SEPARATION OF PIGMENTS FROM PETUNIA'S PETALS USING THIN LAYER CHROMATOGRAPHY

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Abstract

Anthocyanins are a group of water –soluble pigments found in fruits, vegetables and flowers that give these plants their brilliant colors. The separation of the pigments present in different petals of Petunia hybrida was conducted utilizing thin layer chromatography (TLC). The pigments were extracted from different Petunia's petals (purple, white and violet) with ethanol prior to being spoted on the TLC plate. The characteristic color of Petunia's petals is due to a mixture of pigments.

Key words: anthocyanins, pigments, *Petunia hybrida*, TLC

INTRODUCTION

The genus *Petunia*, established by Jussieu in 1803, comprise ~ 30 (sub) species and belongs to the family of the *Solanaceae* (Gerats and Vandenbussche, 2005).

Petunia flower colors, except yellow, are derivated from ***flavonoid*** and ***anthocyanins.*** They are usually localized in the vacuoles of petal epidermal cells (Tsuda et al., 2004). Colour is a result of the preferential absorption of parts of the visible spectrum by suitable compounds. Members of some flavonoid classes, in particular the anthocyanins, play a crucial role in this process.

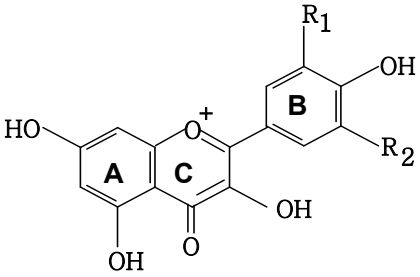
The water soluble flavonoid pigments represent a class of phenylpropanoid secondary plant metabolites. The basic structure of flavonoid consists of two aromatic rings (A and B) and a heterocycle (C) with oxygen. According to the oxidation level of the central pyran ring, the flavonoids are conveniently dividied into some 12 classes. The most important classes with regard to flower color are anthocyanins (**Fig. 1**), flavonols and flavones.

The presence of copigments, such as flavonols and flavones, contributes to a bathochromic shift of anthocyanins and, thus, flower color (Goto 1987). The structural genes encoding the enzymes involved in the hydroxylation, glycosylation, and acylation reactions have been obtained (Tanaka et al. 2004). The genes encoding flavonol and flavone synthases have also been cloned as reviewed (Tanaka et al. 2004). Vacuolar pH and coexisting metal ions are also important in the determination of flower color, but their manipulation is still not feasible because vacuolar pH regulation and metal uptake are not well understood in terms of their

biochemistry and molecular biology. The value of metabolic engineering to modify flower color has been reviewed (Tanaka et al. 2004).

Petunia is a good model for the study of flavonoid/anthocyanin biosynthesis because of its genetics and molecular biology (Holton and Cornish 1995). Many of the genes involved in the pathway have been cloned from petunia for the first time, such as, for example, flavonoid 3',5'-hydroxylase, flavonol synthase, anthocyanidin 3-glucoside rhamnosyltransferase, and flavonoid 3'-hydroxylase. Wild-type petunia flowers contain acylated delphinidin-type anthocyanins (Figure 1) and flavonols, and their color is reddish purple (low pH) or violet (high pH). The flower color becomes redder when the flowers produce cyanidin-type anthocyanins and/or non-acylated anthocyanins (Tsuda et al., 2004).

In addition to their colorful characteristics, anthocyanins possess antioxidant properties that have been implicated in disease prevention (Stintzing et al., 2002; Einbond et al., 2004).



Antocianidine	R1	R2
Pelargonidina	H	H
Cianidina	OH	H
Peonidina	OCH_3	H
Delfinidina	OH	OH
Malvidina	OCH_3	OCH_3
Petunidina	OH	OCH_3

Fig. 1. The chemical structure of anthocyanins

MATERIALS AND METHODS

Plant material: We used the commercial varieties of *Petunia hybrida* with the following color of corollas: purple, white and violet (**Fig. 2**).

Purple petunia | White petunia | Violet petunia

Fig. 2. The commercial varieties of *Petunia hybrida* with different color of petals

This study was conducted in the Biochemistry laboratory of the Faculty of Environmental Protection of the University of Oradea in 2008.
Pigments extraction: pigments were extracted from the corollas of day 0 flowers by homogenizing 1 g of the corolla tissue in 5 ml ethanol. The homogenizing was heated for few minutes, and 100 µl of pigments extracts were applied on the silicagel plates.
TLC technique: Silicagel plates were used (20 x 20 cm, Merck). The solvent mixtures employed were: 1-butanol:acetic acid : water (4:1:5, v/v/v). The Rf value for each pigments is then worked out using the formula:

$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

RESULTS AND DISCUSSION

The TLC chromatogram which we obtained is shown in Fig. 3. The values of Rf of different pigments are shown in the Table 1.
Chapman et al., have identified and proposed structures for the anthocyanins from Blue Wave and Sky Blue Petunias (Table 2).

The anthocyanin pigments (Cyanidin-3-diglucoside-5-glucose and Cyanidin-3-malonyldiglucoside-5-glucose) were found in both Blue Wave and Sky Blue petunias and were both cyanidin based compounds. Cyanidin is typically associated with red-colored flowers and may be present as an artifact of petunia crosses. The remainder of the compounds were divided into delphinidin-based anthocyanins in Sky Blue and malvidin-based anthocyanins in Blue Wave. This observation is well-supported by existing evidence with respect to blue flowers being high in delphinidin-based anthocyanins and purple flowers being rich in malvidin-based anthocyanins.

Table 1

The values of Rf

Type of Petunia	Spot	Rf (cm)	Colour of spot
Purple	P1	0.511	Green-yellow
	P2	0.65	Dark violet
	P3	0.77	Yellow
White	W1	0.67	Light yellow
	W2	0.78	Yellow
Violet	V1	0.43	Yellow
	V2	0.58	Violet

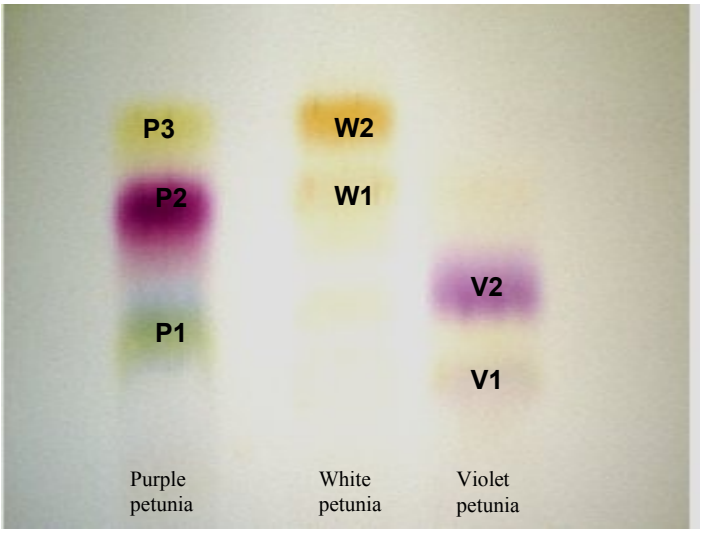


Fig. 3. TLC chromatogram of pigments from different colors of Petunia petals

Table 2

Anthocyanin pigments from Blue Wave and Sky Blue Petunias

Anthocyanin pigments	
Blue Wave Petunia	Sky Blue Petunia
Malvidin-3-coumaryl-5-diglucoside	Delphinidin-3-glucose-5-diglucoside
Malvidin triglucoside	Malvidin-3-coumaryl-5-diglucoside
Cyanidin-3-diglucoside-5-glucose	Cyanidin-3-diglucoside-5-glucose
Malvidin-3-caffeoyldiglucoside-5-caffeolydiglucoside	Delphinidin-3-malonylglucose-5-caffeoylglucose
Cyanidin-3-malonyldiglucoside-5-glucose	Cyanidin-3-malonyldiglucoside-5-glucose
Malvidin-3-caffeoyldiglucoside-5-caffeoylglucose	Delphinidin-3-caffeoylglucose-5-diglucoside
Malvidin-3-caffeoylrhamnosylglucose-5-caffeoylglucosylrhamnose	Delphinidin-3-caffeoyl-5-diglucoside
Malvidin-3-catechin-5-feruloyldiglucoside	Delphinidin-3-caffeoyl-5-glucose
Malvidin-3-caffeoylglucose-5-catechinyldiglucoside	
Malvidin-3-caffeoylglucose-5-catechinyldiglucoside	

CONCLUSION

From the data obtained, we can see that the colors of Petunia flowers are due to a lot of pigments. The TLC chromatogram of Purple Petunia, shown the presence of 4 spots; in the case of white and violet Petunia, the chromatogram shown 2 spots. Some pigments from these different colors of Petunia flowers taken in this study are the same (P3 and W2). From the data of literature the main pigments present in Petunia's petals are: cyanidin, malvidin and delphinidin.

The TLC is a very simple, rapid and economical method, and it can be use for the separation of different pigments from the plants. TLC technique can be a good tool for the screening of pigments from flowers, and utilized for the identification of different species.

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