

Article



## The Regulation of Floral Colour Change in *Pleroma Raddianum* (DC.) Gardner

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## SUPPLEMENTAL MATERIAL

**Figure S1.** Agarose gel (0.8%) showing the cloned fragments. The abbreviations indicate: *PHENYLALANINE AMMONIUM LYASE (PAL), CINAMMATE* 4-HYDROXYLASE (C4H), CHALCONE SYNTHASE (CHS), *FLAVONOL SYNTHASE (FLS)* and *ANTHOCYANIDIN SYNTHASE (ANS), ELONGATION FACTOR* 1- $\alpha$  (*EF1*) and *RIBOSSOMOAL PROTEIN S13 (RPS)* genes.

Figure S2. Phenograms for PHENYLALANINE AMMONIUM LYASE (PAL) (A), CINAMMATE 4-HYDROXYLASE (C4H) (B), CHALCONE SYNTHASE (CHS) (C), FLAVONOL SYNTHASE (FLS) (D), and ANTHOCYANIDIN SYNTHASE (ANS) (E) amino acid sequences. Only sequences from species with full sequenced genomes were included in the analyses: *Eucalyptus grandis* (Eucgr), *Arabidopsis thaliana* (AT), *Brassica rapa* (Brara), *Medicago trunculata* (Medtr), *Trifolium pratense* (Tp) and *Solanum lycopersicum* (Soly). Trees were constructed using the following parameters: Neighbor-joining, Bootstrap of 1,000 replicates and the best model test for each analysis. Grey boxes show the clusters for *Pleroma raddianum* (T.pulchra) and *E. grandis* sequences. Figure S3. *Pleroma raddianum* flowers need light to turn from white to purple colour. Plants were maintained indoor under low light irradiance. After 24 h (A to B) white flowers did not homogenously turn to pink (D) and fell down to following day (B to C). (E). Normal pink flower at S3 stage.

**Figure S4.** Alignments used for primer design to clone the partial gene sequences of *PHENYLALANINE AMMONIUM LYASE (PAL)* (A), *CINAMMATE* 4-HYDROXYLASE (C4H) (B), *CHALCONE SYNTHASE* (CHS) (C), *FLAVONOL SYNTHASE (FLS)* (D) and *ANTHOCYANIDIN SYNTHASE (ANS)* (E). Boxes indicate the primers sequences.

**Figure S5.** RNA integrity. Agarose gel (1%) for analysis of RNA integrity, approximately 500 μg of each sample was loaded. The numbers indicate the biological replicates (1 to 5) for each stage (S1 to S4). S1–buds (day 0), S2–white flowers (day 1), S3–pink (day 2), S4–purple (day 3).

**Table S1.** Sugar, organic acids and phenolic acids analysed by GC-EIMS in each developmental stage of *Pleroma raddianum* flowers. S1–buds (day 0), S2–white flowers (day 1), S3–pink (day 2), S4–purple (day 3).

**Table S2**. Pigment profile along *Pleroma raddianum* flower development. S1–buds (day 0), S2–white flowers (day1), S3–pink (day 2), S4–purple (day 3).

Table S3. Identity of obtained cDNA fragments from Pleroma raddianum with Eucalyptus grandis sequences.

 Table S4. Standard curves parameters.

 Table S5. Primers used for gene cloning and RT-qPCR.

Table S6. RNA quantification by nanodrop. The numbers indicate the biological replicates (1 to 5) for each stage

(S1 to S4). S1-buds (day 0), S2-white flowers (day 1), S3-pink (day 2), S4-purple (day 3).



**Figure S1.** Agarose gel (0.8%) showing the cloned fragments. The abbreviations indicate: *PHENYLALANINE AMMONIUM LYASE (PAL), CINAMMATE* 4-HYDROXYLASE (C4H), CHALCONE SYNTHASE (CHS), FLAVONOL SYNTHASE (FLS) and ANTHOCYANIDIN SYNTHASE (ANS), ELONGATION FACTOR 1-α (EF1) and *RIBOSSOMOAL PROTEIN S13 (RPS)* genes.



Figure S2. Phenograms for PHENYLALANINE AMMONIUM LYASE (PAL) (A), CINAMMATE 4-HYDROXYLASE (C4H) (B), CHALCONE SYNTHASE (CHS) (C),

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were included in the analyses: Eucalyptus grandis (Eucgr), Arabidopsis thaliana (AT), Brassica rapa (Brara), Medicago trunculata (Medtr), Trifolium pratense (Tp) and Solanum

lycopersicum (Soly). Trees were constructed using the following parameters: Neighbor-joining, Bootstrap of 1,000 replicates and the best model test for each analysis. Grey

boxes show the clusters for Pleroma raddianum (T.pulchra) and E. grandis sequences.

6 8 9

10 11



14Figure S3. Pleroma raddiannum flowers need light to turn from white to purple colour. Plants were15maintained indoor under low light irradiance. After 24 h (A to B) white flowers did not16homogenously turn to pink (D) and fell down to following day (B to C). (E). Normal pink flower at S317stage.

A	760	770	780	790	800	810	820	830	840	850	860	870	880	890	900
Eucgr . A01144.1	GCCTCGCCGGCAT-	ACATEGOGE	TICTITCAGC	GCGGCCANA	AGOSCOTO	CACTTGTCAA	CAGCACTECA	GTTGGGTC	GGGCTGGCTGC	GATOSTICTO	TTCGAGACC	ACATTETTO	COTTOTO	GAGAICTTA	COGCA
Eucgr. G02848.1	GCCTTGCCGGGATC	SLGCACGOG-	TICTITGACC	GCAGCCCAAA	GAGGGCCT-G	COCTCGTGAA	TOOCACOUCA	GTCGGGTC	GGGCTCGCTTC	CATTGTCCTG	TACGAOGCOJ	ACATCETGG	COTCETCTC	GAGGTTCTGT	reager
Eucgr. G02849.1	GCCTTGCCGGGATC	ALGCACGGG-	TTCTTTGACC	GCAGCCCAM	GAGGGCCTCG	COATCGTGAA	TEECACOICA	GTCGGGTC	GGGCTCGCTTO	CATTGTCCTG	TTCGAOGCG	ACATCETGG	COTCETCTC	GAGGTTCTGT	reasee
Eucgr.G02850.1 Eucgr.G02851.1	ACCTTGCCGGGATC	ALGCACGGG-	TTCTTTGACC	GCAGCCCAM	GAGGGCCTCG	COATCOTORA COATCOTORA	TGGCACOSCA TGGCACOSCA	GTCGGGTC GTCGGGTC	GGGCTCGCTTC GGGCTCGCTTC	CATTGTCCTG	TTCGA0GTGJ TTCGA0GCGJ	ACATCEAGO	COTCETCTC	GAGGTTCTGT	rcases rcases
Eucyr . 002852 1 Eucyr . J00907.1	AGCTOGCTOGCATO	MOCACOOS-	TICTTCGAGT	GCAGCCGAN	GANGGACTO	CONTROLAD CONTROLAD	TGGCACGICA TGGGACGICA	GTTGGGTC	GCCTTAGCTCC	CATTGTOCTG	TTCGA0GCGJ	ATATCTCA	CONTRETCTOR	GAAGPTCTGT	ICTGCA
Eucgr.J01079.1	GGCTCGCCGGGATT	GA-CACGOGC	TTCTTCGAGC	GCAGCCAAN	GAAGGGTTGO	COCTOGTORA	TEGCACOSCA	GTCGGGTC	GOCCTOSCTTO	CATOGTOCTO	TTCGACGCT	ACATACTOR	COSTCETOTE	GAGGTCCTGT	PCAGOG
	910	920	930	940	950 3	960	970	980	3 990	1000	1013	10:0	1030	1040	1050
Eucgr.A01144.1	ATCTTTSCGGAGCT	CATOCAAGGC	AGCCCGAAT	CACCGACCAT	TTGACOCA"A	AATTGAGGEA	CCATCCTGGT	CACATTGAA	TCTGCGGCTAT	CATOGAACAC	ATTCTOGATO	GAAGCOCCT	ATGTCARAGO	OCAALGAAG	TOCCC
Eucgr. G02848.1	ATCTTC3CCGAGGT	SATOCAAOOG	MGCCGGAGT	CACCGACCA	TTGACCCACA	AGCTCAAGCA	CCATCCCGGA	CANATTGAN	GCTGCAGCTAT	ANTOGASCAC	ATCTTOGATO	GGAGCTCCT	CGTGAAGGA	GCTCANANGO	TCCAC
Eucgr. G02849.1	ATCTTC3CCGAGGT	ATGCAAGGG	ANGCCOGAGT:	CACCGACCA	TTGACCCALA	AGCTCARGER	CCATCOCOGA	CANATIGRA	GCTGCAGCTAT.	ANTOGASCAC	ATCTTOGATO	JOGAGCTCCT/	CGTGARGGA	GCTCAAAAG	TCCAG
Eucgr. G02850.1 Eucgr.G02851.1	ATCTTC3CCGAGGT	SATOCANOGO	MGCCGGAGT	CACTGACCA	TTGACCCACA	AGCTCANGEN	CCATCCOGA	CANATTGAN	GCTGCAGCTAT	ATGGASCAC	ATCTTOGATO	GGAGCTCCT/	CGTGAAGGA	GCTCANANGO	TCCAC
Eucgr.G02852.1 Eucgr.J00907.1	ATATTTSCTGAAGT	CATOCAAOGO CATOCAAOGA	ANTCCOGAGT ANGCCAGAGT	CACCGACCAC	TTGACCCACA	AGCTCARGER RATTGARACA	TCATCCC6GA	CAAATTGAA	GCTGCAGCTAT	CATOGACCAC	ATCTTOGATO	GGAGCTCCT	ACGTGAAGGA	GCTCRARAGO GCCANGRAGT	TOCAT
Eucgr.J01079.1	ATCTTC3CAGAGGT	SATOCAGOOG	MGCCGGAGT	CACAGACCAC	TTGACOCATA	AATTGAAGEA	CCATCCOM	CAGATTGAG	GCTGCGGCTAT	ANTGGASCAC	ATTTTGAT	GAAGCSCTT	ACGTGAAGGC	ICCTARA A AGT	TOCAC
	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
Eucgr.A01144.1 Eucgr.C03570.1	GAGTTTSATCCCTT	CANANGCCG	MGCAAGACC	GTACGCTCTG	AGGACCTCAC	COCAGTOGET	TEGCCCACTE	ATCGAAGTG	ATTOGGTACTO	AACCAMATCO	ATAGAAAGAG	AGATCAACT	CAGTCANTGA	AACCOCCTGA	TCGAT
Eucgr. G02848.1	GAGATGGACCCTCT	CAGAAGCCG	ANGCAGGACCO	ATACGCOCTO	COCACOTOCO	COCAGTOOCT	COOCCCCAG	ATCGAAGTG	ATCAGGGCCGC	CACCAMAATG	ATCGAGAGGG	AGATCAATT	TGTCAACGA	AACCOGCTGA	TCGAC
Eucgr. G02849.1	GAGATGEACCETET	CAGAAGCCG	MGCAGGACCO	ATACGCGCT	CGCACGTOCO	COCAGTOGCT	CGGCCCCCAG	ATCGAAGTG	ATCAGGGCCGC	CACCAMAATG	ATCGAGAGGG	AGATCAACT	TGTCAACGA	AACCOGCTGA	TCGAC
Eucgr. G02851.1	GAGATCIACCETET	CAGAAGCCG	ANGCAGGACCO	ATACGCOCT	COCACOTOCO	COCAGTOOCT	COCCCCCAG	ATCGAAGTG	ATCAGGGCCGC	CACCAMAATG	ATCOACAGOO	AGATCAACTO	TOTCARCOM	AACCOCTG	TCGAT
Eucgr.J00907.1	GATATGEACCCACT	TCAGAAGCCG	MAACAAGATCO	GTACGCGCTG	AGGACTTOCO	CTCAGTGGTT	AGGTCCTCAG	GTGGAAGTG	ATCCGCGCATC	TACCAASTCC	ATTGACCGAC	AGATCAACTO	LAGTTAATGA	AACCENTTGA	TTGAT
A	1210	1000	1230	1040	1250	1240	1070	1200	1090	1300	1310	1320	1300	1340	1350
Eucgr.A01144.1	GTCTCGAGGAACAA	POTCTTOCAC	GITGGCAACT	CCAGGGGACA	COGATTACO	COCCATGIA	CAGCACCCGC	TOGCCATO	GCGTCAATAGG	AAGCTCATG	TOGCACAGT	TETETGAGET	TOTCARCCAC	TTCTACAACA	ATGGC
Eucgr. G02848.1	GTCTCCAGGAACAA	BICTOTOCAT	GAGGGAACT	CCAGGGGACC	CCGATTGOOG	TTCCATGIA	CAACACCCGT	TCGCCATO	GCCTCCATCGG	AAGCTCATG	TOGOGCAGT	TCTCCGAGCT	CGTCAACGAC	TTCTACAACA	ACGG3
Eucgr.G02849.1	GTCTCCAGGAACAA	SCTCTCCAT	GAGGGAACT	CCAGGGGACO	CCGATTGGCG	TTCCATGIA	CAACACCCOT	TCGCCATO	GOCTOCATOGO	AAGCTCATG	TTGCGCAGT	TCTCCGAGCT	COTCARCEAC	TTCTACAACA	AC003
Eucgr.G02850.1 Eucgr.G02851.1	GTCTCCAGGAACAA GTCTCCAGGAACAA	DICTOTOCAT DICTOTOCAT	GEAGOGAACTI GEAGOGAACTI	CCAGGGGACC	CCGATTGGOG	TTCCATGIA	CAACACCOGT	CTOGECATO	GOCTOCATOGOK GOCTOCATOGOK	AAGCTCATG	TOGOGCAGT	TCTCC6AGCT TCTCC6AGCT	CGTTANCGAC CGTTANCGAC	TTCTACAACA TTCTACAACA	ACGG3 ACGG3
Eucgr. G02852.1 Eucgr. J00907.1	GTCTCCAGGAACAAI GTCTCCAGGAACAAI	SCTCTCCAT	GTAGGGAACT	CCAGGGGAC	CCGATTGGCG	TTCCATGIA	CAACACCCOT	TOGECATO	GOCTOCATOGGG	AGGCTCATG	TTOGOGEAGT	TCTCCGAGCT	CGTCAACGAC TGTCAATGAC	TTCTACAACA TTCTATAACA	ACGG3 ATGG3
Eucgr. J01079.1	GTCGCGAGGAACAA	BOCCTOCAC	GITGGGAACTI	CCAGGGGACC	CCGATTGGTG	ICTCCATGIA	CAACACTOCO	TGGCGATT	GCGTCCATAGG	AAGCTCATG	TCGCGCAGT	TCTCMAGCT	TGTCAACGAC	TTCTACAACA	ACGGG
	1360	1370	1380	1390	1400	1410	1420	1430	1440	1450	1460	1470	1400	1490	1500
Eucgr. A01144.1	TTGCCTTCCAATCT	ACTOCTOCT	ASCARCCAR	CCTAGACTAC	GOCTTCAACG	BAGCCGAGAT	AGCCATGOCA	CUTACTO	TOTGAGTTICCA	TACTTOGCA	ATCCAGTCA	CARCENTET	GCAGAGTGCG	GAGCAACACA	ACCAS
Eucyr. 602848.1	CTGCCCTCTAACCT	TCC0G0GGA	CHARCECAN	CTTOGATTA	GOCTTCANOG	AGCTGAGAT	AGCCATGGCC	CATACTOC	TCGGAGCTGCAG	TTTCT:GOC	ACCOUNTER	CCARCEATGT	CCAGASCOCO	GAGCAACACA	ACCAS
Eucgr. G02849.1	CTOCCCTCTAACCT	TOCOGOGGA	CICAACCCCA	CCTOGATTAT	GOCTTCAAOG	AGCCGAGAT	CGCCATGOCC	CATATTOC	TEGGAGETGEA	TTCCTIGCC	ACCURTCA	CCAACCATGT	CGAGAGCGCG	GAGCAACACA	ACCAS
Eucgr. G02851.1	CTGCCCTCTAACCT	TCCGGGGGA	CIGAACCCGAG	CCTOGATTAT	GOCTTCANOG	BAGCCGAGAT	COCCATGOCC	CATACTOC	TCGGAGCTGCA	TTCCTTGCC	ACCUTTER	CCARCEATGT	CGAGAGCGCG	GAGCAACACA	ACCAS
Eucgr. G02852.1 Eucgr. J00907.1	TTOCCTCTAACCT	TCTGGCGGT	CEARCOCARC	CCTOGATTA: CTTOGATTAI	GOTTTTAAAGO	JAGCTGAGAT	AACAATGOCC	CTTATIC	TCOGAGCTOCA	TTCCTGGCC	ACCOUNTER	CCARCCATGT	CCAGAGCGCG ACAAAGTGCT	GAGCAACACA	ACCAS
Eucgr. J01079.1	CTGCCCPCAAATCT	21000000000	CSCARCCCTAC	CCTOGACTA:	GOCTTCAROON	DTOCAGAGAT	COCCATOCCO	PEATACTOR	TCOGAGCECCAJ	TTCCTCGCC)	UATCOOPTA	CCARTCRTOT	CCAGAGOGGG	GAGENCERCE	ACCAS
	1510	1520	1530	1540	1550	1560	1570	1580	1590	1600	1610	1620	1630	1640	1450
Eucgr.A01144.1 Eucgr.C03570.1	GACGTGAACTCCCT GATGTGAACTCTCT	DOCCTGATC	TECTERAGEN/	GACOGCCGA3	GCAGTCGACA' OCCATCGATA'	CCTGCAGTT	CATGTCGACT	ACCTTCTTO	GTGGCGCTCTG( GTGGCACTITG)	CAGOCOGTO	ACCTGAGGC	ATCTCG AGGA	ARATCTORAG	AGCGCAGTGA	AGAGC
Eucgr. G02848.1 Eucgr. G02848.2	GACGTGAACTCCTT	SOCCTGATC SOCCTGATC	TEGTEGAGCA/	GACCGCCGA3	GCCGTCGACG	ICCTGAACT	CATGTCCTCC	ACCTTCCTO	GTCGCCCTGTGC GTCGCCCTGTGC	CAGGCCATCO	ACCTGAGGC	ACCTGE AAGA	GAACCTCAN	AGCGTGGTCA AGCGTGGTCA	AGAAC
Eucgr. G02849.1	GACGTGAACTCCTT	BOCCTGATC	TEGTEGAGCA	GACTGOCGAS	GCCGTCGACG	TOCTGAAGET	CATGTCCTCC	ACCTTCCTG	GTCGCCCTGTGC	CAGOCCATCO	ACCTGAGGC	ACCTGG AGA	GAACCTCAAG	AGCGTGGTCA	AGAGE
Eucgr. 902851.1	GAUGTGAACTOUTT	AUCUTUATU	TUTUSAGUA	ACTUCUAS	GCCGTTGACS	ISCTORAG.T	CATGTOCICC	CETTEET	STUBELLISTER	CAGGCCATCI	ACCTORAGE	ACCTUS	BAACUTUAM	AGESTOSTCA	AGAAC
Eucgr.J00907.1	GATGTGAACTCATT	MGATTGATC	TETGETAGAN	AACAGOGGAA	GCAGTCGACA	TCTACAGET	GATGTCASCA	COTTICIA	GTTGCACTITG	CANOCIATA	ACCTGAGGC	ATTTGE AAGA	ARACTTORAG	AGCACOGTCA	AGAAC
Eucgr.J01079.1	GATGTCAACTCCCT	MOCCTGATC	TEATCAAGGAI	GACCGCCGAS	GOCATOGACA	TATTGAAGET	CATGTCTICC	ACTITICCTO	arrectcister 2	CAGOCAGTT	ACCTGAGGC	ATCTGS AGGA	GANCCTGANG	AGCACGGTGA	AGAAC
	1660	1670	1680	1690	1700	1710	1720	1730	1740	2750	1760	1770 3	1700	1790	1800
Eucgr.A01144.1 Eucgr.C03570.1	COGTGACCAAGT TCGGTCTTCCAAGC	DOCCARGAOG AGTGARGARA	ACOCTTACCAO	GTCAGAAGAT	GGGGAGCTOCI GGCTTGCTOC	ACCCATCGAC TTGAATCGCG	GTTCTGCGAG	AAGGATCTO	CTCAAAGTGGTC TTGCAOGTCGT	GATCOOGAG GAACATGAG	COGTOTICA	CTTATCTOGA CCTATCTAGA	CGACCECTGO	AATCOTTOCT.	ACGTT
Eucgr. G02848.1 Eucgr. G02848.2	ACGGTGAACCAAGT	DICCANGANG	GTOCTCTACGT GTOCTCTACGT	COGOTOCAAC	GOCGAGCTCC	ACCCGTCGTG	GTTCAGCERG	AAGACCTG	ATCAAOGTOGT	GACCOGAR	ACGTCTTCG	CCTACATOGA CCTACATOGA	TGACCECTGO	AGCGCCACGT.	ACCCC
Eucgr. G02849.1	ACOGTGAACCAAGT	BOCANGANO	GTOCTCTACG	COGOTOCAA	GOCGAGCTCC	ACCOUNTED	GTTCAGCGAG	AAGACCTG	ATCAAOGTOGTO	GACCOGGAG	ACGTCTTCG	CCTACATTGA	TGACCCCTG	AGCGCCACGT	ACCCC
Eucyr. G02851.1	ACGGTGLACCAAGT	SCCANGANG	GTOCTCTACG	TEGGTCCAL	GGCGAGCTCC	CCCGTCGIG	GTTCAGCCAG	AAGACCTG	ATCAAOGTOGT	GACCOGAG	ACGTOTTO	CCTACATTGA	TGACCECTGO	AGCGCCACGT	ACCCC
Eucgr . J00907.1	ACOGTALGECAGAT	COCANGANO	GTGTTAACCA	GOGACOCAA	GGAGAGCTCC	TCCGTCALG	ATTCTGCCAG	AAGATCTO	TCAAAGTGGT	GACCOTGAA	ATGTATTCO	CATACATTOA	TGATCEATO	AGTGCGACTT.	ACCCA
augr:0010/9.1	AND TO POLICIAL		a chicki (G)			10/-	1010 I GI GAG	1000	1000	1007	A. DIGITCA	1000	1000 IGC	and an and a state of the	manus .
	1810	1820	1830	1840	1850	1860	1870	1880	1890	1900	1910	1920	1930	1940	1950

Figure S4. Alignments used for primer design to clone the partial gene sequences of
 *PHENYLALANINE AMMONIUM LYASE (PAL)* (A), *CINAMMATE 4-HYDROXYLASE (C4H)* (B),
 *CHALCONE SYNTHASE (CHS)* (C), *FLAVONOL SYNTHASE (FLS)* (D) and *ANTHOCYANIDIN* SYNTHASE (ANS) (E). Boxes indicate the primers sequences.

В	10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Eucgr.C00065.1.egr.23569548/1- Eucgr.J01844.1.egr.23599860/1-	ATGGCTCGTCTTACCTCCTTCGTTATCCAACTCCTCTCGAACTACCCCCTCGACAACTACCTCTCCAACTACCTTTCGCCGCGCGCG
Eucgr.C00065.1.egr.23569548/1- Eucgr.J01844.1.egr.23599860/1-	160 170 180 190 200 210 220 20 240 250 260 270 260 270 280 290 300 TACCTATTTTCGACGTGGGGGGGACATCCCCGGGGGGGGG
Eucgr.C00065.1.egr.23569548/1- Eucgr.J01844.1.egr.23599860/1-	
Eucgr.C00065.1.egr.23569548/1- Eucgr.J01844.1.egr.23599860/1-	460 470 480 490 500 510 520 530 540 550 560 570 580 590 600 GRICALTGGCGCALAATGCGTCGCATCALGACGACGACGACGACGACGACGACGACGACGACGACGACG
Eucgr.C00065.1.egr.23569548/1- Eucgr.J01844.1.egr.23599860/1-	610         620         630         640         650         660         670         680         690         700         710         720         730         740         750           GGGATCGTTATCAGGATCGAGTCGAGATGATGCTCTACAGACATCATGTATCGGATCATGTTCGACTCCAAGTCCAAGTCGAGTCGCCAGTCGAGCGAG
Eucgr.C00065.1.egr.23569548/1- Eucgr.J01844.1.egr.23599860/1-	760         770         780         770         800         810         820         830         840         850         860         870         880         890         900           TTCGACTACAATTACCGCGATTTCATTCCCATGCTCAGGCCTTCTTCGAAAGGTTACTTGAACGAAGGCGCAAGGCGCCTTGCCTTTTTTTGACAACTACTGTCGACGAAGAGCGAAAATAATGGCAGCGGAAT         TTCGACTACAACTACGGCGCTTGCCTTTCTGAAGGACGACGAAATCAGCGCGGCTTGCCTTTCTGAGGACGCGCCTTGCCTTTCTGAGGACGAGAAGGAGAAGGAAAATAATGGCAGCGGAAT           TTCGACTACAACTACGGCGATTTCATTCCCCATGCTCAGGGCTAGCTGCAGCTGCCTGC
Eucgr.C00065.1.egr.23569548/1- Eucgr.J01844.1.egr.23599860/1-	910 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040 1050 GOGGACAGAC/ACCAGATIAACACCCCCCACAACCCCCCACAACCCCCACAACCCCCACA
Eucgr.C00065.1.egr.23569548/1- Eucgr.J01844.1.egr.23599860/1-	1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200 GEAGAGETEGETEARTECTECCARTEC
Eucgr.C00065.1.egr.23569548/1- Eucgr.J01844.1.egr.23599860/1-	1210 1220 1230 1240 1250 1260 1270 1280 1270 1280 1290 1300 1310 1320 1330 1340 1350 1350 1350 1350 1350 1350 1350 135
Eucgr.C00065.1.egr.23569548/1- Eucgr.J01844.1.egr.23599860/1=	1360         1370         1380         1390         1400         1410         1420         1430         1440         1450         1460         1470         1480         1490         1500           AGGTTCT TGGAGGGGGGCGAGGTCGGAGGTCGGAGGTCGGACTCAGGTACTTCAGGTACTCCGGTTCGGGGTGGGAGGGGGCGGGGGGGG
Eucgr.C00065.1.egr.23569548/1- Eucgr.J01844.1.egr.23599860/1-	1510 1520 1530 1540 1550 1560 1570 1580 1590 1600 1610 1620 1630 1640 1650
Eucgr.C00065.1.egr.23569548/1- Eucgr.J01844.1.egr.23599860/1-	

Figure S4. (Continued)



**Figure S4.** (Continued)

D Eucgr.F03761.1 Eucgr.F03763.1 Eucgr.L00738.1 Eucgr.L00738.2	10  20  30  40  50  60  70  80  90  100  110  120  130  140  150
Eucgr.F03761.1 Eucgr.F03763.1 Eucgr.L00738.1 Eucgr.L00738.2	160       170       180       190       200       210       220       230       240       250       260       270       280       290       300         CCCCACCACGCTCTTATCCGGGAAATCCACGAGGCGTCCCCGCACTGGGGATTCTTCCTTGTGACCGATCACGGGATACCACCGGAGCTGATCGGCAGCTGCAGGGGGCCGGCGGCGGCGGCGGCGGCGGCGCGGGAGCTCCCCGCAGGAGGAGGAGAGAG       CCCCACCACGCTCTCATCCGGGAAATCCACGAGGCATGCCGCGACTGGGGGATTCTTCCTTGTGACCGATCACGGGATACCACGGGATCCGCGGGAGCTGAGGGGGGCCGTGAGGTCCTCAGGGGAGCCGGCGGGGCCGTGGGCGGCCGGGAGGCCGGGGGCCGCGGGAGGCCGCGGGGGG
Eucgr.F03761.1 Eucgr.F03763.1 Eucgr.L00738.1 Eucgr.L00738.2	310 320 330 340 350 360 370 380 390 400 410 420 430 440 450 366 370 380 390 400 410 420 430 440 450 366 366 366 366 366 366 366 366 366 36
Eucgr.F03761.1 Eucgr.F03763.1 Eucgr.L00738.1 Eucgr.L00738.2	460 470 480 490 500 510 520 530 540 550 560 570 580 590 600 CACCCCCCCTTCTTACAGGGAAGTCACTGAAGAATACAACAAGGAGATACTGAAAGTGACAGAGGTGCTGCTGGAGCTGATCTCGGAGGGCCTAGGAGAAAAATGTCTTGAAAGCAAGTTTGGGAGGTGACCAGATGGAGCTAGAG CACCCCCCCTTCTTACAGGGAAGTCACTGAAGAATACAACAAGGAGACACTGAAAGTGACAGAGGTGCTGCTGGAGGTGATCTCCGAGGGCCTAGGACTGGAGGAAAAATGTCTTGAAAGCAAGTTTGGGAGGTGACCAGATGGAGGTGACCAGATGGAGGTGACCAGATGGAGGTGACCAGATGGAGGTGACCAGATGGAGGTGCTGCTGGAGGCGAGCTGATCTCCGAGGCCTAGGACTGGAGGAAAAATGTCTTGAAAGCAAGTTTGGGAGGTGACCAGATGGAGGTGCTGCTGGAGGTGATCTCCGAGGCCTAGGACTGGAGGAGAACGGCCGCGGGGAGGAGGCGACCAGATGGAGGCGACCAGATGGAGGCGACCAGATGGAGGAGGCGGCGGACAAGCTGTTCCTCTGCCTCTCCCTGGGCCTGGGCGGGGGGGCGGC
Eucgr.F03761.1 Eucgr.F03763.1 Eucgr.L00738.1 Eucgr.L00738.2	610 620 630 640 650 660 670 680 690 700 710 720 730 740 750 ATGAAATAAACTGTACCACCTGCCCCACACCTCGCCTCG
Eucgr.F03761.1 Eucgr.F03763.1 Eucgr.L00738.1 Eucgr.L00738.2	760       770       780       790       800       810       820       830       840       850       860       870       880       890       900         TACTTGCCCGATGCTCTTTTCTGCCACGTCGCCGATCAAATAGAGGTTCTAAGCAACGGAAAGTACAAAAGTGTACTTCATAGGAGCTTGGTGAACAAGGGAAAAGACGCGAATGTCATGGGGAGTGTTTGTGCACCCCCACAGAGGCA       760       770       780       770       780       800       810       820       830       840       850       860       870       880       890       900         TACTTGCCCGATGCTCTTTTCTGCCACGTGGCGAGTGTCAAAGGGGTTCTAAGGCACGGAAAGTACAAAAGTGTACTTCATAGGAGCTTGGTGAACAAGGCAAAAGGCGCGAATGTCATGGGGCAGGTTTGTTGCACCCCCGCAAAGGCAA       760
Eucgr.F03761.1 Eucgr.F03763.1 Eucgr.L00738.1	910 920 930 940 950 960 970 980 990 1000 

**Figure S4.** (*Continued*)

E	10	20	30	40	50	60	70	30	90	100
Eucgr.D01945.1.egr.23575751/1- GSVIVT01019892001.vvi.17829430	ATGGTGAGCGTTGTGG	CTGGTAGAST	GAGAGCTTG	TCCAGCAGTG	CATTCAGTCO GATCCAGTCO	ATCCCCAR	CAGTATOTGAC	GCCGAAGGAG	GAGCTCACA	AGCA
	110	5'—	130	140	3'	160	170	180	190	200
Eucgr.D01945.1.egr.23575751/1- GSVIVT01019892001.vvi.17829430	TTGGCGACATCTICGA TTGGCAATGTCTITGA	GGAGGAGAAG GGAGGAGAAG	AGCATGAGG	GCCCTCAGGT GCCCTCAGGT	TCCAACTATT	ACCTCGAGG	ACATAGCGTC1	GAGGACGAG	TGGTCAGGG	MGAG
Rucgr.D01945.1.egr.23575751/1- G&VIVT01019892001.vvi.17829430	210 GTGCCACGAGGAGCTC ATGCCOGGAGGAGTTC	220 AGSAAGGCTGC	230 CACCGACTG	240 GGCGTCATG	250 CACCTOGTCAL CACCTTGTCAL	260 ACCATGOGAT	270 CCCCAACGACC CTCTGATGACC	280 TCATTGAGCO	290 TIGTCAAGAA TIGTTAAGGT	300
Eucgr.D01945.1.egr.23575751/1- GSVIVT01019892001.vvi.17829430	310 GGCGAGGTGTTCTTCJ GGAGAGACCTTTTTCJ	320 ACCTCCCGATC	330 	340 6AGAAGCATG 6AGAAGTATG	350 CCAACGACCAC CTAATGACCAC	360 	370 AAGATCCAGGO AAGATCCCTGO	380 CIACGGGAGG	390 AAGCTTGCA	400   AACA 
Eucgr.D01945.1.egr.23575751/1- GSVIVT01019892001.vvi.17829430		420 CGASTGGGAG TGAGTGGGAG	430 SACTACTTCT	440 TCCACCTOST TCCACCTCAT	150 TTACCCTGAG	460 SACAAGCGTG SACAAGCGCG	470 ACTTGTCCATC	480 TEGCCCANG	490 	500 ATTA

**Figure S4.** (Continued)



33 34 Figure S5. RNA integrity. Agarose gel (1%) for analysis of RNA integrity, approximately 500 µg of each sample was loaded. The numbers indicate the biological replicates

(1 to 5) for each stage (S1 to S4). S1-buds (day 0), S2-white flowers (day 1), S3-pink (day 2), S4-purple (day 3).

TableS1. Sugar, organic acids and phenolic acids analysed by GC-EIMS in each developmental stage
 of *Pleroma raddianum* flowers. S1-buds (day 0), S2-white flowers (day 1), S3-pink (day 2), S4-purple
 (day 3).

Carbohydrates	S1 (mg g <sup>-1</sup> DW)	S2 (mg g <sup>-1</sup> DW)	S3 (mg g <sup>-1</sup> DW)	S4 (mg g-1 DW)
1- Glycerol	$4.34 \pm 0.59$	$2.98 \pm 1.49$	$2.76\pm0.74$	$3.78 \pm 1.05$
2- Myo-inositol	$25.00 \pm 2.76^{a}$	$21.93 \pm 4.51^{ab}$	$19.52 \pm 4.03^{ab}$	16.59 ± 1.83 <sup>b</sup>
Total sugar alcohol <sup>np</sup>	<b>22.61 ± 13.28</b> <sup>a</sup>	$24.91 \pm 3.58^{a}$	$22.28 \pm 3.42^{ab}$	$20.37 \pm 1.42^{b}$
3- Ribose	$0.42 \pm 0.12$	$1.33 \pm 0.10$	$0.71 \pm 0.38$	$0.92 \pm 0.70$
4- Xylose <sup>np</sup>	2.79 ± 1.25ª	$6.85 \pm 0.44^{b}$	$3.02 \pm 2.13^{a}$	$5.72 \pm 1.40^{ab}$
5- Fructose	$199.58 \pm 60.88^{b}$	500.06 ± 61.65ª	474.17 ± 85.72ª	492;13 ± 124.60ª
6- Glucose <sup>np</sup>	25.07 ± 12.99 <sup>b</sup>	$75.91 \pm 2.53^{ab}$	82.75 ± 2.41 <sup>a</sup>	$72.66 \pm 3.58^{a}$
7- Galactose	$112.08 \pm 44.44^{b}$	324.82 ± 35.46 <sup>a</sup>	<b>294.86</b> ± 52.55 <sup>a</sup>	279.70 ± 62.15 <sup>a</sup>
Total soluble sugar	361.92 ± 112.79 <sup>ь</sup>	902.71 ± 81.55ª	876.29 ± 133.88ª	857.64 ± 209.19ª
Organic acids	S1 (mg g <sup>-1</sup> DW)	S2 (mg g <sup>-1</sup> DW)	S3 (mg g <sup>-1</sup> DW)	S4 (mg g <sup>-1</sup> DW)
8- Succinic acid	$0.08 \pm 0.04^{b}$	$0.24 \pm 0.05^{a}$	$0.28 \pm 0.03^{a}$	$0.22 \pm 0.05^{a}$
9- Malic acid	20.83 ± 12.97°	92.52 ± 18.00 <sup>b</sup>	$116.66 \pm 16.51^{ab}$	<b>129.41 ± 27.11</b> ª
10- Citric acid	$4.71\pm2.46$	$5.81 \pm 1.16$	$6.40\pm0.63$	$6.96\pm0.73$
Total	25.60 ± 14.87°	98.48 ± 18.41 <sup>b</sup>	$121.68 \pm 17.89^{ab}$	136.55 ± 26.81ª

38 DW–Dry weight. Compounds highlighted in bold showed statistically significant differences between stages.

39 Letters indicate statistically significant different values. Parameters indicated with a superscript np (np)

40 had non-parametrical distribution and the Kruskal-Wallis test was applied.

Table S2 Pigment profile along Pleroma raddianum	flower development S1-	-buds (day 0) S2-white flowers	(day 1) S3-pink (day 2) S4-purple (day 3)
Table 52. I ignient prome along Fleromu ruuuunun	<i>i</i> nower development. 31-	-buus (uay 0), 32–wille nowers	(uay 1), 55-pilik (uay 2), 54-pulpie (uay 5)

Phenolic acids	S1 (mg g <sup>-1</sup> DW)	S2 (mg g <sup>-1</sup> DW)	S3 (mg g <sup>-1</sup> DW)	S4 (mg g <sup>-1</sup> DW)
1- Phenolic acid	$4.43 \pm 2.96$	$5.9 \pm 2.67$	$3.76 \pm 2.46$	7.6 ± 3.08
2- Cinnamic acid derivative <sup>np</sup>	$3.89 \pm 2.28$	$4.18\pm3.85$	$6.16 \pm 1.50$	$3.53 \pm 3.41$
3- Phenolic acid <sup>np</sup>	$0.84\pm0.91$	$1.55\pm0.17$	$1.85\pm0.20$	$1.92 \pm 0.21$
4- Phenolic acid <sup>np</sup>	$0.38 \pm 0.75$	$0.14 \pm 0.22$	$0.14 \pm 0.21$	$0.15 \pm 0.20$
5- Cinnamic acid derivative <sup>np</sup>	$0.23 \pm 0.23$	$0.57\pm0.62$	$0.22 \pm 0.16$	$0.26 \pm 0.27$
6- Phenolic acid	$0.90 \pm 0.61^{b}$	5.24 ± 1.33ª	$4.19 \pm 1.08^{a}$	$4.23 \pm 0.94^{a}$
Total phenolic acids	$10.69 \pm 3.43^{\text{b}}$	$17.58 \pm 2.66^{a}$	$16.39 \pm 2.24^{a}$	$17.69 \pm 0.49^{a}$
Flavonols	S1 (mg g <sup>-1</sup> DW)	S2 (mg g <sup>-1</sup> DW)	S3 (mg g-1DW)	S4 (mg g-1DW)
7- N.I.	$0.40\pm0.41$	$0.70\pm0.13$	$0.49\pm0.40$	$0.57\pm0.34$
8- Myricetin hexoside	$0.77\pm0.47$	$0.56\pm0.19$	$0.56\pm0.08$	$0.58\pm0.18$
9- Myricetin hexoside	$0.29\pm0.21$	$0.22\pm0.13$	$0.23\pm0.07$	$0.27\pm0.06$
10- Quercetin galloylhexoside	$0.41\pm0.30$	$0.22\pm0.10$	$0.23\pm0.06$	$0.23\pm0.16$
11- Quercetin hexoside	$1.26 \pm 0.65$	$1.02\pm0.37$	$0.98\pm0.19$	$0.94 \pm 0.16$
12- Quercetin glucuronide <sup>np</sup>	$0.16\pm0.07$	$0.06\pm0.03$	$0.07\pm0.04$	$0.11\pm0.06$
13- Kaempferol galloylhexoside <sup>np</sup>	$1.61\pm0.85$	$1.36\pm0.61$	$1.34\pm0.22$	$1.24\pm0.33$
14- N.I. <sup>np</sup>	$0.19\pm0.12$	$0.14\pm0.08$	$0.14\pm0.08$	$0.17\pm0.09$
15- Kaempferol hexoside	$3.40 \pm 1.30$	$3.20\pm0.91$	$3.12\pm0.55$	$2.92\pm0.42$
16- Kaempferol galloylhexoside <sup>np</sup>	$2.16\pm0.94$	$1.78\pm0.72$	$1.78\pm0.46$	$1.70\pm0.23$
17- Kaempferol 3-O-β-D-glucopyranoside/ kaempferol-(2"-O-methyl)-4'-O-α-D-glucopyranoside	$6.48 \pm 1.51$	$6.32\pm0.85$	$6.44\pm0.50$	$6.21 \pm 0.47$
18- Kaempferol pentoside	$2.75 \pm 1.43$	$2.41 \pm 1.07$	$2.23\pm0.63$	$1.96\pm0.30$
19- Kaempferol galloylhexoside	$0.24\pm0.14$	$0.22 \pm 0.11$	$0.18\pm0.07$	$0.16\pm0.04$
20- Kaempferol pentoside	$0.23\pm0.15$	$0.15\pm0.10$	$0.14\pm0.07$	$0.11 \pm 0.05$
21- Kaempferol <i>p</i> -coumaroylhexoside	$0.53\pm0.29$	$0.46\pm0.26$	$0.40\pm0.18$	$0.29\pm0.07$
23- Kaempferol 3-O-glucuronide-6"-O-methylester	$0.28 \pm 0.15$	$0.29\pm0.09$	$0.21\pm0.01$	$0.17 \pm 0.05$

Table S2. (Continued)

Flavonols	S1 (mg g-1DW)	S2 (mg g-1DW)	S3 (mg g-1DW)	S4 (mg g-1DW)
25- Quercetin 3-O-(6"-O-p-coumaroyl)- β-D-glucopyranoside <sup>np</sup>	$0.68 \pm 0.38$	$0.52\pm0.19$	$0.44\pm0.08$	$0.40\pm0.05$
26- N.I.	$0.43\pm0.21$	$0.35\pm0.20$	$0.31\pm0.13$	$0.26\pm0.04$
27-Kaempferol 3-O-(6"-O-p-coumaroyl)-β-D-glucopyranoside <sup>np</sup>	$1.44\pm0.65$	$1.20\pm0.46$	$1.05\pm0.00$	$0.94\pm0.12$
28- Kaempferol <i>p</i> -coumaroylhexoside	$0.19\pm0.09$	$0.16\pm0.06$	$0.10\pm0.03$	$0.08\pm0.02$
29- Kaempferol	$0.94\pm0.48$	$1.05\pm0.44$	$0.83 \pm 0.20$	$0.71\pm0.08$
30- Kaempferol <i>p</i> -coumaroylhexoside <sup>np</sup>	$0.00 \pm 0.00^{b}$	$0.08 \pm 0,00^{\text{b}}$	$0.13 \pm 0.03^{a}$	$0.13 \pm 0.03^{a}$
Total Flavonols	$24.99 \pm 10.31$	$22.17 \pm 6.66$	$21.25 \pm 3.13$	$19.94 \pm 2.64$
Anthocyanins	S1 (µg g-1DW)	S2 (µg g-1DW)	S3 (µg g-1DW)	S4 (µg g-1DW)
22- Petunidin <i>p</i> -coumaroylhexoside acetylpentoside <sup>np</sup>	$0.00 \pm 0.00^{\circ}$	$0.00 \pm 0.00^{\circ}$	8.43 ± 2.21 <sup>b</sup>	$22.42 \pm 8.00^{a}$
24- Malvidin <i>p</i> -coumaroylhexoside acetylpentoside <sup>np</sup>	$0.00 \pm 0.00^{\circ}$	$0.00 \pm 0.00^{\circ}$	$19.87 \pm 5.28^{b}$	$53.72 \pm 20.18^{a}$
Total Anthocyanins <sup>np</sup>	$0.00 \pm 0.00^{\circ}$	$0.00 \pm 0.00^{\circ}$	$28.29 \pm 7.47^{b}$	<b>76.14 ± 28.11</b> <sup>a</sup>

DW–Dry weight. NI- not identified. Compounds highlighted in bold showed statistically significant differences between stages. Letters indicate statistically significant different values in each line. Parameters indicated with a superscript np ( $^{np}$ ) had non-parametrical distribution and the Kruskal-Wallis test was applied.

Gene	Enzyme	Nucleotides number in partial sequence of <i>P. raddianum</i>	Ammino acids number in partial sequence of <i>P. raddianum</i>	Identity of ammino acids (%) with <i>E. grandis</i>
PAL	PHENYLALANINE AMMONIA-LYASE	633	206	95
C4H	CINNAMATE 4-HYDROXYLASE	1,073	357	87
CHS	CHALCONE SYNTHASE	621	207	88
FLS	FLAVONOL SYNTHASE	817	272	69
ANS	ANTHOCYANIDIN SYNTHASE	361	120	83
RPS	<b>RIBOSOMAL PROTEIN S13</b>	371	123	98
EF1	ELONGATION FACTOR $1\alpha$	694	231	93

Table S3. Identity of obtained cDNA fragments from *Pleroma raddianum* with *Eucalyptus grandis* sequences.

Table S4. Standard curves parameters.

Compound	R <sup>2</sup>	equation
<i>p</i> -coumaric	0.99	y=12955x
kaempferol	0.97	y=49285x
cyanidin	0.99	y=364963x

Gene	Description	Forward	Melting temperature	Reverse	Melting temperature	Amplicon	Use
PAL	flavonoid biosynthesis	MTYGTSAAYRGCACBGSWGTYGGGTC	65.3°C	CSRTGCCTSAGGTCDAYBGCYTG	63.4°C	813	Cloning
C4H	flavonoid biosynthesis	CTGGCTCCAGGTSGGCRAYGACCTC	65.4°C	GKCCWCCYTTCTCRSTSGTGTCGAGC	64.3°C	1277	Cloning
CSH	flavonoid biosynthesis	GGYCTSMGCCCCWCCGTCAAG	65.0°C	CGCCCCAYTCSARCCCCTC	63.5°C	661	Cloning
FLS	flavonoid biosynthesis	GGARGTGGARAGARTKCARGC	58.1°C	GACATYCKCGYCTTKTCCTTGTTCAC	56.2°C	863	Cloning
ANS	flavonoid biosynthesis	GAGTYGAGAGCTTGTCSAGCAGTGG	62.7°C	GTAGTCCTCCCACTCRAGCTGSCC	64.7°C	385	Cloning
EF1a	reference gene	GGGTAARGARAAGGTTCACATC	54.9°C	CCRATACCACCAATCTTGASAC	53.7°C	740	Cloning
RPS13	reference gene	GTCGCATGCACAGYCGMGG	61.9°C	CCARACRGGWGGKAGCTTCTTGG	58.1°C	412	Cloning
PAL	flavonoid biosynthesis	GCACGAGATGGACCCGTTGC	61.3°C	GTGCCTTGGAAATTCCCGCC	59.6°C	158	RT-qPCR
C4H	flavonoid biosynthesis	GGCGAGATCAACGAGGACAACGTCC	63.2°C	GAGGTTCATGTGGGGCACGAGG	62.4°C	229	RT-qPCR
CSH	flavonoid biosynthesis	GAACAACAAGGGGGCTCGTGTCC	62.4°C	CCGAGTCAGGCAGGATGGTCTG	61.6°C	168	RT-qPCR
FLS	flavonoid biosynthesis	GCTGACCACGAGGGATTGGTGCG	64.5°C	CTTCTGGAGCCGAGTCCCGTAACC	64.5°C	166	RT-qPCR
ANS	flavonoid biosynthesis	CGGTTGTGCGTGAGACCTGCC	63.3°C	GCCTTGGATCTTCCCCGACCCC	64.3°C	150	RT-qPCR
EF1a	reference gene	GAGGAGCGTCACGGGAAGCAAG	62.3°C	GTAGTAGCGAGCCAGCCTGTGG	61.8°C	142	RT-qPCR
RPS13	reference gene	GGATGGACAGACCCGTGAGCACG	63.8°C	GGGACAAACGGGATCTTCTCGGGG	63.3°C	155	RT-qPCR

 Table S5. Primers used for gene cloning and RT-qPCR.

Stage	Biological replicate	ng/µL	260/280	260/230
	1	875.70	2.11	2.30
	2	494.40	2.07	1.93
S1	3	629.80	2.15	1.78
	4	3,173.30	2.12	2.23
	5	418.70	2.10	2.21
	1	1,012.80	2.13	2.25
	2	847.70	2.10	2.01
S2	3	499.30	2.10	1.96
	4	1,039.80	2.13	2.32
	5	1,135.40	2.13	2.30
	1	888.20	2.11	2.20
	2	1,136.90	2.12	2.37
S3	3	890.30	2.13	2.36
	4	1,026.60	2.12	2.29
	5	702.70	2.16	2.21
	1	1,030.30	2.13	1.93
	2	534.20	2.15	2.08
S4	3	1,262.70	2.14	2.35
	4	1,565.40	2.12	2.31
	5	954.20	2.12	1.92

**Table S6**. RNA quantification by nanodrop. The numbers indicate the biological replicates (1 to 5) for each stage (S1 to S4). S1- buds (day 0), S2- white flowers (day 1), S3- pink (day 2), S4-purple (day 3).