



FLAVONOIDS IN SAINTPAULIA IONANTHA EXPRESSING THE FANTASY MUTATION

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Key Word Index—*Saintpaulia ionantha*; Gesneriaceae; African violet; anthocyanin; peonidin; malvidin.

Abstract—The Fantasy mutation in *Saintpaulia ionantha* produced flowers that had a purple background color with pink sectors. The purple background tissue contained kaempferol 3-*O*-glucoside and malvidin 3-rutinoside-5-glucoside. The pink sector tissue contained kaempferol 3-*O*-glucoside and peonidin-3-rutinoside-5-glucoside. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genetics of flower color in *Saintpaulia ionantha* or African violet has been extensively studied by Reed [1, 2]. He identified eight genes (*I*, *S*, *W*, *Bd*, *Bw*, *P*, *R*, and *A*) that controlled flower color. Two of these genes (*I* and *S*) were responsible for inhibiting pigmentation. The presence of a dominate allele of either gene resulted in white flowers. A dominate *W* allele was required for flower pigmentation. The intensity of pigmentation was controlled by the *Bw*, *Bd* and *A* genes. Either a *Bd*-, *Bw*- or *aa* genotype resulted in less intensely colored flowers. In *ii ss W- P- R-* genotypes, the flowers were purple; while in *ii ss W-pp R-* genotypes, the flowers were pink. In *ii ss W- P- rr* genotypes, the flowers were red. An unstable allele at the *P* locus resulted in flowers that were pink with purple flecks or sectors. This phenotype was called Fantasy.

Khokhar *et al.* [3] found that purple flowers contained the anthocyanin malvidin-3-rutinoside-5-glucoside; while pink or red flowers contained pelargonidin-3-rutinoside-5-glucoside or peonidin-3-rutinoside-5-glucoside, respectively. This study was undertaken to determine the biochemistry of the Fantasy mutation.

RESULTS AND DISCUSSION

The co-pigments were purified by preparative HPLC. The purple (RHS Colour Chart 89B) sectors

and the pink (RHS 66C) background tissue contained one major co-pigment that was identical in both tissues. Six other co-pigments were present in trace amounts. Base hydrolysis of the major co-pigment did not yield an acyl moiety. Controlled acid hydrolysis yielded only the flavonoid aglycone kaempferol and glucose. UV spectral properties [4], R_fs values on TLC [5], and HPLC retention time [6] indicated that the major co-pigment was kaempferol 3-*O*-glucoside.

The anthocyanin pigments extracted from flower petal tissue were purified by preparative HPLC. A single major anthocyanin was present in the pink background tissue and the purple sectors. The major anthocyanin in the pink background tissue was also present as a minor pigment in the purple sectors. Base hydrolysis of the two major anthocyanins did not yield an acyl moiety. Controlled acid hydrolysis yield several intermediates which were identified as the 5-glucoside, 3-glucoside, 3-rutinoside, 3-rutinoside-5-glucoside and the 3,5-diglucoside. Spectral properties, R_fs and retention time indicated that the major anthocyanin present in the pink background tissue was peonidin-3-rutinoside-5-glucoside. Malvidin-3-rutinoside-5-glucoside was the major anthocyanin in the purple sectors (Table 1).

Three different phenotypes (solid purple, solid pink and sectored) were observed in the population of plants that arose through the self-pollination of "Fantasy Charm". All the descendants contained kaempferol 3-glucoside as the major co-pigment. The anthocyanin in the solid purple descendants was the same as that present in the purple sectors (malvidin-3-rutinoside-5-glucoside). The solid pink descendants contained the same anthocyanin that was found in the pink background tissue (peonidin-3-rutinoside-5-

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Table 1. Analytical HPLC data for the anthocyanins and flavonols from a fantasy mutant of *Saintpaulia ionantha* and its descendants that arose through self-pollination

	Mv-3-rut-5-glu	Pn-3-rut-5-glu
Fantasy Charm		
background	14.4	85.6
sectors	75.3	24.7
Purple descendant	88.3	11.7
Pink descendant	12.5	87.5

Values are reported as the percentage of the total anthocyanin present.

Abbreviations—Mv = malvidin, Pn = peonidin, rut = rutinoside, glu = glucoside.

glucoside). In the population, the ratio of solid purple seedlings to solid pink to sectoring was 18:18:12. This ratio did not differ statistically from 3:3:2 ($\chi^2 = 0.035$).

EXPERIMENTAL

Plant Material

Plants of *S. ionantha* "Fantasy Charm" expressing the fantasy mutation, were obtained from Fisher Greenhouses in Linwood, NJ. Progeny from self-pollinating "Fantasy Charm" were raised and analyzed for flower sectoring and flavonoid composition. Plants were grown during the summer in a greenhouse under standard cultural practices at Beltsville, Maryland.

The purple sectors were dissected and separated from the pink background tissue. Approximately 1 g of fresh weight petal tissue was extracted with 1% HCl-MeOH and the extracts reduced to dryness at 40° under red. pres. The residue was dissolved in 1% HCl-MeOH and filtered.

Isolation and characterization of the anthocyanins

The anthocyanins in each conc. extract were analyzed by high resolution HPLC on a 7.8 × 300 mm column of Bondapak C-18 using a 30 min linear gradient of 0–10% MeCN in aqueous 15% HOAc–1.5% H₃PO₄, followed by a 10 min linear increase to 20% MeCN and finally held at 20% MeCN for an additional 10 min. Flow rate was 1.0 ml min⁻¹ and detection was by visual absorption at 540 nm. The individual anthocyanins were characterized by co-elution with known standards [6].

The anthocyanins were purified through prep. HPLC on a Bondapak C-18 column using the same procedure described for high resolution HPLC except H₃PO₄ was not used in aqueous solvent. The isolated anthocyanins were base hydrolyzed in aqueous 1% (w/v) KOH for 30 min at room temp. under N₂, acidified and the acyl moiety extracted with Et₂O [7, 8]. The deacylated anthocyanins were partially hydrolyzed at 70° in EtOH–aqueous 10% HCl (1:1) for the characterization of the intermediates formed [7, 8]. Complete acid hydrolysis was obtained at 100° after 2 h. Isolated compounds were characterized, along with known standards, by HPLC [6], Rf's [5], UV and Vis spectral analysis [4] and by the products of controlled acid or base hydrolysis.

Isolation and characterization of the co-pigments

The co-pigments in each conc. extract were analyzed by high resolution HPLC on a 7.8 × 300 mm column of Bondapak using a 20 min linear gradient of 0–20% MeCN in aqueous 1% TEAP buffered to pH 3.0 with H₃PO₄ and held at 20% MeCN for an additional 20 min. Flow rate was 1.0 ml min⁻¹ and detection was by UV absorption at 340 nm. The individual co-pigments were characterized by co-elution with known standards.

The co-pigments were purified through prep. HPLC on a Bondapak C-18 column using the same procedure as described for high resolution HPLC except aqueous 15% HOAc was substituted for TEAP. The isolated co-pigments were base and acid hydrolyzed using the procedures described for the anthocyanins.

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