A survey of anthocyanins in *Petunia*

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I. INTRODUCTION

The varieties of garden petunia (*Petunia hybrida* hort.) present a wide variation of flower colours, ranging from red through violet and purple to blue.

Five common anthocynidins have been found in petunia flowers, namely delphinidin, petunidin, malvidin cyanidin and peonidin.

In a previous report, the author analysed the anthocynidin composition in flowers of a number of garden varieties of petunia (Muszyński 1964). The present paper gives the results of anthocyanin analyses of the same varieties.

II. METHODS

The anthocyanins present in the flowers of petunia were analysed by methods developed together with Nybom (1963 and 1964). The pigments were extracted with 0.1 per cent methanolic hydrochloric acid by desintegration of freshly collected flowers in a mortar. The extracts were filtered and evaporated with the help of an air-fan. The dried extracts can be kept at room temperature over a long period of time. For chromatographic analysis the anthocyanins are dissolved again in methanolic hydrochloric acid as used for the extraction.

Two-dimensional thin-layer chromatography on cellulose-coated plates was used for the separation of the anthocyanins. The chromatograms were run in the first direction in hydrochloric acid: formic acid:water (4:1:8 v/v). After drying overnight the chromatograms were run in the second direction in butanol-acetic acid:water (6:1:2 v/v). After final drying the plates were photo-copied by the Agfa-Copy-Rapid process.

The individual anthocyanins were identified by comparing their R_f values with those of the known pigments and with the data found in literature (Birkofer et al. 1963; Harborne 1958). As reference substances the anthocyanins of *Ribes*, *Rubus* and other plants were used.

Table 1

Anthocyanins present in the varieties of garden petunias

No.	Variety	Anthocyanidins	Anthocyanis Glicosidation type	Acylation	Main pigment
1	Alpha Blue	Mv Pt	3RGac5G(a); 3RG5G(c); 3GG(c) 3RGac5G(b);	+	Mv3RGac5G
2	Alpha Indigo	Mv Pt	3RGac5G(a); 3RG5G(c); 3GG(d); 3RGac5G(b); 3RG5G(c);	+	Mv3RGac5G
3	Blue Magic	Mv Pt	3RGac5G(a); 3RG5G(c); 3RG(c); 3GG(c); 3RGac5G(b); 3RG5G(d); 3RG(d); 3GG(d);	+	Mv3RGac5G
4	Black Night	Mv Pt	3RGac5G(b); 3RG5G(c); 3G(d); 3RG(d); 3RGac5G(a); 3RG5G(b); 3G(d); 3GG(d);	+	Pt3RGac5G
5	Blue Lustre	Dp Mv Pt	3RG5G(d); 3GG(d) 3RGac5G(a); 3RG5G(c); 3RGac5G(b); 3RG5G(d);	+	Mv3RGac5G
6	Deep Purple	Mv Pt	3RGac5G(a); 3RG5G(c); 3RGac5G(a); 3RG5G(c); 3GG(d);	+	Mv3RGac5G Pt3RGac5G
7	Nocturne	Mv Pt	3RGac5G(a); 3RG5G(c); 3GG(d); 3RGac5G(b); 3RG5G(c);	+	Mv3RGac5G
8	Rapsody	Pn Mv Pt	3RGac5G(c); 3RGac5G(a); 3RG5G(c); 3G5G(d); 3RGac5G(c);	+	Mv3RGac5G
9	Rotes Meer	Mv Pt	3RGac5G(a); 3RG5G(c); 3G5G(d); 3RGac5G(b); 3RG5G(d);	+	Mv3RGac5G
10	Plum Dandy	Mv Pt	3RGac5G(a); 3RG5G(c); 3RGac5G(b); 3RG5G(c);	+	Mv3RGac5G
11	Flaming Velvet	Mv Pt Dp	3RGac5G(b); 3RG5G(d); 3RGac5G(a); 3RG5G(c); 3G5G(d); 3GG(d);	+	Pt3RGac5G
12	Rotkäppchen	Pn Cy	3RGac5G(a); 3RG5G(c); 3G(d); 3GG(d); 3RGac5G(b);	+	Pn3RGac5G
13	Rose Lustre	Pn Mv	3RGac5G(a); 3RG5G(c); 3RGac5G(b); 3RG5G(d);	+	Pn3RGac5G
14	Light Pink	Pn Cy Mv	3RGac5G(a); 3RG5G(c); 3RGac5G(c); 3RGac5G(c);	+	Pn3RGac5G
15	Himmelröschen	Pn Cy	3RGac5G(a); 3RG5G(c); 3RGac5G(b);	+	Pn3RGac5G
16	Dawn Satin	Pn Cy Dp	3G(c); 3G(a); 3GG(b); 3RG5G(d); 3G(d); 3GG(d);	—	Cy3G
17	Salmon Red	Pn Cy Dp	3G(c); 3G(a); 3GG(b); 3RG5G(d); 3GG(d);	—	Cy3G

No.	Variety	Anthocyanidins	Anthocyanidins Glicosidation type	Acylation	Main pigment
18	Fire Gleam	Pn	3G(c);	—	Cy3G
		Cy	3G(a); 3GG(b); 3RG5G(d);		
		Dp	3G(c); 3GG(c);		
19	Cardinal	Pn	3G(c); 3RG(d);	—	Cy3G
		Cy	3G(a); 3RG(d); 3GG(b); 3G5G(d); 3RG5G(d);		
		Dp	3G(d); 3GG(d); 3G5G(d);		
20	Red Satin	Pn	3G(b);	—	Cy3G
		Cy	3G(a); 3GG(b); 3G5G(d); 3RG5G(d);		
		Dp	3G(d); 3GG(c);		
		Rs	3G(d); 3RG(d);		
		Pt	3G(d); 3GG(d);		
21	Scarlet Lustre	Pn	3G(c);	—	Cy3G
		Cy	3G(a); 3RG(d); 3GG(b); 3G5G(d); 3RG5G(d);		
		Pt	3GG(d);		
		Dp	3G(c); 3GG(c); 3G5G(d);		
		Rs	3G(d);		
22	Scarlet Red	Pn	3G(c);	—	Cy3G
		Cy	3G(a); 3GG(b); 3G5G(d); 3,5RG(d);		
		Dp	3G(c); 3GG(c);		
23	Red Cascade	Pn	3G(c);	—	Cy3G
		Cy	3G(a); 3GG(b); 3G5G(d); 3RG5G(d);		
		Dp	3G(d); 3GG(c);		
24	Local variety	Pn	3G(b); 3RG5G(d);	—	Cy3G
		Cy	3G(a); 3RG(d); 3G5G(c); 3GG(b); 3RG5G(d);		
		Pt	3G(d); 3GG(d); 3RG5G(d);		
		Dp	3G(c); 3GG(d); 3RG5G(d);		

(a) = main pigment; (b) = by-pigment; (c) = small amount; (d) = traces.

III. RESULTS

The analyses performed showed that the red flowering varieties of garden petunias contain mono- and di-glucosides of the anthocyanidins present, the mono-glucosides being in prevailing (Table 1, figs. 4—5). Among the great number of varieties analysed it was only one which contained rhamnosides solely (fig. 6 — *Petunia hybrida superbissima purpurea*).

The blue- and purple flowering varieties contain acylated rhamnoglucosides as the main pigments and small amounts of unacylated pigments (Tabl. 1, figs. 1—3).

It is worth underlining that only the blue- and purple flowering varieties contained the acylated pigments.

Figs 1—6. Photocopies of chromatograms developed in two directions as described in text:
 Varieties: 1 — Królowa targu, 2 — Blue Lustre, 3 — Gottfried Michaelis, 4 — Cardinal, 5 — Scarlet Red, 6 — *P.h. superbissima purpurea*

The identification of anthocyanins present in the flowers of garden petunias is not difficult by thin-layer chromatography as the pigments form very regular and well separated spots (fig. 7).

In order to identify the individual acylated anthocyanins it was necessary to perform saponification with the aim to release acyl-free pigments. This process was done according to Abe and Hayashi (1956). For this purpose the extracts obtained in the usual way as described above were treated for two hours with 10 per cent methanolic potassium hydroxide in an atmosphere of hydrogen. Then the solution was acidified with anhydrous 7 per cent methanolic hydrochloric acid, and the saponified anthocyanins were precipitated by the addition of ether. For chromatography, anthocyanins were re-extracted from the precipitate with 0.1 per cent methanolic hydrochloric acid.

IV. DISCUSSION

As can be seen from the table, about half of the varieties analysed have acylated pigments. Only the main pigments are acylated, although they are accompanied by small amounts of non-acylated pigments. It is necessary to underline the fact that the acylation is found in the blue flowered varieties only.

Fig. 7. Schematic presentation of positions of some anthocyanins on the thin-layer chromatograms developed in two directions.

Pn — peonidin, Mv — malvidin, Cy — cyanidin, Pt — petunidin, Dp — delphinidin, 3G — 3-glucoside, 3GG — 3-di-glucoside, 3RG5G — 3-rhamnoglucoside-5-glucoside

In another series of experiments — (unpublished data) we have found that the blue colour occurring in the F_1 hybrids is due to the presence of acylation.

Further research on the relation between acylation and the flower colour in *petunia* is foreseen.

Most varieties analysed contain glycosides of delphinidin, but there are varieties with glycosides of cyanidin and peonidin only, in the absence of delphinidin.

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Przegląd antocyjanin petunii

Streszczenie

W kwiatach petunii ogrodowych występuje pięć antocyjanidyn, jakie są najczęściej spotykane u roślin, a mianowicie delfinidyna, malwidyna, petunidyna, cyjanidyna i peonidyna. Analiza chromatograficzna, przeprowadzona przy pomocy chromatografii cienkowarstwowej, wykazała, że w kwiatach odmian niebieskich i purpurowych barwnikami głównymi są acylowane 3-ramnogluko-5-glukozydy, natomiast w kwiatach odmian czerwonych barwnikami głównymi są nieacylowane mono- i di-glukozydy. Na szczególne podkreślenie zasługuje fakt wykrycia ścisłej korelacji między niebieszczeniem barwy kwiatów petunii, a występowaniem acylacji. Okazało się przy tym, że niebieszczenie barwy mieszańców związane jest również z występowaniem acylacji.

Chromatografia cienkowarstwowa stanowi łatwą i dogodną metodę identyfikacji barwników antocyjanowych petunii, dając dobre rozdzielenie (por. schemat na rys. 7).