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Anthocyanins in *Salvia*—Their Significance in Species Relationship and Evolution

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The species relationship in 10 species and varieties of *Salvia* was studied on the basis of anthocyanin pigments distribution patterns. The identified pigments fall under three groups. All red, scarlet and pink-flowered varieties contained pelargonidin, all blue flowered varieties contained delphinidin and amethyst and grape violet coloured varieties contained cyanidin derivatives. Glycoside 3-rhamnoside occurs frequently in most of the species. The flower colour of F_1 intervarietal hybrid of *S. coccinea* was found to be the colour of one of its parents. In both the parents and their hybrid the pigment identified was pelargonidin 3-rhamnoside. *S. coccinea* is more closely related to *S. splendens* var. Fireball. All these species and varieties contain pelargonidin as their principal anthocyanidin. Two other varieties of *S. splendens* e.g. amethyst and grape violet may be related to *S. coccinea* and *S. grahamii* as they also contain pelargonidin apart from having cyanidin as their main pigment. Three other species i.e. *S. farinacea*, *S. pratensis* and *S. hispanica* may be closely related to one another having delphinidin as their main pigment. The flowers of *S. farinacea* are found to be visited by bees, which indicates primitiveness. While considering the evolutionary aspect, it is assumed that the blue flowered species are the most primitive as is shown by their pollination mechanism as well as by the presence of the pigment delphinidin. In course of time, these may have given rise to the scarlet flowered varieties, the intermediate step being the species and varieties containing cyanidin as the main pigment. Some varieties of the same species have been found to contain both cyanidin and pelargonidin. The former varieties appear as an intermediate stage through which the highly evolved pelargonidin containing varieties have evolved.

Key Words: **Anthocyanins, *Salvia*, Distribution, Species relationship, Evolution**

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Introduction

Within the generic limits of *Salvia*, the colour variation is astonishing. The flower colour ranges from scarlet through purple and violet to azure-blue, white and even pale yellow. The colour variation of flowers is due to the presence of intensely coloured anthocyanin pigments. It has been clearly shown that anthocyanins have considerable potentialities as taxonomic markers in plant classification (Alston et al. 1963, Swain 1963, Harborne 1967). Although the data so far accumulated are very limited, significant correlations between anthocyanin distribution patterns and morphological features in several instances have been noted and these promise to make an important contribution to plant classification and phylogeny.

Salvia is a very large genus with more than 700 species distributed in both the hemispheres. However, excepting a few reports (Asen 1961, Shibata et al. 1966, Cornu & Paynot 1969) works on anthocyanins of this genus are rather scanty. For this reason, the present investigation was undertaken to identify the major anthocyanins present in different species and varieties of *Salvia*. Such information, apart from providing a general distribution pattern, may help in understanding their interrelationships and evolution.

Materials and Methods

The plant materials on which the present work was done are listed and described in table 1. The plants were grown in pots and fresh petals were collected from about twenty plants of the same age for each species and varieties.

Extraction and Chromatography: Five to ten grams of petals were macerated in 20 ml of methanolic-HCl (1% V/V) for 5 min, gently mashed at room temperature for 5 min and filtered in vacuo. The mashed petals were once again treated with a fresh 10 ml portion of the solvent, as before and vacuum filtered. The filtrates were mixed together and concentrated by vacuum distillation under 40°C in dark.

The pigments were separated as broad bands in 3 mm filter papers by chromatography for 6–8 hr. The colour bands were eluted out and the eluates chromatographed in Whatman No. 1 paper by descending chromatography for 8 to 10 hours in dark at a temperature of 21 ± 1°C.

The solvents described below were used for each identification.

Solvents	*Percentage composition*
1. BAW	*n*-butanol (40%), acetic acid (10%), water (50%) (top layer)
2. Bu–HCl	*n*-butanol (50%), 2N HCl (50%)
3. Acetic-Acid–HCl	acetic acid (15%), hydrochloric acid (3%), water (82%).

Spectrophotometry: Spectral curves of the extracted anthocyanins in the visible range (400–750 nm) were obtained for knowing the wavelengths of the absorption maximum which characterise the anthocyanins to some extent.

The concentrated extracts used in the chromatography are diluted 10 to 20 times with methanolic-HCl. The optical densities are determined by taking the extracts in cells 1 cm or 1 mm path-length, according to the colour intensities. The observed λ_{max} values are given in table 2.

Results

The reported Rf values (table 2) in a single solvent were derived from the results of four sets of chromatograms each bearing six original spots of a single eluate. The calculated Rf values did not differ significantly

Table 1 *The plant materials*

Species and varieties	Flower colour	Source of collection
Salvia coccinea Juss. var. Red Indian	Deep crimson	Suttons and Sons, P. Ltd., Calcutta
S. coccinea Juss. var. Pink Pearl	Pink	
S. farinacea Benth var. Royal Blue	Blue	
S. splendens Sell var. Fireball	Scarlet	
S. splendens Sell var. Grape Violet	Grape Violet	M/s. N. Cooper & Co., Poona Isolated from a mixed population of *S. splendens* and the names were given by the authors according to flower colour
S. splendens Sell. var Amethyst	Amethyst	
S. grahamii Benth	Deep Crimson	Zoo and Botanic Garden, Holland
S. hispanica Linn.	Blue	
S. pratensis Linn.	Blue	
F_1 hybrid of *S. coccinea* var. Red Indian $\times$ *S. coccinea* var. Pink Pearl	Crimson	Produced by crossing

from one another. Conclusions regarding the nature of anthocyanins were arrived at after comparing the Rf values in three solvents (two in some cases) with those listed by Harborne (1967). The observed Rf values were found to be in such good agreement with Harborne's data that satisfactory conclusion regarding the identity of anthocyanin could be drawn from those values alone.

However, Harborne observed (1967) that usually pelargonidin-3 glycosides have their λ_{max} at about 505 nm and corresponding glycosides of cyanidin and delphinidin have their λ_{max} at 520–526 nm and 532–537 nm respectively. These values do agree with our observed values (table 2) for pigments identified as pelargonidin, cyanidin and delphinidin glycoside from their Rf values.

The two varieties of *S. coccinea*, F_1 hybrid of these varieties and the species *S. grahamii* all possess pelargonidin; 3-rhamnoside.

Of the three varieties of *S. splendens* studied, the variety Fireball possesses pelargonidin, 3-glycosides whereas varieties amethyst and grape violet both contain pelargonidin glycosides besides cyanidin, 3-rhamnosides as the chief pigment.

The remaining three species viz., *S. farinacea* var. Royal Blue, *S. pratensis* and *S. hispanica* were all found to contain delphinidin, 3-rhamnoside.

Table 2 *Chromatographic and spectrophotometric results*

Species/varieties	λmax in Me-OH-HCl	E 400 / E vis max (as %)	Rf Values BAW	BU-HCl	HOAC-HCl	Pigment
Salvia coccinea var. Red Indian	510 nm	20	73	60	—	Pg, 3-rhamnoside
Salvia coccinea var. Pink Pearl	510 nm	22	71	61	53	Pg, 3-rhamnoside
Salvia farinacea var. Royal Blue	540 nm	17	38	51	38	Dp, 3-rhamnoside
Salvia splendens var. Fireball	505 nm	21	69	61	—	Pg, 3-rhamnoside
Salvia splendens var. Grape Violet	535 nm	21	62	65	50	Cy, 3-rhamnoside
			46	26	—	Pg, 3-rhamnoside 5-glucoside
Salvia splendens var. Amethyst	525 nm	17	62	65	50	Cy, 3-rhamnoside
			46	26	—	Pg, 3-rhamnoside-5-glucoside
Salvia grahamii	510 nm	20	71	62	53	Pg, 3-rhamnoside
Salvia hispanica	540 nm	19	38	49	34	Dp, 3-rhamnoside
Salvia pratensis	535 nm	17	37	52	—	Dp, 3-rhamnoside
F_1 hybrid of *Salvia coccinea* var. Red Indian × *Salvia coccinea* var. Pink Pearl	510 nm	19	70	60	53	Pg, 3-rhamnoside

Pg, Pelargonidin; *Cy*, Cyanidin; *Dp*, Delphinidin

Discussion

The ten species and varieties of *Salvia* in which anthocyanins were identified fall under three groups. All red, scarlet and pink flowered varieties contain pelargonidin. all blue flowered varieties contain delphinidin and amethyst and grape violet coloured varieties contain cyanidin derivatives. It has also been found that the glycoside 3-rhamnoside occurs frequently in most of the species of *Salvia*.

The flower colour of the F_1 hybrid plant was red which is the colour of one of its parents. No new pigment in the F_1 plant itself was expected and by chromatographic as well as spectral properties this assumption was found to be correct. In both the parents and their hybrid, the pigment identified was pelargonidin, 3-rhamnoside. Harborne (1967) states that dominant-recessive relationship controls flavonoid production in the flower and F_1 plants have the same pigments as the dominant parent, only in the F_2 are new substances likely to be synthesised.

On the basis of studies on the distribution pattern of anthocyanins in different species, a conclusion regarding species relationship may be drawn. *S. coccinea* is more closely related to *S. grahamii*. These two species are also related to *S. splendens* var. Fireball as all of them contain pelargonidin as their principal anthocyanidin. The two other varieties of *S. Splendens* i.e. amethyst and grape violet may be related with those of *S. coccinea* and *S. grahamii*, because these varieties, apart from containing cyanidin as the main pigment, also have pelargonidin. Three species e.g. *S. farinacea*, *S. pratensis* and *S. hispanica* may be closely related with each other having delphinidin as the main anthocyanidin.

In practically all plants studied, mutations occur in the direction delphinidin——→ cyanidin——→pelargonidin (Harborne 1967). Furthermore, dominant-recessive relationships operate in the same direction, delphinidin being dominant over cyanidin and cyanidin being dominant over pelargonidin production (Beale 1941). The dominance of delphinidin can clearly be traced to natural selection among temperate plants, for a blue flower-colour is flavoured by bee pollinators. According to Harborne (1973), "anthocyanin type is correlated with natural selection for flower colour. Thus, selection for blueness in the Boraginaceae, a family which has mainly bee-pollinated flowers, has led to delphinidin being the principal plant pigment, whereas selection for red in the bird-pollinated Gesneriaceae and Bignoniaceae has produced plants with pelargonidin and epigenidin as flower pigments. Further proof of this point is that in wind-pollinated families such as Gramineae and Cyperaceae there is no selection for inflorescence colour, for example, cyanidin, the biosynthetically simplest anthocyanidin is uniformly present in the Gramineae." Indeed, practically the only exception to the above rule occurs in a species of *Salvia*, *S. splendens*. According to Beale (1941) this species contains a pelargonidin derivative in the presumably wild form, and has given rise to a violet mutant containing a delphinidin derivative. The latter is apparently dominant judged by the appearance of the hybrid between the two forms (Beale, unpublished). Beale obtained authentic wild material of *S. splendens* from Brazil.

In the present experimental material, out of three *Salvia* species with blue flowers studied, only *S. farinacea* was found to be pollinated by bees (*Apis* spp.). However, the other species e.g. *S. hispanica* and *S. pratensis* were not bee-pollinated. This may be due to their floral structure, because the mouth of the corolla tube is such that the bees cannot enter it. In case of *S. splendens*, three varieties were studied. One had large bright scarlet corollas and the other

two had amethyst and grape violet coloured corollas. But surprisingly no bird was found to visit the flowers. On the other hand, all the varieties were regularly visited by several kinds of ants and other insects. As regards pigmentation with the varieties, scarlet flowers have pelargonidin and the other varieties have cyanidin. If the pathway of mutation is delphinidin——→cyanidin——→ pelargonidin, it is surprising whether the varieties with cyanidin have given rise to pelargonidin in scarlet variety or vice versa. Because as reported earlier, if the scarlet variety with pelargonidin is the wild type, then mutations must have occurred in the direction that has given rise to cyanidin. If it is so, then it is a special kind of mutation which does not follow the usual pathway. However, there is a report of such unusual mutation from pelargonidin to cyanidin which has been noted in *Rosa polyantha* (Scott Moncrieff 1936), and that is due to a back mutation and is probably of cytoplasmic origin.

From the point of view of evolution, it appears that the blue flowered species are the most primitive as shown by their pollination mechanism as well as by their containing delphinidin. In course of time, these have given rise to the scarlet types, the intermediate step being the cyanidin containing varieties. It has been found that some varieties of the same species contain both cyanidin and pelargonidin, so it may be stated that these former varieties may represent the intermediate stage through which the highly evolved pelargonidin containing varieties have evolved.

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