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A Gene for Flower Colour Fading in *Petunia hybrida*

P. de Vlaming and J. E. M. van Eekeres
Department of Genetics and Plantphysiology, Universiteit van Amsterdam, Amsterdam (the Netherlands)

H. Wiering
Hortus Botanicus, Universiteit van Amsterdam, Amsterdam (the Netherlands)

Summary. Full coloured *Petunia hybrida* flowers lose their colour and become completely white if the gene *Fa* is dominant. This gene is only expressed in mutants with the pH gene *Ph4* homozygous recessive. It is shown by genetical experiments that the fading is restricted to the 3-rutinosido(p-coumaroyl)-5glucoside glycosylation pattern of anthocyanins. The 3-glucosides and 3-rutinosides show a weak fading only.

Key words: *Petunia hybrida* – Flower colour – Fading gene

Introduction

Flowers of *Petunia hybrida* plants homozygous recessive for the pH genes *Ph3* or *Ph4* sometimes show a remarkable fading of colour of the flower limb (Wiering 1974). About two or three days after the fullcoloured flower buds are open, anthocyanin disappears gradually and before the flowers wither, they are completely white (Fig. 1). This fading is restricted to the flower limb; the flower tube and pollen maintain their full colour.

From preliminary experiments it is clear that the difference between fading and not fading is monofactorial and that fading is dominant over not fading. These experiments also showed that only flowers of plants homozygous recessive for the genes *Ph3* or *Ph4* can fade. These flowers are of the so-called purplish type in which the pH of flower homogenates is about 6.0; reddish types, dominant for the genes *Ph3* and *Ph4*, whose flower homogenates show a pH of about 5.5, never fade.

An additional condition for fading may be the presence of a special glycosylation and methylation pattern of the anthocyanin: flowers containing malvidin 3-(p-coumaroyl)rutinosido-5-glucoside fade to white but those containing cyanidin-3-glucoside only fade very weakly.

Preliminary experiments also indicate that fading causes only a difference in quantity of anthocyanin; there is no difference in the quality of these pigments.

In this paper genetical experiments will be described which give more information about the specificity of the fading process in relation to the gene *Ph4*. The effect of the fading gene is shown on plants differing in the glycosylation and methylation pattern of anthocyanins.

Materials and Methods

In two of the three crosses necessary to obtain the -3G, -3RG and -3RGac5G glycosylation types of delphinidin, the use of parents homozygous dominant for *Ph4* and of an unknown genotype for *Fa* could not be avoided. However, the other parent is a line homozygous recessive for *Ph4* and homozygous dominant for *Fa*. This means that in the F1 a dominant allele of *Fa* is present and that in the F2 the gene *Ph4* segregates. This could result in F1's homozygous dominant or heterozygous for *Fa*.

The genotypes and phenotypes of the parents and their F1's are given in Table 1. In all parents the anthocyanin genes necessary for a coloured flower limb are homozygous dominant. A short description of segregating flower colour genes, used in this study, is given here (see for full description Wiering 1974):

Hfl– 3′–5′ hydroxylated anthocyanins (delphinidin, petunidin or malvidin) present
hflhfl only 3′ hydroxylated anthocyanins (cyanidin or peonidin) present
Rt– 3 rhamnosylated anthocyanins (cyanidin-3 rutinoside or delphinidin-3 rutinoside) present
rtrt cyanidin-3 glucoside or delphinidin-3 glucoside present
Gf– 5 glucosylated 3 acylated anthocyanins (cyanidin-3RGac5G, peonidin-3RGac5G, delphinidin-3RGac5G, petunidin-3RGac5G or malvidin-3RGac5G) present
gfgf cyanidin-3 rutinoside or delphinidin-3 rutinoside present
Mt– 3′methylated anthocyanins (peonidin or petunidin) present
mtmt cyanidin or delphinidin present
Mf– 5′ methylated anthocyanin (malvidin) present

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Fig. 1. A stalk of *Petunia hybrida* with a young not yet faded flower and an older completely faded flower

mfmf petunidin present
Ph– all Ph genes dominant; flower colour of reddish type
phph with at least one Ph gene homozygous recessive; flower colour of purplish type
Fa– fading of the anthocyanins in the flower limb
fafa no fading of the anthocyanins

The order of action of the above mentioned glycosylation and methylation genes is given in Fig. 2.

The position of *Fa* in the *Petunia* genome is not yet known; the other above mentioned genes have been located (Wiering et al. 1979). The analysis of anthocyanins and anthocyanidins by thin layer chromatography was performed as described by Wiering and de Vlaming (1973).

The P values in the tables are at the X^2 level.

Results and Discussion

The Cross V55 × R93

In both parents of this cross the gene *Ph4* is homozygous recessive, resulting in progenies with flowers of the purplish type only. The results of the F2 and B1 are given in Table 2. Due to the segregation of the genes *Hf1* and *Rt*, four flower colour classes can be observed. Because both parents are homozygous dominant for the glycosylation gene *Gf* and have the same methylation gene *Mt*, the gene *Rt* gives a segregation in the two types of plants not only differing in glycosylation pattern but also in methylation and acylation.

Fading types are present in the colour classes with peonidin-3RGac5G and petunidin-3RGac5G. Flowers accumulating cyanidin-3G and delphinidin-3G demonstrate only a very weak fading. However, the *rtrt* plants could not be divided into clear fading and not fading phenotypes. Supposing all *rtrt*-plants are of the not fading type, it could be thought that this is the result of an absolute linkage between *Rt* and *Fa*. However, the clear 3:1 ratio in F2 and the 1:1 ratio in B1 for fading and not fading types in the *Rt-* genotypes indicate an independant inheritance of *Rt* and *Fa*. Two of the not fading delphinidn-3G plants are crossed with a not fading peonidin-3RGac5G purplish type (*ph4ph4*) plant: fading plants are found in the progenies. Thus the dominant allele of *Fa* must be present in the tested delphinidin-3G plants. In the F2 and B1, the genes *Hf1*, *Rt* and *Fa* segregate in the expected ratios. From this cross we conclude that:

Table 1. Genotype and phenotype of inbred lines and their F1's

Line code	Colour of the flower limb		Main anthocyanin[a]	HCC[b]	Genotype[c]							
					Hf1	*Rt*	*Gf*	*Mt*	*Mf*	*Ph1*	*Ph4*	*Fa*
R93	red 1	purplish type not fading	cyanidin-3G	0023/2	–	–	+	×	×	+	–	–
Vu6	grey 2	reddish type not fading	delphinidin-3RG	024/1	+	+	–	×	–	+	+	?
V32	purple	purplish type not fading	delphinidin-3RGac5G	35/1	+	+	+	–	–	–	+	?
V55	purple	purplish type fading	malvidin-3RGac5G	733	+	+	+	×	× / –	+	–	+
V55 × R93	purple	purplish type fading	malvidin-3RGac5G	733	+ / –	+ / –	+	×	× or × / –	+	–	+ / –
V55 × Vu6	purple	reddish type not fading	petunidin-3RGac5G	30	+	+	+ / –	×	–	+	+ / –	+ or + / –
V32 × V55	purple	reddish type not fading	petunidin-3RGac5G	30	+	+	+	× / –	–	+ / –	+ / –	+ or + / –

[a] G = glucose, R = rhamnose, RG = rutinose, ac = p-coumaric acid
[b] Colour according to the Horticultural Colour Chart of the British Colour Council
[c] + = homozygous dominant; – = homozygous recessive; × = one or both of the genes *Mt1* and *Mt2* homozygous dominant, one or both of the genes *Mf1* and *Mf2* homozygous dominant; + / –, × / – = heterozygous

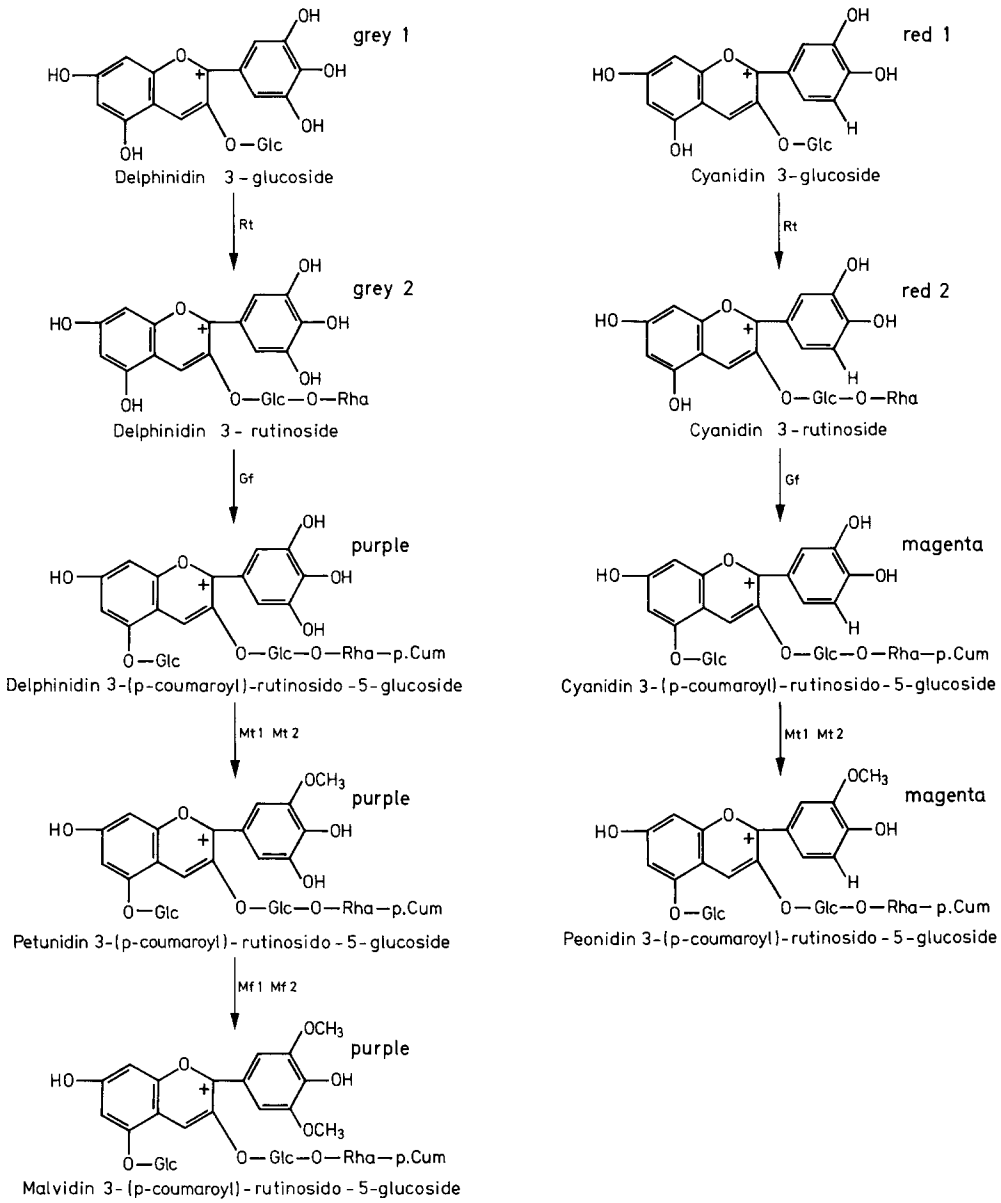

Fig. 2. Order of action of glycosylation and methylation genes in *Petunia hybrida:* left column *Hf1* –, right column *hf1hf1*

1. *Fa* is not expressed in flowers accumulating delphinidin-3G and cyanidin-3G
2. There is no indication for linkage between *Fa, Hf1* and *Rt*
3. It is confirmed that the difference fading/not fading is monofactorial

The Cross V55 × Vu6

The results of the F2 of this cross are given in Table 3. In contrast with the previous experiment there is a segregation of reddish and purplish types, due to the gene *Ph4*. By the segregation of the genes *Gf* and *Ph4* four colour classes can be found. Because both parents are homozygous dominant for the glycosylation gene *Rt* and have the same methylation gene *Mt*, the gene *Gf* gives a segregation in two types of plants not only differing in glycosylation and acylation pattern but also in methylation. The genotype of the parent Vu6 with respect to *Fa* is unknown so there are two possibilities for the F1: *Fa* is heterozygous or homozygous dominant. All plants of the class purple purplish type fade to white, the plants of the grey 2 purplish type class show a

Table 2. F2 and B1 of the cross V55 × R93; F1 $\frac{Hf1}{hf1}\frac{Rt}{rt}\frac{Fa}{fa}$; B1 × R93; genetical background *ph4ph4 GfGf MtMt*

Expected segregating types				Results	
Genotype	Phenotype Main anthocyanin	Colour of flower limb		Number of plants	
				F2	B1
Hf1 – Rt –	malvidin-3RGac5G	purple purplish type	fading	103	31
			not fading	45	26
Hf1 – rtrt	delphinidin-3G	grey 1 purplish type	fading	0	0
			not fading	38	65
hf1hf1 Rt –	peonidin-3RGac5G	magenta purplish type	fading	25	44
			not fading	4	21
hf1hf1 rtrt	cyanidin-3G	red 1 purplish type	fading	0	0
			not fading	12	62
			total	227	249

		F2		B1	
Segregation for	*Hf1*:	186:41	$P_{3:1} = 0.02$	122:127	$P_{1:1} = 0.75$
	Rt:	177:50	$P_{3:1} = 0.68$	122:127	$P_{1:1} = 0.75$
	Fa[a]:	128:49	$P_{3:1} = 0.41$	75: 47	$P_{1:1} = 0.02$
Linkage	*Fa-Hf1*[a]:	103:25:45: 4	$P_{2\times2} = 0.08$	31:44:26:21	$P_{2\times2} = 0.13$
	Hf1–Rt:	148:38:29:12	$P_{2\times2} = 0.22$	57:65:65:62	$P_{2\times2} = 0.48$

[a] in *Rt* – plants only

Table 3. F2 of the cross V55 × Vu6; F1 $\frac{Gf}{gf}\frac{ph4}{Ph4}\frac{Fa}{?}$; genetical background *Hf1 Hf1 RtRt MtMt*

Expected segregating types				Results
Genotype	Phenotype Main anthocyanin	Colour of flower limb		Number of plants
Gf – Ph4	petunidin-3RGac5G	purple reddish type	fading	0
			not fading	125
gfgf Ph4 –	delphinidin-3RG	grey 2 reddish type	fading	0
			not fading	45
Gf – ph4ph4	petunidin-3RGac5G	purple purplish type	fading	39
			not fading	0
gfgf ph4ph4	delphinidin-3RG	grey 2 purplish type	fading	0
			not fading	16[a]
			total	225

Segregation for	*Gf*: 164:61	$P_{3:1} = 0.47$	
	Ph4: 170:55	$P_{3:1} = 0.84$	

[a] Weak fading

weak fading. As expected, plants of the two reddish type colour classes do not fade.

The results of this cross with respect to *Fa* can be explained in two ways: a) the F1 is homozygous dominant for the gene *Fa* which can not express themselves in the grey 2 purplish type class, b) The F1 is heterozygous for the gene *Fa* but there is also a very strong or absolute linkage between *Gf* and *Fa*.

To check which of these two theories is correct, two of the weak fading plants of the grey 2 purplish type

containing delphinidin-3RG, were crossed with a not fading peonidin-3RGac5G purplish type (*ph4ph4*) plant. Since only fading plants are found in the progenies of these crosses and as the colour class purple purplish type of the F2 V55×Vu6 reveals only fading plants, it is clearly indicated that all plants of the F2 must be homozygous dominant for *Fa*. We conclude that:

1. The F1 was homozygous dominant for *Fa*
2. In delphinidin-3RG containing flowers which are homozygous recessive for the gene *Ph4*, *Fa* is expressed slightly
3. The expression of the dominant allele of *Fa* is possible in petunidin-3RGac5G containing flowers homozygous recessive for the gene *Ph4*.

The Cross V32×V55

The results of the F2 of this cross are given in Table 4. Because the genotype of the parent V32 with respect to the gene *Fa* is unknown, for this F1 there are also two possibilities.

The segregation of the gene *Fa* is troubled by the fact that the parents differ in two pH genes, *Ph1* and *Ph4*. There is no difference in flower colour between purplish types with either the genotype *Ph1-ph4ph4*, *ph1ph1Ph4-* or *ph1ph1ph4ph4*, but preliminary experiments have shown that *ph1ph1Ph4-* genotypes do not fade.

A number of plants were chromatographed to obtain the segregation in plants with delphinidin and with petunidin (segregation for *Rt*). It is striking that in the purple purplish type class not only fading plants are found with petunidin-, but also plants with delphinidin-3RGac5G as the main pigment.

Mt segregates in a 3:1 ratio, the segregation for *Ph1* and *Ph4* together does not deviate significantly from the expected 9:7 ratio. The segregation for fading/not fading in the purplish types is very hard to explain. It could be excluded that *Fa* is homozygous dominant in the F1. This is supported by the result of a test cross of a fading purplish type plant which shows that this plant is heterozygous for *Fa*. Thus, the expected ratio fading: not fading in the purplish types should be 3:4. The results (23:76) deviate strongly from the expected ratio ($P<0.001$) and can probably be explaned by the presence of a certation or semi lethality gene linked with *Ph4*.

From this cross the conclussions can be drawn that

1. The F1 is heterozygous for *Fa*
2. Fading can also occur in flowers containing delphinidin-3RGac5G

The overall conclusion from the three crosses is that the dominant allele of the fading gene *Fa* expresses themselves only in flowers accumulating 3RGac5G anthocyanins and that this expression is independent of the methylation pattern. It is not known if the acylation on position 3 or the glycosylation on position 5 is essential for fading because neither the acylated delphinidin-3RG, nor the non acylated -3RG5G glycosylation type, is ever found as the main flower pigment in *Petunia hybrida*.

In the literature a correlation has been described between ethylene production and fading in the *Vanda orchid* (Burg and Dijkman 1967) but when the ethylene production was measured in both fading and not-fading flowers of *Petunia*, no significant differences in quantity were found (results not given).

Table 4. F2 of the cross V32×V55; F1 $\frac{mt}{Mt}\ \frac{ph1}{Ph1}\ \frac{Ph4}{ph4}\ \frac{?}{Fa}$ genetical background *Hf1Hf1 RtRt GfGf*

Expected segregating types			Results			
Genotype	Phenotype Main anthocyanin	Colour of flower limb	Fading[a]	Not fading[b] total		Total
Mt – Ph[a]*–*	petunidin-3RGac5G	purple reddish type	0	168	56[b]	168
mtmt Ph –	delphinidin-3RGac5G		0		17[b]	
Mt – phph[a]	petunidin-3RGac5G	purple purplish type	17[b]	76	18[b]	99
mtmt phph	delphinidin-3RGac5G		6[b]		15[b]	
		total	23	244		267

Segregation for *Ph1+Ph4*: 169:99 $P_{9:7}=0.04$
Mt: 91:38 $P_{3:1}=0.25$

[a] *Ph –=Ph1 – Ph4 –*; *phph=ph1ph1 Ph4 –*, *Ph1 – ph4ph4*, *ph1ph1 ph4ph4* (no difference in phenotype)
[b] Number of plants chromatographed for segregation delphinidin/petunidin

Isolated pieces of flower limb fade, so it seems that the fading process takes place in the epidermis cells of the flowers independent of the green parts of the plant.

In a following paper further results about the mechanism of fading will be presented.

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P. de Vlaming
J. E. M. van Eekeres
Research Section Biosynthesis of Flavonoids
Department of Genetics and Plant Physiology
Universiteit van Amsterdam, Kruislaan 318
1098 SM Amsterdam (the Netherlands)

Drs. H. Wiering
Hortus botanicus
Universiteit van Amsterdam
Plantage Middenlaan 2
1018 DD Amsterdam (the Netherlands)