



Figure S1. Heatmap of almond flower buds DEGs based on scaled TPM. Phenological states and cultivars of almond flower buds are represented by colors. A, AB and B corresponded to extra-early Desmayo Largueta (D1, D4, D7), extra-late Penta (P1, P10, P14), and ultra-late Tardona (T1, T14, T17) cultivars samples. Each cell is colored from green to red based on the level of expression of that gene in a given sample. Genes (rows) are ordered according to their cluster (*K*-means clusters 1 to 8). Clusters are ordered from *EndoGS* and *EcoGS* (top) to 1-6 (bottom) and are represented as a color bar on the left side.



Figure S2. GO categories representation related to the enrichment score in clusters 1-6: Biological processes (red bars), cellular components (green bars) and molecular functions (blue bars).

Table S1. Experimental design for RNA sequencing and validation of almond flower buds: Samples (15 flower buds/tree/cultivar) from three cultivars with contrasting flowering time and chilling requirement (highlighted) were selected for RNA extraction and sequencing (A, AB, B) collected on Season 1 dates indicated. Samples collected during Season 2 were used together with those from Season 1 for RNA-seq validation by qRT-PCR. Million reads and reads conted (%) per sequenced sample. CP: Chill Portions.

Cultivar	Flowering time (date)		Flower bud samples		Chill accumulated (CP)		RNAseq output data (Season 1)	
	Season 1	Season 2	State	Sampling date (Season 1)	Season 1	Season 2	Million reads	% reads counted
Desmayo			A	11/10/2015	0	5.0	106.2	73.6
Desmayo	January 28th	January 27th	A-B	12/1/2015	7.1	9.1	121.3	73.4
Desmayo			B	12/21/2015	16.1	20.1	108.3	74.5
Penta			A	11/10/2015	0	5.0	91.9	76.8
Penta	February 10th	February 2nd	A-B	1/12/2016	25	29.2	115	74.3
Penta			B	2/10/2016	41.2	53.8	96.9	74.6
Tardona			A	11/10/2015	0	5.0	90	75.4
Tardona	February 23rd	February 10th	A-B	2/10/2016	41.2	44.8	105.3	74.4
Tardona			B	3/1/2016	49.2	60.6	99.5	75.3

Table S6. qRT-PCR primers sequence, melting temperature and amplicon size of DEGs used for RNA-seq validation. Tm: Melting temperature.

DEG name	Forward primer (5'-3')	Tm (°C)	Reverse primer (5'-3')	Tm (°C)	Amplicon size (bp)
<i>XET</i>	GGTTTGGTTCTGGGTCT	56.16	GCTTTGTCCTTTACTCGGT	56.8	115
<i>4CLL1</i>	G TTCAGCAGGAGCCACTT	59.1	ACAAGAATGGATGGCTTCA	56.00	123
<i>NIP7</i>	TTGAAGTTTGAGGAGATGGAACAG	59.73	GGCCAATAAAGACTCGTTCAA	60.02	111
<i>GLUCAN ENDO-β-1,3-GLUCOSIDASE</i>	AACTCCCTCCCTCTCCATC	59.09	CGGTGAGGTGGTTCTGGA	60.46	94
<i>EG1</i>	CTCCAGGGGTATATTGGG	54.82	AAGAGACCTGGAACAAAAG	53.99	148
<i>SWEET10</i>	GTGATGAGAAAAGTGGCAT	54.88	CCAGTGCCAACATTTTATAC	53.95	152
<i>AIP2</i>	ATGCAAGCGTCGTGGTAA	59.25	TGGGGTTTGTGTTCCGTTT	59.23	93
<i>DAM-like 1</i>	CCTCCACTTCTTAACCAGA	55.02	TGCTCCCAGTCTCTCTCT	58.00	93
<i>DAM-like 2</i>	AGGAACTTGAGGAGAAGAG	54.72	TCAGTTCTTCTCAGTTTCA	55.31	141
<i>LOX3.1</i>	TACGTAGCCAATGGTGTA	54.33	CGAGATCAAAGCGTGT	55.26	126
<i>RS5</i>	CTAGAAGGGGGAGAACGA	55.28	CCTAGAGAACGAGACCCA	54.94	80
<i>ATHB12</i>	CTGCAACTGAATAACCAAGG	56.01	TACAGCATACTCAGAGCC	54.86	90
<i>NCED5</i>	CTTTGGGGTGTGTTTTTCT	55.64	CTCTTCTCTCTTCTTCT	56.00	80
<i>OEP16</i>	CCACGGACCCTTTCTAAA	55.63	TGAGCACACTTTGAAGAAG	54.77	99
<i>60S</i>	ATACCAGTTGAAGGATCGT	55.45	GGAATATGAGTTGCTAAGAAGG	55.44	105

Table S7. Pearson correlation (r) values of gene expression data calculated between RNA-seq TPMs and qRT-PCR data from sequenced samples (A, AB and B) of season 1 (highlighted in grey) and the r values calculated between qRT-PCR data from season 1 and season 2 (A, A2, AB, AB2, B).

	Season 1 samples (RNAseq vs qRT-PCR)			Season 1 vs Season 2 samples (qRT-PCR)		
	Desmayo	Penta	Tardona	Desmayo	Penta	Tardona
<i>RS5</i>	0.98	0.99	1.00	0.91	0.35	0.93
<i>ATHB12</i>	1.00	0.00	0.98	0.20	0.01	1.00
<i>NCED5</i>	1.00	1.00	1.00	0.95	0.99	0.93
<i>LOX3.1</i>	1.00	0.98	1.00	0.83	0.22	0.98
<i>DAM-like 1</i>	1.00	0.98	0.96	0.84	1.00	1.00
<i>DAM-like 2</i>	1.00	1.00	0.99	0.87	0.98	1.00
<i>AIP2</i>	0.96	0.99	1.00	0.89	0.91	0.97
<i>SWEET10</i>	1.00	1.00	0.88	0.90	0.70	0.94
<i>4CLL1</i>	1.00	1.00	1.00	0.18	0.95	0.84
<i>XET2</i>	1.00	1.00	0.97	1.00	1.00	(-0.24)
<i>GLUCAN ENDO-β-1,3-GLUCOSIDASE</i>	1.00	0.96	1.00	0.98	0.97	0.73
<i>EG1</i>	1.00	1.00	1.00	0.58	0.97	0.89
<i>NIP7</i>	0.99	0.99	0.99	0.98	0.98	0.76