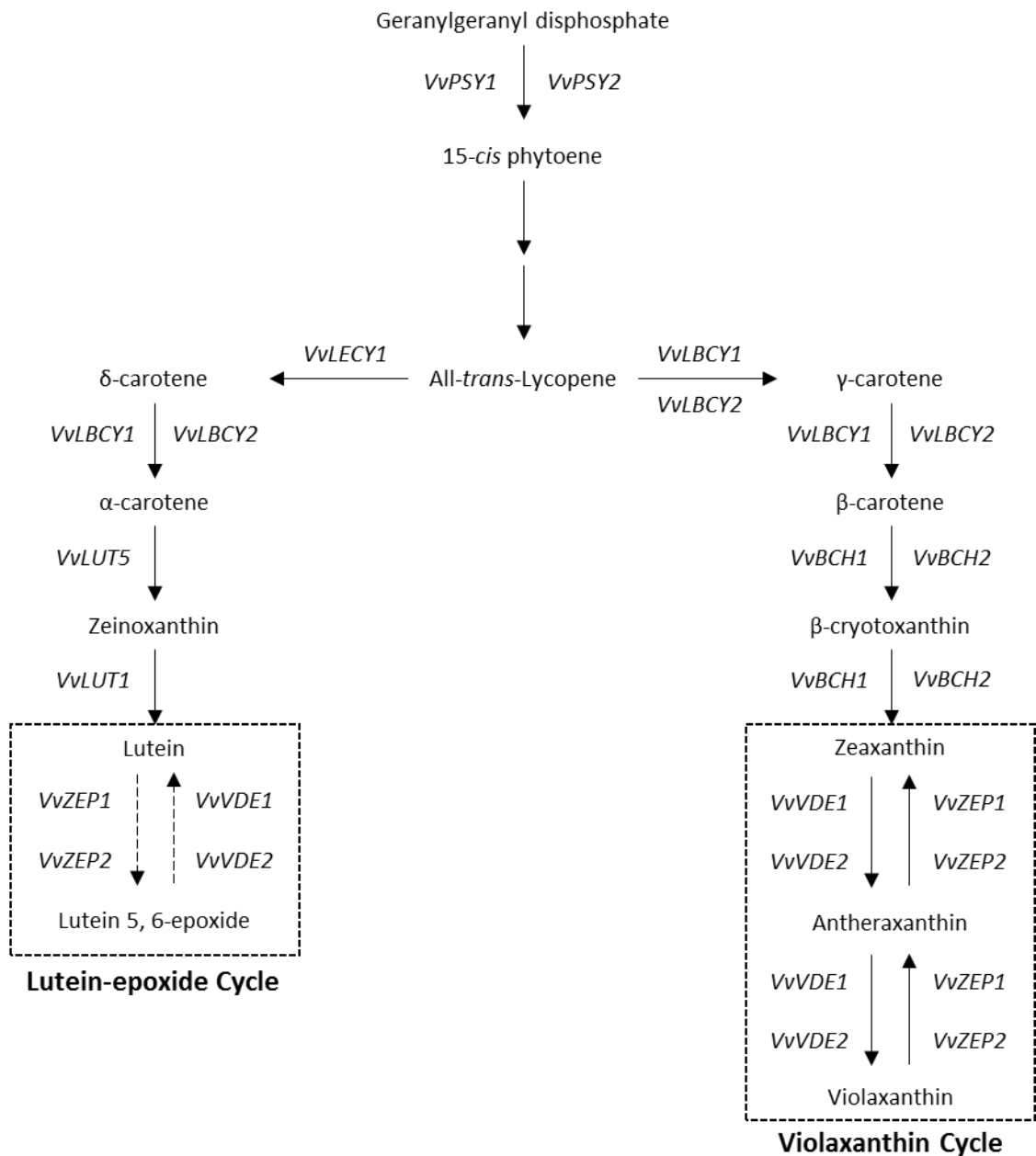
† Equal senior authorship.

## Supplementary Materials

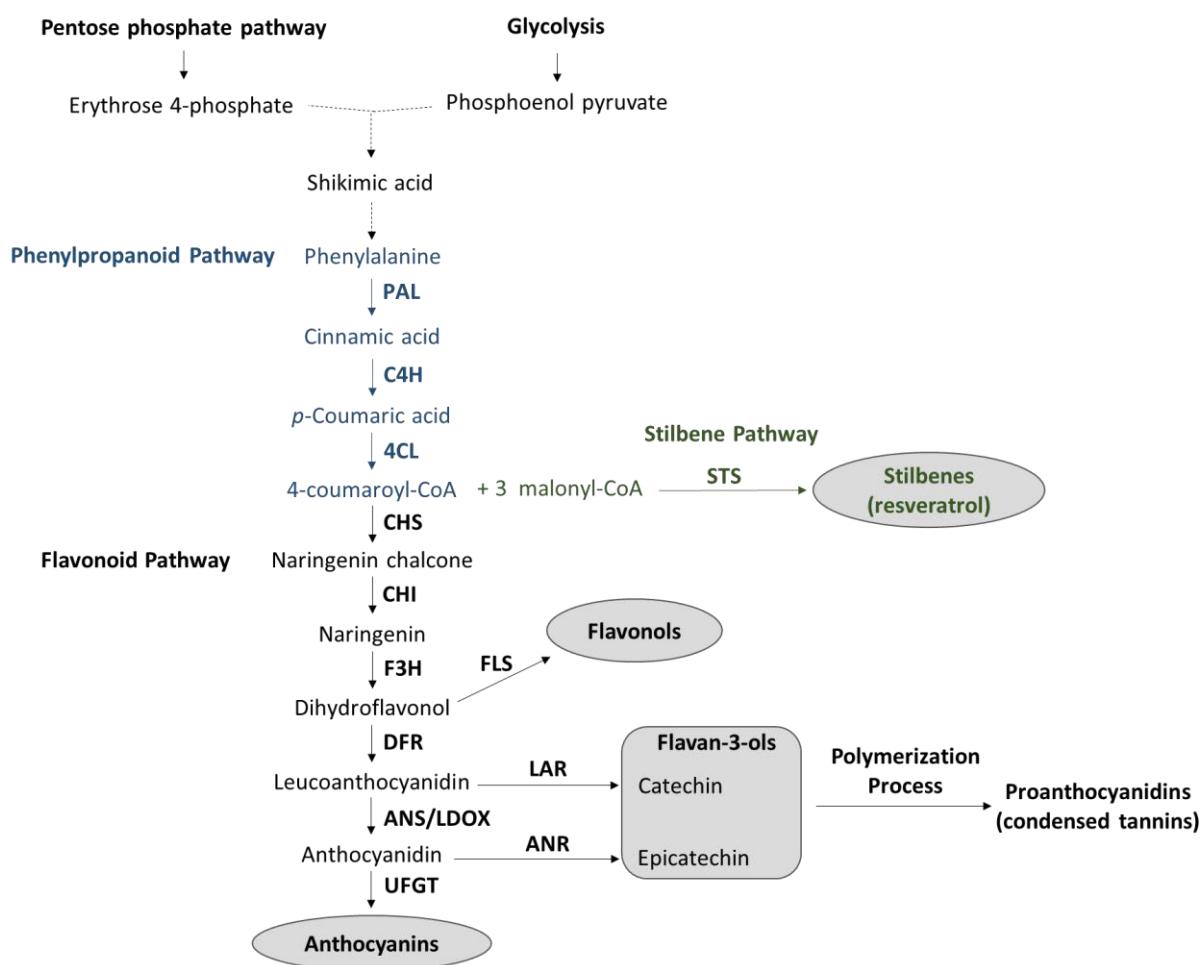
**Table S1.** Forward (F) and reverse (R) primers used for gene expression analysis by real-time PCR. Sequences accession numbers were obtained through Grape Genome Browser 12x or, for the carotenoid metabolism genes, from Plaza 1.0 (April, 2009; [http://bioinformatics.psb.ugent.be/plaza\\_v1/](http://bioinformatics.psb.ugent.be/plaza_v1/)), or Grape Genome Database (<http://genomes.cribi.unipd.it/grape/>) for *VvLAR2* and *VvRubisCO*.

Gene	Accession number (Genoscope)	Primers
<i>VvACT1</i> (reference gene)	GSVIVT01026580001	F: 5'-GTGCCTGCCATGTATGTTGCC-3'
		R: 5'-GCAAGGTCAAGACGAAGGATA-3'
<i>VvGAPDH</i> (reference gene)	GSVIVT00009717001	F: 5'-CACGGTCAGTGAAGCATCAT-3'
		R: 5'-CCTTGTCTCAGTGAACACACCAG-3'
<i>VvPAL1</i>	GSVIVG01025703001	F: 5'-CCGAACCGAATCAAGGACTG-3'
		R: 5'-GTTCCAGCCACTGAGACAAT-3'
<i>VvSTS1</i>	GSVIVT01010590001	F: 5'-CGAAGCAACTAGGCATGTGT-3'
		R: 5'-CTCCCAATCCAATCCTTCA-3'
<i>VvCHS1</i>	GSVIVT01032968001	F: 5'-GTCCCAGGGTTGATTTCAA-3'
		R: 5'-TCTCTCCTTCAGACCCAGTT-3'
<i>VvCHS3</i>	Lac14-SSH (CX126991)	F: 5'-TCGCATCACAAATAGCGAAC-3'
		R: 5'-CAGGGAAGCTGCCATGTATT-3'
<i>VvFLS1</i>	GSVIVT01008913001	F: 5'-CAGGGCTTGCAGGTTTTAG-3'
		R: 5'-GGGTCTTCTCCTTGTTACG-3'
<i>VvMYBPA1</i>	GSVIVT01010590001	F: 5'-AGATCAACTGGTTATGCTTGCT-3'
		R: 5'-AACACAAATGTACATCGCACAC-3'
<i>VvDFR</i>	GSVIVT01009743001	F: 5'-GGCTTTCTAGCGAGAGCGTA-3'
		R: 5'-ACTCTCATTTCCGGCACATT-3'
<i>VvANR</i>	GSVIVG01035256001	F: 5'-CAATACCAGTGTCTCTGAGC-3'
		R: 5'-AAACTGAACCCCTCTTCAC-3'
<i>VvLAR1</i>	GSVIVT01024419001	F: 5'-CAGGAGGCTATGGAGAAGATAC-3'
		R: 5'-ACGCTTCTCTGTACATGTTG-3'
<i>VvLAR2</i>	VIT_217s0000g04150	F: 5'-CAAATCGCTTCATTTCCGACCTCCC-3'
		R: 5'-GTCATCTTCTTCCACGGTTACACGG-3'
<i>VvLDOX</i>	GSVIVT01009743001	F: 5'-ACCTTCATCCTCCACAACAT-3'
		R: 5'-AGTAGAGCCTCCTGGGTCTT-3'
<i>VvChlSyn</i>	GSVIVT01011187001	F: 5'-GCTGAGACTGATGCAAATGAAGCC-3'
		R: 5'-TGCCGAGAAGCTGATTGAAGTTCCG-3'
<i>VvRubisCO</i>	VIT_13s0019g02050	F: 5'-TTCAAAGCTCTGCGCGCTCTAC-3'
		R: 5'-TCTCTCAACTTGGATGCCATGAGG-3'
<i>VvSPS1</i>	GSVIVT01012825001	F: 5'-GGTTTGCCTATTGTTGCCACCAG-3'
		R: 5'-CAAGAAGGCCATTGTCAAGTACCC-3'
<i>VvSuSy1</i>	GSVIVT01015018001	F: 5'-TGTTAAGGCTCCTGGATTCAATTA-3'
		R: 5'-AGCCAAATCTTGGCAAGCA-3'
<i>VvPSY1</i>	VV00G37410	F: 5'-TCGATGTGGTGAAGTTTGTGCAG-3'
		R: 5'-TCAGGTGTCATCAGCATTTGTTCCC-3'
<i>VvLBCY2</i>	VV08G15130	F: 5'-AGGCTGGGTGGTTGAGAATTGG-3'
		R: 5'-TCCTCAACGACAAACCGAAGTGG-3'
<i>VvLECY1</i>	VV11G01840	F: 5'-TGGATGTGGCAGGGATTCTTGG-3'
		R: 5'-TAGCATGGTTGCTCCAGTAGGG-3'

<i>VvLUT1</i>	VV08G10880	F: 5'-TGCTTGCTAGCAGAGAAGAGGTG-3'
		R: 5'-ACTGCCATTGAGTGCATCAGTTC-3'
<i>VvVDE1</i>	VV00G14320	F: 5'-GTTGTCTATGGTTGGTGGTTTGGC-3'
		R: 5'-ATGCCAGTGGGTCTCCATTTC-3'
<i>VvZEP1</i>	VV07G11310	F: 5'-AGCGTGCTATTGGTGGAGAGTG-3'
		R: 5'-CCCGATTATGCAGGGTTTGTCTC-3'



**Figure S1.** Simplified carotenoid metabolic pathway. *PSY1/PSY2*, phytoene synthase; *LEC1/LEC2*, lycopene epsilon cyclase; *LBCY1/LBCY2*, lycopene  $\beta$ -cyclase; *LUT5/LUT1*, lutein synthase; *BCH1/BCH2*,  $\beta$ -carotene hydroxylase; *VDE1/VDE2*, violaxanthin de-epoxidase; *ZEP1/ZEP2*, zeaxanthin epoxidase. Adapted from Young et al. [39].



**Figure S2.** Biosynthetic pathways of phenolic compounds in grape berry. PAL, phenylalanine ammonia lyase; C4H, cinnamate-4-hydroxylase; 4CL, 4-coumaroyl:CoA-ligase; CHS, chalcone synthase; STS, stilbene synthase; CHI, chalcone isomerase; F3H, flavonone 3-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol reductase; ANS, anthocyanidin synthase; LDOX, leucoanthocyanidin dioxygenase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase; UFGT, flavonoid glucosyltransferase. Full lines represent direct enzymatic conversion and dashed lines represent omitted intermediates. Adapted from Ageorges et al. [88] and Teixeira et al. [89].

**Table S2.** Matrix with Pearson correlation coefficients ( $r$ ), calculated for selected genes (*VvFLS1*, *VvDFR*, *VvLDOX*, *VvLAR1*, *VvLAR2*, *VvANR* and *VvMYBPA1*) in the exocarp, considering all samples collected in both light microclimate and developmental stages ( $n = 18$ ). The coefficient values in bold are statistically significant ( $p \leq 0.05$ ).

	<i>VvFLS1</i>	<i>VvDFR</i>	<i>VvLDOX</i>	<i>VvLAR1</i>	<i>VvLAR2</i>	<i>VvANR</i>	<i>VvMYBPA1</i>
<i>VvFLS1</i>	1.000						
<i>VvDFR</i>	<b>-0.770</b>	1.000					
<i>VvLDOX</i>	-0.419	-0.104	1.000				
<i>VvLAR1</i>	-0.439	0.456	-0.021	1.000			
<i>VvLAR2</i>	-0.452	<b>0.842</b>	-0.414	<b>0.567</b>	1.000		
<i>VvANR</i>	<b>-0.504</b>	<b>0.845</b>	-0.381	<b>0.567</b>	<b>0.982</b>	1.000	
<i>VvMYBPA1</i>	<b>-0.519</b>	<b>0.864</b>	-0.335	<b>0.557</b>	<b>0.962</b>	<b>0.979</b>	1.000

**Table S3.** Matrix with Pearson correlation coefficients ( $r$ ), calculated for selected genes (*VvFLS1*, *VvDFR*, *VvLDOX*, *VvLAR1*, *VvLAR2*, *VvANR* and *VvMYBPA1*) in the seed, considering all samples collected in both light microclimate and developmental stages ( $n = 18$ ). The coefficient values in bold are statistically significant ( $p \leq 0.05$ ).

	<i>VvFLS1</i>	<i>VvDFR</i>	<i>VvLDOX</i>	<i>VvLAR1</i>	<i>VvLAR2</i>	<i>VvANR</i>	<i>VvMYBPA1</i>
<i>VvFLS1</i>	1.000						
<i>VvDFR</i>	-0.379	1.000					
<i>VvLDOX</i>	<b>0.877</b>	-0.399	1.000				
<i>VvLAR1</i>	-0.239	0.367	-0.342	1.000			
<i>VvLAR2</i>	-0.167	<b>0.752</b>	-0.138	0.327	1.000		
<i>VvANR</i>	<b>0.556</b>	0.098	0.357	0.420	0.212	1.000	
<i>VvMYBPA1</i>	0.125	<b>0.698</b>	0.167	0.215	<b>0.782</b>	0.404	1.000