Supporting information

Changes in the Proanthocyanidin Composition and Related Gene Expression in Bilberry (*Vaccinium myrtillus* L.)

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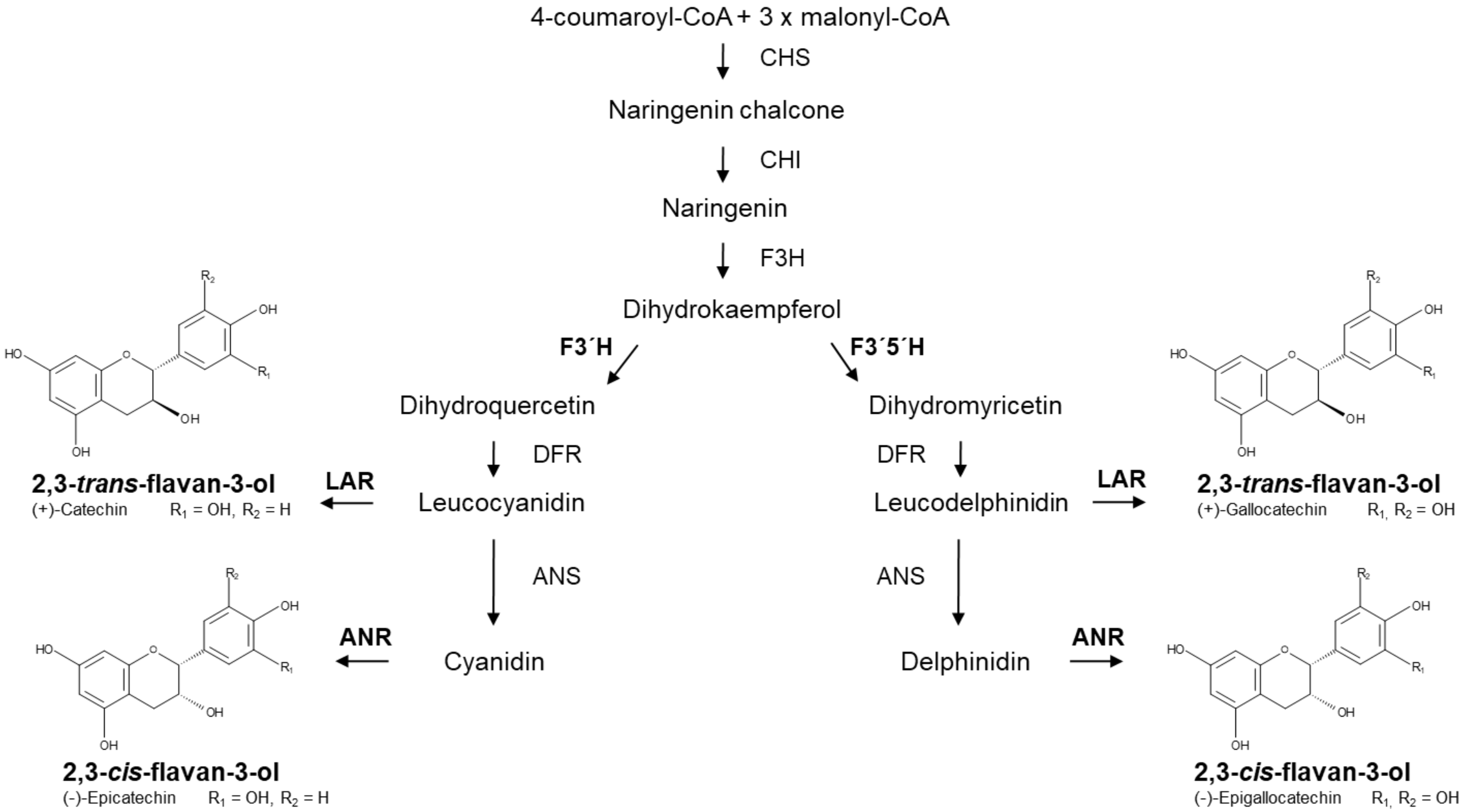


Figure S1. The PA biosynthetic pathway. CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3′H, flavonoid 3′-hydroxylase; F3′5′H, flavonoid 3′5′-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase.

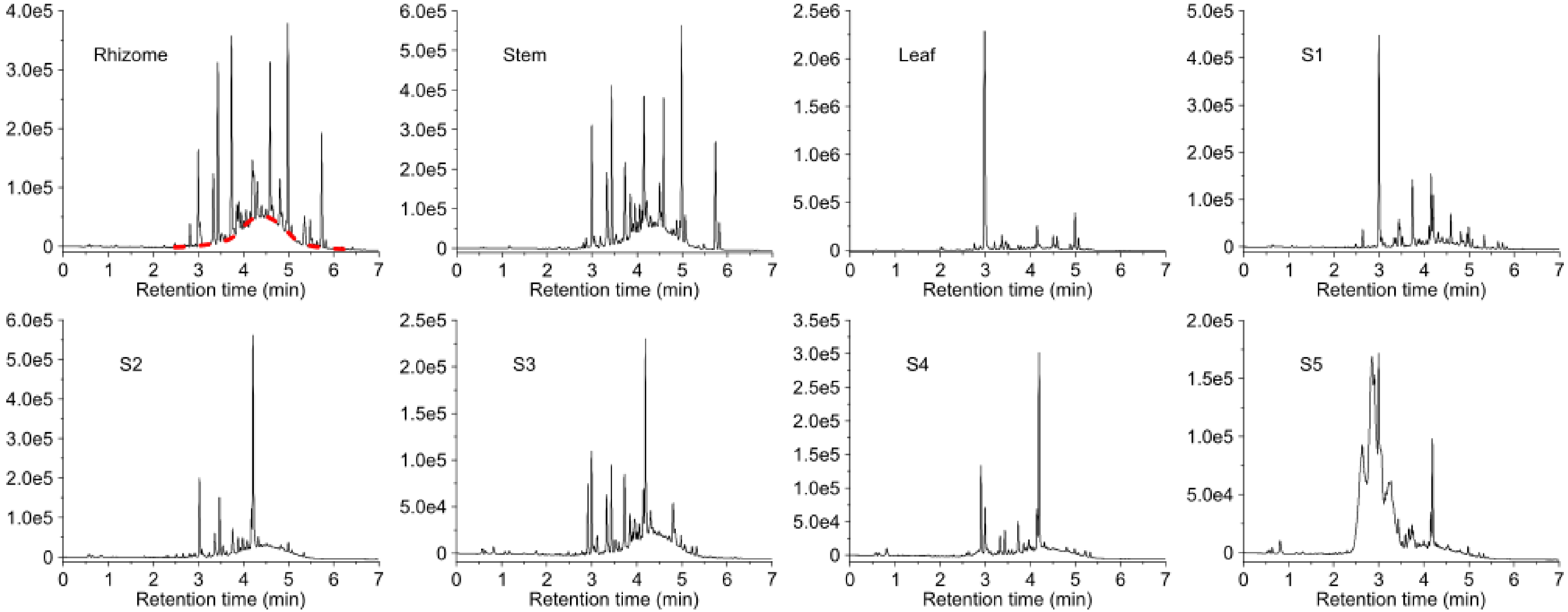


Figure S2. UV chromatograms (λ=280 nm) of the bilberry tissue samples from the UHPLC-DAD–ESI-3Q-MS analyses. The dashed red line in the rhizome chromatogram shows an example of the chromatographic hump caused by PAs, which are not separated from one another when using reversed-phase liquid chromatography.

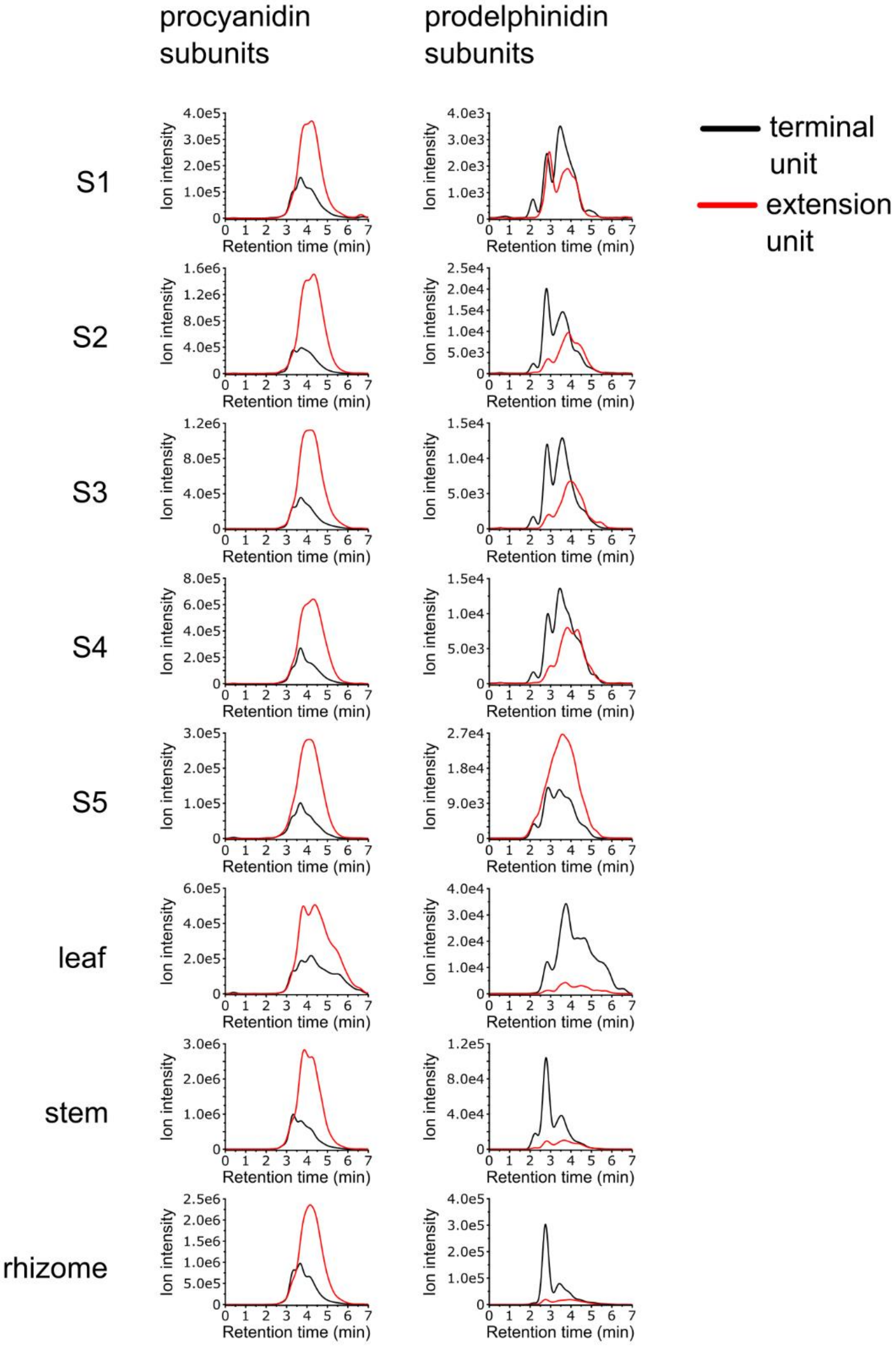


Figure S3. Fingerprints of PCs and PDs in bilberry tissues determined from the UHPLC-DAD–ESI-3Q-MS analyses, showing terminal and extension units separately.

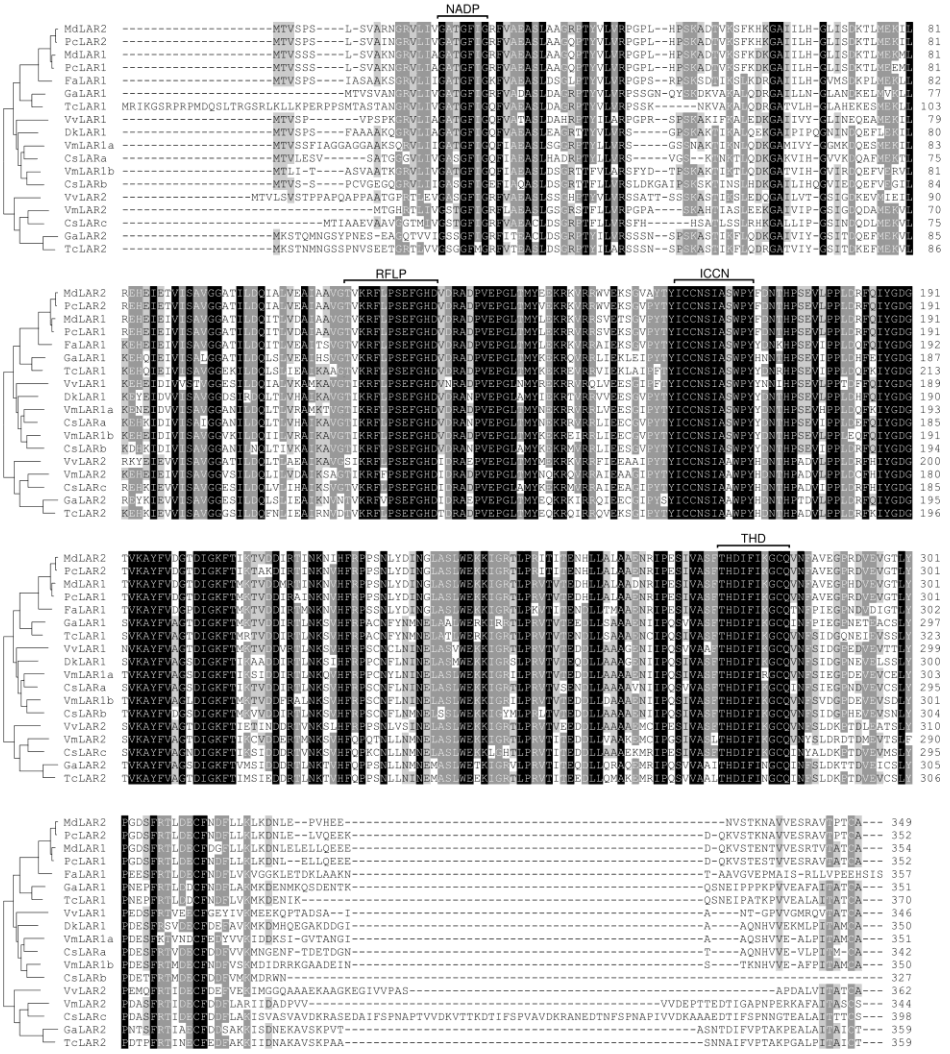


Figure S4. Comparison of the deduced amino acid sequences of *LAR* genes from bilberry with other species. Characteristic domains found in LAR sequences, such as NADP-binding domain and RFLP, ICCN, and THD motifs, are shown. The GenBank accession numbers are as follows: *Camellia sinensis* CsLARa (ASU87429), CsLARb (ASU87431), CsLARc (ASU87430); *Diospyros kaki* DkLAR1 (BAH89267); *Fragaria × ananassa* FaLAR1 (AAZ78662); *Gossypium arboreum* GaLAR1 (CAI56319), GaLAR2 (CAI56323), *Malus × domestica* MdLAR1 (AAX12185), MdLAR2 (AAX12186); *Pyrus communis* PcLAR1 (ABB77696), PcLAR2 (ABB77697); *Theobroma cacao* TcLAR1 (XP_007046315), TcLAR2 (ADD51358); *Vitis vinifera* VvLAR1 (CAI26310), VvLAR2 (KY615698). The *VmLAR1a, VmLAR1b,* and *VmLAR2* sequences have been deposited in GenBank under accession numbers MT013357, MT013358, and MT013359, respectively.

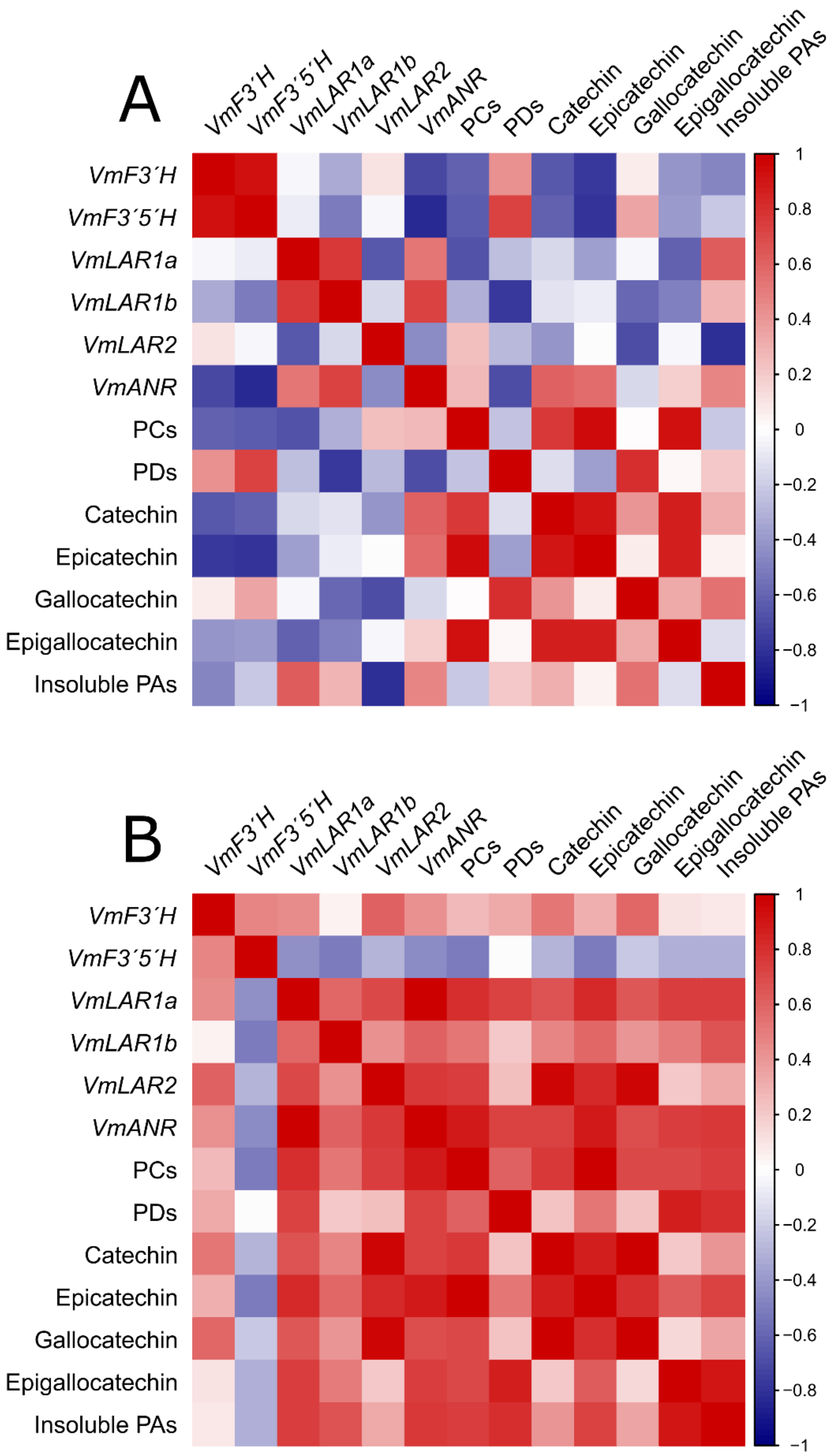
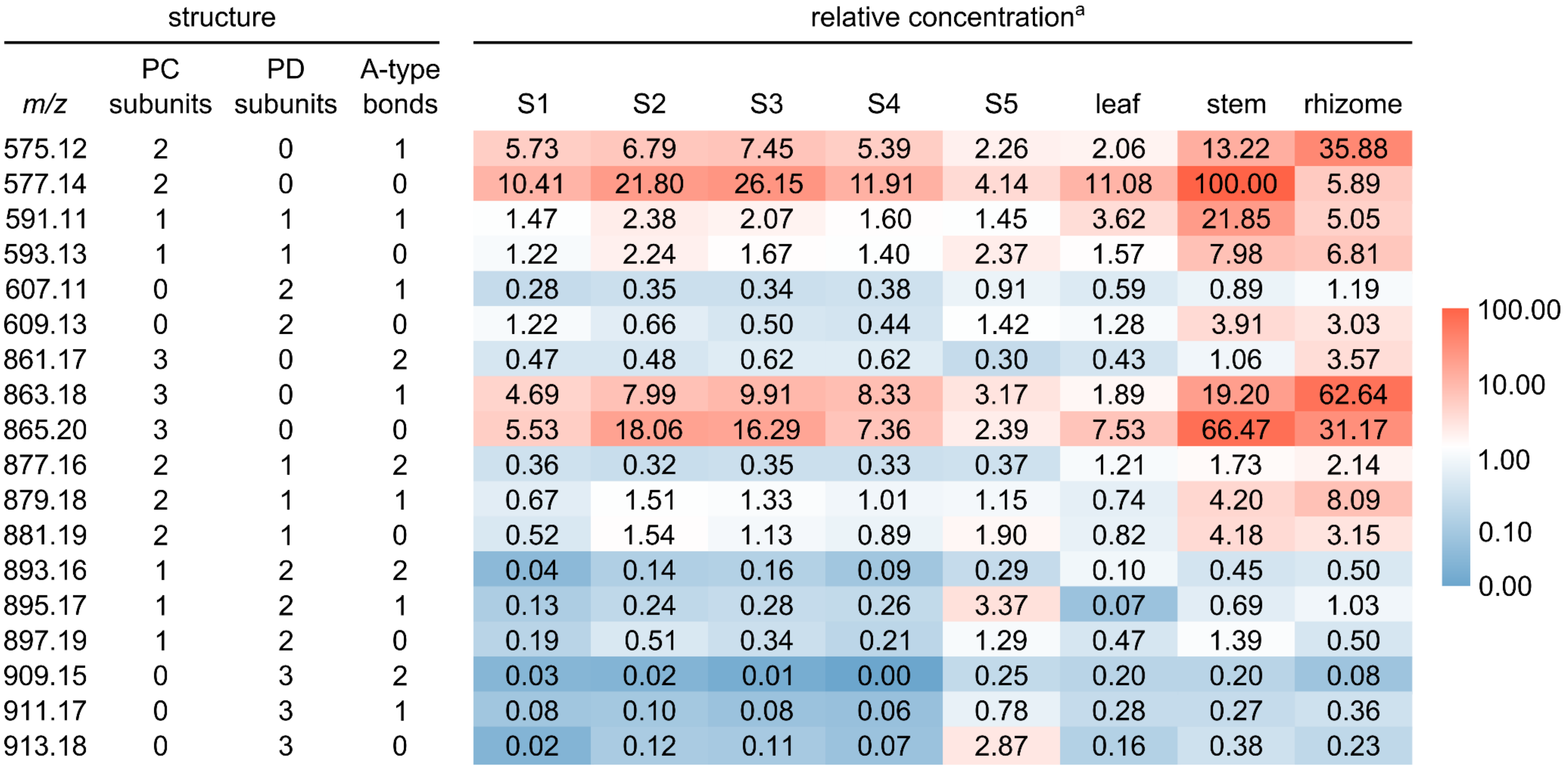


Figure S5. Correlation matrices of measured concentrations and expressions among berry (A) and all tissue (B) samples.

Table S1. Gene-specific primers used for quantitative real-time PCR analysis.

Gene	Primer sequence 5´-3´
VmF3´H	TTCTTCGACACCCGAAAGTC (forward) TCGAACCCTTTGGAATGAAG (reverse)
VmF3´5´H	GATTGCGTGGATGGACTTACA (forward) AAATCTGGGTTCCCTTTACGC (reverse)
VmLAR1a	CAGAGGGTCCTGATAATTGGAG (forward) CATTGGAAGAACCAGACCTGAC (reverse)
VmLAR1b	CGTTGATCACAGCTTCTGTTGC (forward) CGCCTTTGTCTTGGAGAGTCTT (reverse)
VmLAR2	GGGTGGTGTTAGCATCTTGGAC (forward) CCAGCTGCTTCTATAGCCCTTC (reverse)
VmANR	GCTGGTGTTTCTCCCACAAT (forward) AAATATATGGGCGCGACAAA (reverse)
VmGAPDH	CAAACTGTCTTGCCCCACTT (forward) CAGGCAACACCTTACCAACA (reverse)

Table S2. A heatmap of the semiquantitative relative concentrations of dimeric and trimeric PAs with A- and B-type bonds.



structure				relative concentration[a]							
m/z	PC subunits	PD subunits	A-type bonds	S1	S2	S3	S4	S5	leaf	stem	rhizome
575.12	2	0	1	5.73	6.79	7.45	5.39	2.26	2.06	13.22	35.88
577.14	2	0	0	10.41	21.80	26.15	11.91	4.14	11.08	100.00	5.89
591.11	1	1	1	1.47	2.38	2.07	1.60	1.45	3.62	21.85	5.05
593.13	1	1	0	1.22	2.24	1.67	1.40	2.37	1.57	7.98	6.81
607.11	0	2	1	0.28	0.35	0.34	0.38	0.91	0.59	0.89	1.19
609.13	0	2	0	1.22	0.66	0.50	0.44	1.42	1.28	3.91	3.03
861.17	3	0	2	0.47	0.48	0.62	0.62	0.30	0.43	1.06	3.57
863.18	3	0	1	4.69	7.99	9.91	8.33	3.17	1.89	19.20	62.64
865.20	3	0	0	5.53	18.06	16.29	7.36	2.39	7.53	66.47	31.17
877.16	2	1	2	0.36	0.32	0.35	0.33	0.37	1.21	1.73	2.14
879.18	2	1	1	0.67	1.51	1.33	1.01	1.15	0.74	4.20	8.09
881.19	2	1	0	0.52	1.54	1.13	0.89	1.90	0.82	4.18	3.15
893.16	1	2	2	0.04	0.14	0.16	0.09	0.29	0.10	0.45	0.50
895.17	1	2	1	0.13	0.24	0.28	0.26	3.37	0.07	0.69	1.03
897.19	1	2	0	0.19	0.51	0.34	0.21	1.29	0.47	1.39	0.50
909.15	0	3	2	0.03	0.02	0.01	0.00	0.25	0.20	0.20	0.08
911.17	0	3	1	0.08	0.10	0.08	0.06	0.78	0.28	0.27	0.36
913.18	0	3	0	0.02	0.12	0.11	0.07	2.87	0.16	0.38	0.23

[a] The values are normalized (0–100) peak areas per dry weight of the corresponding calculated m/z (±0.10) values of the theoretical singly charged ions from extracted ion chromatograms. Red and blue colour indicate high and low quantities, respectively, on a logarithmic scale.