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6 **Supplemental Figure S1. Expression profiles of flavonoid biosynthetic structural genes**
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8
9 **in wild-type tobacco flowers**

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11 15 a) Tobacco flowers used for northern blot analysis. Bar indicates 1 cm.
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13
14 b) Northern blot analysis was performed using total RNAs from isolated petal samples at four
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16 different flower developmental stages (S1-S4) as defined by Nishihara et al. (2005). Total
17
18 RNA, blotted onto nylon membrane, was hybridized by each DIG-labeled gene probe.
19
20 Ethidium bromide stained ribosomal RNA band (rRNA) are shown as a control.
21
22
23
24
25 20 PAL: phenylalanine ammonia lyase (accession number AB289451), CHS: chalcone synthase
26
27 (AF311783), CHI: chalcone isomerase (AB213651), F3H: flavanone 3-hydroxylase
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29 (AB289450), F3'H: flavonoid 3'-hydroxylase (AB289449), DFR: dihydroflavonol
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31 4-reductase (AB289448), ANS: anthocyanidin synthase (AB289447), FLS: flavonol synthase
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33 (AB289452), UBI: ubiquitin (DQ138111).
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41 **Supplemental Figure S2. Alignment of deduced amino acid sequences of two tobacco**

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43
44 5 ***DFR* genes**

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46 The Genbank accession numbers of *DFR* genes were shown as follows; tobacco 1
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48 and 2 (present study), petunia (accession number X79723), gentian (D85185), maize
49
50 (X05068), gerbera (Z17221).
51

- 52
53
54 Asterisks indicate common amino sequences. Triangle and gray box show the
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56
57 10 important amino acid residues that *DFR* enzyme distinguishes DHK with other
58
59 dihydroflavonols as a substrate.
60

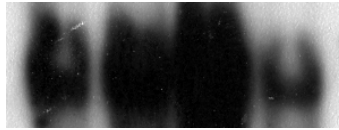
a



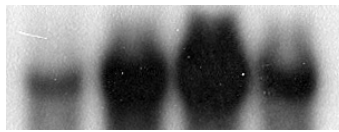
S1 S2 S3 S4

b

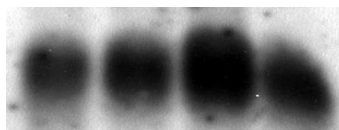
NtPAL



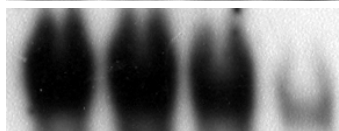
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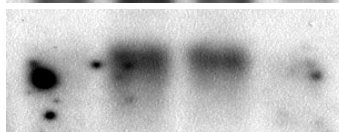
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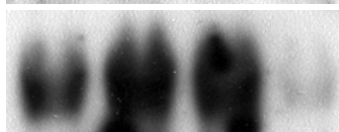
NtF3H



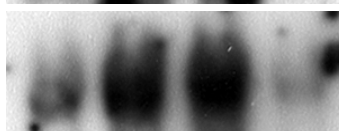
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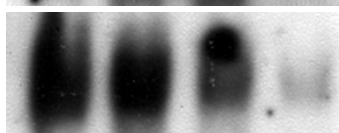
NtDFR



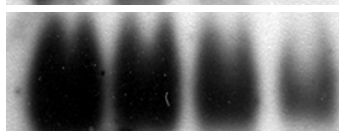
NtANS



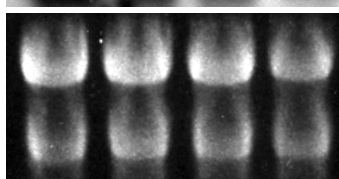
NtFLS



NtUBQ



rRNA



Supplemental Figure S1

Tobacco1	1	MASEAHAHVHAP-PPVA-PTVCVTGAAGFIGSWLVMRLLERGVNHATVVRDPENKKKVKHLFELPKADTNLTLWKADLSV	78
Tobacco2	1	MASEGHAHVHAPSPPAA-PTVCVTGAAGFIGSWLVMRLLERGVNHATVVRDPENKKKVKHLELEPKADTNLTLWKADLSV	79
Petunia	1	---MASEAVHAPSPPVAVPTVCVTGAAGFIGSWLVMRLLERGVNHATVVRDPENKKKVKHLELEPKADTNLTLWKADLTV	77
Gentian	1	-----MEGGILSNATTVCVTGASGYIGSWLAMRLLERGYTVRATVVRDPGNLKKVQHLELEPKASTNLTLLKADLTE	71
Maize	1	-----MERGAGASEKGTVLVTGASGFGVSWLVMKLLQAGYTVRATVVRDPANVGKTKPLMDLPGATERLSIWKADLAE	72
Gerbera	1	-----MEEDSPATVTCVTGAAGFIGSWLVMRLLERGVVHATVVRDPGDLLKVKHLELEPKAQTNLKLWKADLTQ	68
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		▽	
Tobacco1	79	EGSFDEAIQGCQGVFHVATPDMDFESEDPENEVIKPTVGRGLSIIESCACANTVKRLVFTSSAGTLDAQEHQKLFYDETS	158
Tobacco2	80	EGSFVEAIQGCQGVFHVATPDMDFESEDPENEVIKPTVGRGLSIIESCACANTVKRLVFTSSAGTVDVQEHQKLLYDETS	159
Petunia	78	EGSFDEAIQGCQGVFHVATPDMDFESKDPENEVIKPTVGRGLSIIESCACANTVKRLVFTSSAGTLDVQEQKLFYDQTSW	157
Gentian	72	EGSFDEAIHGCHGVFHVATPDMDFESKDPKNEVIKPTIDGFLSIIIRSCVKAQTVKVLVFTSSAGTVDVQEQKPVYDENDW	151
Maize	73	EGSFHDAIRGCTGVFHVATPDMDFLSKDPENEVIKPTVEGMSIIMRACKEAGTVRRIVFTSSAGTVNLEERQRPVYDEESW	152
Gerbera	69	EGSFDEAIQGCQGVFHVATPDMDFESKDPENETIKPTIEGVLISIIIRSCVKAQTVKVLVFTSSAGTVNGQEQQLHVYDESHW	148
		****.*..**.*..*****.*..**.*..**.*..**.*..* ..**.*..* ..**.*..* ..**.*..*	
Tobacco1	159	SDDLFIYAKKMTGWMYFVSKILAEEAAMEAAKKKNDFISIIPLVVGPFITPTFPPLSLITALSITGNEAHYCIKQGG	238
Tobacco2	160	SDDLFIYAKKMTGWMYFVSKILAEEAAMEAAKKKNIDFISIIPLVVGPFILPTFPPLSLITALSITGNEAHYSIIKQGG	239
Petunia	158	SDDLFIYAKKMTGWMYFASKILAEEAAMEAAKKKNIDFISIIPLVVGPFITPTFPPLSLITALSITGNEAHYCIKQGG	237
Gentian	152	SDDLFINSTKMTGWMYFVSKILAEEAAMEAVTKPNIDGFIISIIPLVVGPFITPTFPPLSLITALSITGNEAHYGIKQGG	231
Maize	153	TDVDFCRVVKMTGWMYFVSKTLAEEAALAYAAEHGLDVTIIPTLVVGPFISASMPPLSLITALALITGNAPHYSILKQVQ	232
Gerbera	149	SDDLFIYSKMTAWMYFVSKTLAEEAADWATKGNISFISIIPLVVGPFITSTFPPLSLITALSITGNEAHYSIIKQGG	228
		*..**.*..*..**.*..**.*..**.*..**.*..* ..**.*..* ..**.*..* ..**.*..*	
Tobacco1	239	YVHDDLCEAHIFLYEQPKAEGRFICASHHAIIDYVAKMVREKWPPEYVPTFVKGDIDKDLPVVYFSPKCLTDMGFQFKY-	317
Tobacco2	240	YVHDDLCEAHIFLYEHPKAEGRFICASHHAIIDYVAKMVQEKWPPEYVPTFVKGDIDKDLVSVYFSSKCLTDMGFQFKY-	318
Petunia	238	YVHDDLCEAHIFLYEHPKADGRFICSSHHAIIDYVAKMVREKWPPEYVPTFVKGDIDKDLPVVYFSSKCLTDMGFQFKY-	316
Gentian	232	FVHDDLCEAHIFLYEHPKAEGRYICSSHDTTIDHLAKMIRQNWPEYYIPTKLGIDEDIPVVSFSNNKLDLGFQYKY-	310
Maize	233	LIHDDLCDAEIFLFFENPAAAGRYVCSHDTIHLAAMLRDRYPEYDVPQRFPGIQDDLQVRFSSKCLQDLGFTRYK	312
Gerbera	229	YVHDDLCECHIIYLYENPKAGRYICSSHDTIHQLAKIIKDWPEYYIPTKFGIDEELPIVVSFSNNKLDLGFQYKY-	307
		..*****..**.*..**.*..**.*..**.*..**.*..* ..**.*..* ..**.*..* ..**.*..*	
Tobacco1	318	TLEDMYKGAIECTCRQKQLLPFSTQSTADNDRDKETIPLSAENYASGKENS PVANGTGSSTNGEI	381
Tobacco2	319	TLEDMYKGAIECTCRQKQLLPFSTRSTADNVRDKEAIPLSTENYASGKENS PVANGTGSSTNGEI	382
Petunia	317	TLEDMYKGAIDTCRQKQLLPFSTRSAEDNGHNREAIISAQNYASGKENAPVANHTEMLSNVEV	380
Gentian	311	TLEDMFRGAIDTCCKEKRMLPLSIGHQKESTDPEVDEVVEMELIQDSL-----	359
Maize	313	TLEDMFDAAIRTCQEKGLIPLATAAGGDGFASVRAPGETEATIGA-----	357
Gerbera	308	NLEDMFKAIDTCREKGLLPYSTIKNHINGNHVGVHVIKNNGDDQEKGLLCCSKEGQ-----	366
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Supplemental Figure S2