

Genetic Control of Flavonoid Biosynthesis in Flower Petals of Soybean

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Introduction

Soybean flowers have various colors including purple, white, near white, dilute purple, pale, pink, magenta, purple blue, and light purple (Fig. 1). Six genes (*W1*, *W2*, *W3*, *W4*, *Wm* and *Wp*) primarily control flower color in soybean (Fig. 2). This study was conducted to determine the structure and amount of flavonoids in flower petals.

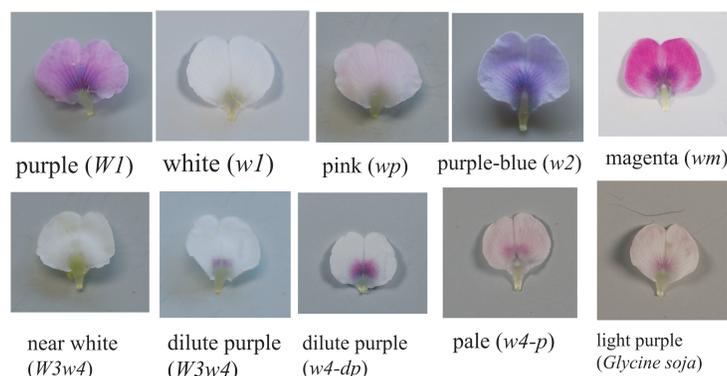


Fig 1. Banner petals of flower color variants in soybean and its wild relative *Glycine soja*.

Materials and methods

Plant materials

Ten cultivars and lines with various flower colors (Table 1) were grown in fields. 200mg of banner petals were collected in three replications.

HPLC analysis

200 mg of petals was extracted with 2 ml of MeOH containing 0.1% HCl for anthocyanin or in 2 ml of MeOH for flavonols and dihydroflavonol. HPLC separation of the isolated anthocyanins, flavonols and dihydroflavonol was performed with Shimadzu HPLC systems using Shim-pack CLC-ODS at a flow rate of 1.0 ml/min, detection wavelength was 190-700 nm and eluent was MeCN/HOAc/H₂O/H₃BO₃ (6:8:83:3) for anthocyanins and MeCN/H₂O/H₃BO₃ (22:78:0.2) for flavonols and dihydroflavonols. The amount of flavonoids were estimated by measurement of peak area (detection wavelength of anthocyanins = 530 nm; flavonols= 351 nm; dihydroflavonols = 290 nm).

Table 1. Flower color and genotypes of flower color genes in soybean lines and a *Glycine soja* line used in this study.

Line name	Flower color	Genotype
Clark	purple	<i>W1 W2 w3 W4 Wp Wm</i>
Clark- <i>w1</i>	white	<i>w1 W2 w3 W4 Wp Wm</i>
Clark- <i>w4</i>	near white	<i>W1 W2 w3 w4 Wp Wm</i>
Clark- <i>W3w4</i>	dilute purple	<i>W1 W2 W3 w4 Wp Wm</i>
Nezumisaya	purple-blue	<i>W1 w2 w3 W4 Wp Wm</i>
Harosoy- <i>wm</i>	magenta	<i>W1 W2 w3 W4 Wp wm</i>
T321	dilute purple	<i>W1 W2 w3 w4-dp Wp Wm</i>
T369	pale	<i>W1 W2 w3 w4-p Wp Wm</i>
LD05-15019-pink	pink	<i>W1 W2 w3 W4 wp Wm</i>
B09021(<i>G. soja</i>)	light purple	<i>w1-lp(t) W2 w3 W4 Wp Wm</i>

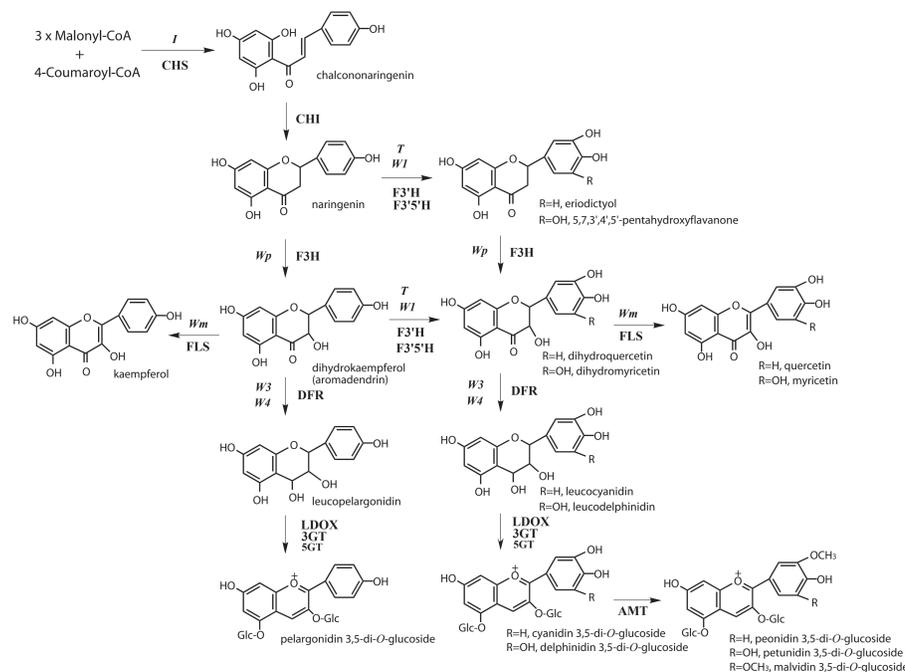


Fig. 2. Schematic diagram of anthocyanin and flavonol synthetic pathway. Enzyme names are abbreviated as follow: chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), flavanone 3-hydroxylase (F3'H), dihydroflavonol 4-reductase (DFR), flavonoid 3'-hydroxylase (F3'H), flavonoid 3'-hydroxylase (F3'S'H), flavonol synthase (FLS), leucoanthocyanidin dioxygenase (LDOX), flavonoid 3-glucosyltransferase (3GT), anthocyanin 5-O-glucosyltransferase (5GT) and anthocyanin methyltransferase (AMT). Soybean genes encoding the enzymes are shown on or to left of the arrows in italics.

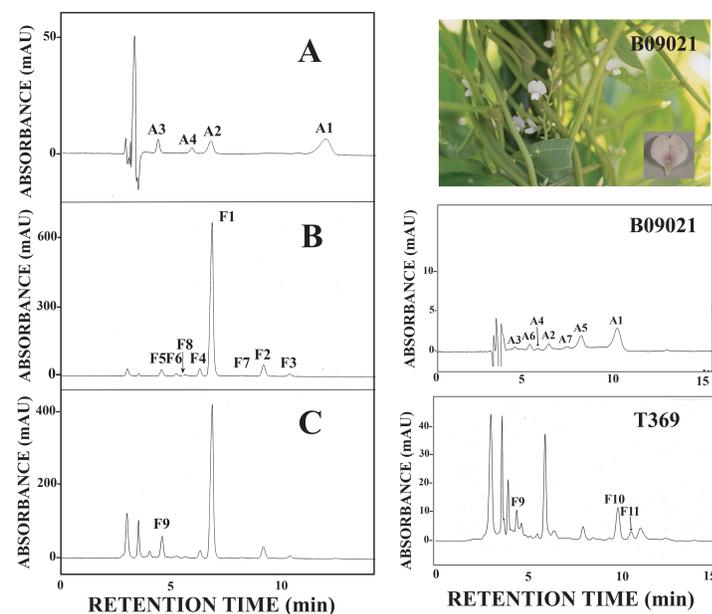


Fig. 3. HPLC chromatogram of anthocyanins (A), flavonols (B) and dihydroflavonols (C) in flower petals of Clark. A1 = malvidin 3,5-di-*O*-glucoside, A2 = petunidin 3,5-di-*O*-glucoside, A3 = delphinidin 3,5-di-*O*-glucoside, A4 = delphinidin 3-*O*-glucoside, A5-A7 = unidentified anthocyanins, F1 = kaempferol 3-*O*-gentiobioside, F2 = kaempferol 3-*O*-rutinoside, F3 = kaempferol 3-*O*-glucoside, F4 = kaempferol 3-*O*-glucoside, F5 = kaempferol 3-*O*-rhamnosyl-(1 2)-[glucosyl-(1 6)-galactoside], F6 = quercetin 3-*O*-gentiobioside, F7 = kaempferol 7-*O*-glucoside, F8 = kaempferol 7-*O*-diglucoside, F9 = aromadendrin 3-*O*-glucoside, F10 and F11 = unidentified dihydroflavonols.

Table 2. Contents of anthocyanins, flavonol glycosides and dihydroflavonols in HPLC analysis ($\times 10^3$) in flower petals of soybean and *Glycine soja* with various flower color.

Lines	Anthocyanins							Total	Flavonol glycosides								Dihydroflavonols			Total	
	A1	A2	A3	A4	A5	A6	A7		F1	F2	F3	F4	F5	F6	F7	F8	Total	F9	F10		F11
2003	Clark	981	715	529	515	-†	-	2,741	16,334	886	192	707	863	451	53	516	20,002	1,498	-	-	1,498
	Clark- <i>w1</i>	-	-	-	-	-	-	-	14,467	1,238	385	740	883	478	201	459	18,851	1,319	-	-	1,319
	Clark- <i>W3w4</i>	†	-	-	-	-	-	-	14,056	1,153	384	787	807	507	90	643	18,427	1,102	-	-	1,102
	Harosoy- <i>wm</i>	1,734	1,052	854	739	-	-	4,378	872	790	273	376	-	296	341	318	3,265	4,167	-	-	4,167
2007	Clark	766	282	399	191	-	-	1,638	7,581	792	221	300	323	162	88	144	9,612	789	-	-	789
	Clark- <i>w4</i>	80	53	-	-	-	-	133	8,170	1,011	377	391	410	182	393	313	11,247	714	-	-	714
	LD05-15019pink	536	275	214	154	-	-	1,179	199	121	-	97	150	77	101	117	862	220	-	-	220
	Nezumisaya	1,439	378	410	218	-	-	2,445	7,480	821	147	181	304	111	41	126	9,210	725	-	-	725
	B09021	317	172	147	116	287	160	1,422	13,465	776	279	-	745	376	-	228	15,868	2,010	-	-	2,010
2008	Clark	933	538	399	255	-	-	2,125	9,433	772	177	441	353	138	12	128	11,454	843	-	-	843
	T321	369	255	178	148	-	-	951	9,418	805	159	371	523	100	174	287	11,838	647	-	-	647
	T369	513	347	233	178	-	-	1,272	703	214	102	135	243	152	130	158	1,837	96	154	54	304

†No pigment was detected.

‡Trace amount of pigment was detected.

Results

1. The recessive alleles at the *W1* (white flower) or the *W4* locus (near white flower) substantially reduced the amount of anthocyanins without affecting the contents of flavonol glycosides or dihydroflavonols.
2. The recessive allele at the *Wp* locus (pink flower) reduced anthocyanins, flavonol glycosides, and dihydroflavonols. The recessive allele of the *Wm* locus (magenta flower) substantially reduced the amount of flavonol glycosides and increased dihydroflavonols without affecting the amount of anthocyanins.
3. The recessive allele of the *W2* locus (purple-blue flower) did not affect amount of flavonoids, but it increased vacuolar pH of flower petals (pH =6.10) compared to purple flower of Bay (pH = 5.73), suggesting that *W2* is responsible for vacuolar acidification in flower petals.
4. A new flower color phenotype (light purple) was discovered in a Japanese accession of *Glycine soja*. Complementation analysis suggested that a new allele at the *W1* locus is responsible for light purple flowers. Light purple flowers contained three novel anthocyanins together with lower amounts of the four anthocyanins present in purple flowers. Structure of these anthocyanins is under investigation.
5. Pale flowers (*w4-p*) contained two additional dihydroflavonols. Structure of these dihydroflavonols is under investigation.

Acknowledgements

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Postdoc/Ph.D. student wanted

We are engaged in soybean research areas including flavonoids, transposable elements, flooding tolerance, seed coat cracking, cleistogamy and maturity genes. If there is anyone interested in working with us as a postdoc fellow or a Ph.D. student, please make contact with R. Takahashi at masako@affrc.go.jp.



Variegated flowers of *G. soja* Flooding treatment of RILs Seed coat cracking Cleistogamous flower (right)