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PRECURSORS AND GENETIC CONTROL OF PIGMENTATION

1. INDUCED BIOSYNTHESIS OF PELARGONIDIN, CYANIDIN AND DELPHINIDIN IN *ANTIRRHINUM MAJUS*

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Summary

Anthocyanidins have been synthesised in various acyanic genotypes of *Antirrhinum majus* following the administration of three dihydroflavonols.

1. Introduction

THE pathway of biosynthesis of flavonoids is now believed to be that outlined in fig. 1 (Harborne, 1967; Grisebach, 1972). The first flavonoid formed from cinnamic acid derivatives is flavanone (dihydroflavone). Introduction of an hydroxyl group in the 3-position gives the flavanonol (dihydroflavonol), which it is postulated rearranges to the flavenol, and this, by losing an OH^-, can form anthocyanidin. Flavone and flavonol can be formed by oxidation of the corresponding dihydro-compounds. Flavanone can also

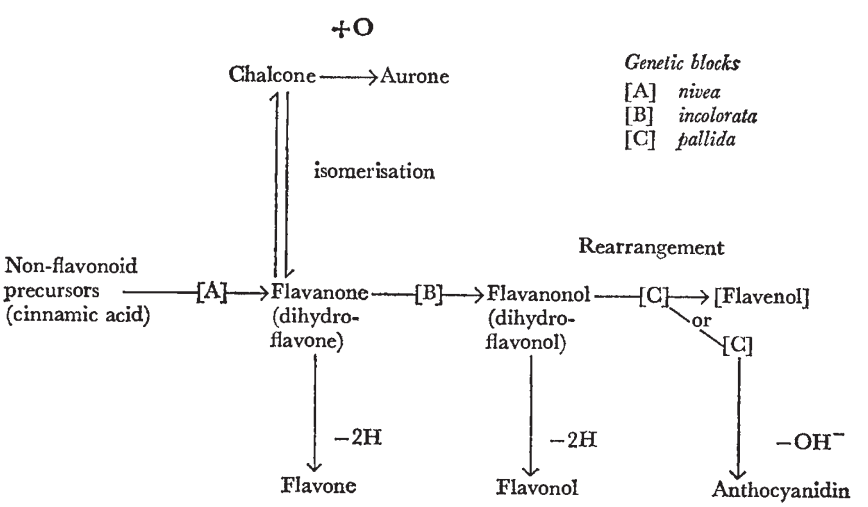


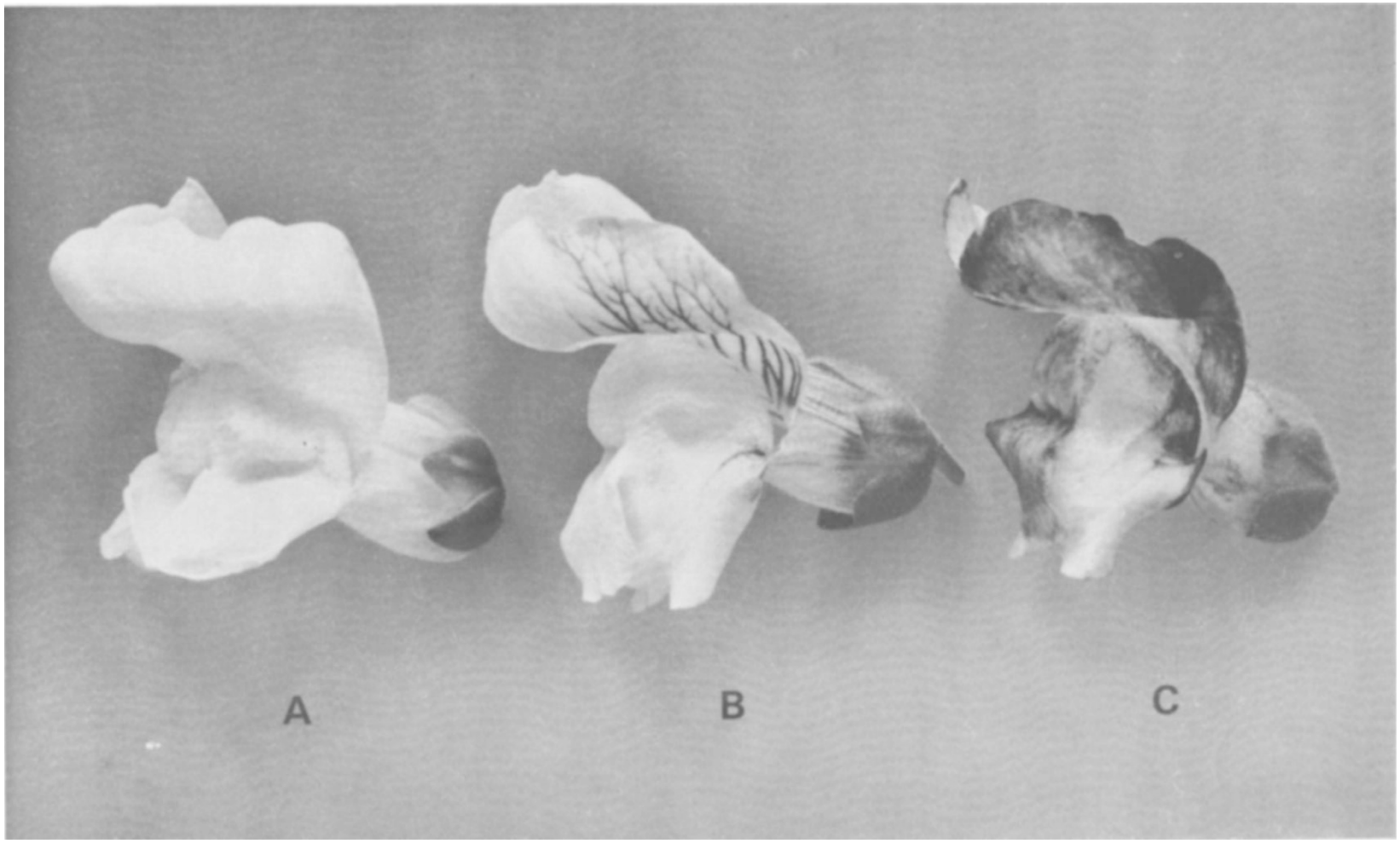
FIG. 1

Plate 1

Three similar flowers of composite genotype

incolorate: *Nivea*: *Pallida*: *Eosinea*

A. Untreated.
B. Dihydroquercetin imbibed through cut stem of inflorescence for 48 hours.
C. Dihydroquercetin imbibed through immersion of corolla tube in 0·1 per cent aqueous solution for 16 hours.


A
B
C

isomerise to the chalcone, which on oxidation yields the aurone. With the exception of flavononol and flavenol, examples of all these compounds have been found as glycosides in *Antirrhinum majus* (Harborne, 1967; Gilbert, 1973). The glycosides of the anthocyanidins are usually the 3-rutinosides, but the occasional presence of the 3-glucoside has been detected (Gilbert, 1971, 1972).

The identification of the flavonoid compounds in some *Antirrhinum majus* mutant genotypes can indicate the positions in the pigment pathway at which the genetic blocks occur (fig. 1). Thus, in the albino flower (homozygous for *nivea* (*niv*)) no flavonoid compounds can be detected and the block is presumably before the flavanone stage. Plants homozygous for *incolorata* (*inc*) produce flavones and aurone but no flavonols or anthocyanidin and must be blocked between flavanone and flavanonol. Flavonols, but no anthocyanidins, are present in an unmutated *pallida-recurrens* (*pal-rec*) genotype which is thus presumably blocked after the flavanonol stage, but whether this is before or after flavenol production cannot, as yet, be deduced. Anthocyanidins are naturally produced only when both the *niv* and *inc* genes are presented by at least one dominant wild-type allele. The series of *pallida* (*pal*) alleles includes many that reduce the quantity and variously determine the areas of anthocyanidin production on the corollas. The only completely acyanic allele at this locus is the unmutated unstable *pal-rec* (Fincham and Harrison, 1967).

In addition to the three genes mentioned above is the *eosinea* (*eos*) gene. When this is represented by the dominant allele *Eosinea* (*Eos*), the flavonoids formed have 3′,4′-dihydroxy substitution (*eg.*. cyanidin), but when recessive (*eos*) there is only one (4′) hydroxyl group (*e.g.* pelargonidin).

Support for the positions allocated to these genetic blocks might well be obtained by administering possible intermediates in the biosynthetic pathway to the various genotypes. The use of certain precursors could also initiate new pigments foreign to the species. These possibilities have been investigated and the results are reported in this paper, and briefly in Stickland and Harrison (1973).

2. Materials and methods

Dihydrokaempferol, prepared from the rhamnoside extracted from the leaves of *Exocarpus cupressiformis* (Cooke and Haynes, 1960), was kindly presented to us by Dr R. G. Cooke. Dihydroquercetin (chromatographically pure) was obtained from Koch-Light Laboratories Ltd. Dihydromyricetin was prepared from myricetin (obtained from Fluka A.G.) by reduction with sodium dithionite by the method employed by Geissman and Lischner (1952) for reduction of quercetin. The hydroxyl substitution pattern of the above flavanonols and of the corresponding anthocyanidins is given in table 1.

Table 1

Positions of OH groups	Flavanonol	Anthocyanidin
3, 5, 7, 4′	Dihydrokaempferol	Pelargonidin
3, 5, 7, 3′, 4′	Dihydroquercetin	Cyanidin
3, 5, 7, 3′, 4′, 5′	Dihydromyricetin	Delphinidin

Pigments were extracted from the flowers by immersion in cold (2° C.) methanol containing 1 per cent (w/v) HCl for 1-2 days. These were then separated on two-dimensional chromatograms essentially as described by Harborne (1963), except that cellulose thin-layers were used and the butanol:acetic acid:water solvent was modified to the proportions 4:0·6:5 in order to produce R_F values closer to those obtained with paper chromatography.

The *niv*, *inc* and *pal* lines of *A. majus* have been inbred and are regularly used as standard genetic stocks in this Institute.

Several techniques for administering the precursors to flowers were tried. Young flowering shoots were cut from the plant and the stems placed in a 0·1 per cent aqueous solution of dihydroquercetin; a strong production of pigment in the veins of the corolla occurred (plate I, B). However, when single flowers with their calyces and pedicels attached were immersed in the same concentration so that the liquid reached half-way up the corolla tube a more general synthesis of pigment occurred throughout the lobes (plate I, C). This synthesis of pigment was usually seen within 24 hours, depending on the genotype of the flower and the precursor used; some were slower and the pigment was less readily identified except by chromatography. A fine spray of dihydroquercetin (0·1 per cent solution) on an acyanic (*inc*) inflorescence still attached to the plant and enclosed in a polythene bag to minimise evaporation produced pigmentation similar to plate I, C within 2 hours.

The shortage of dihydromyricetin and dihydrokaempferol precluded the use of the techniques and concentrations mentioned above. More dilute solutions could only be tested on limited material and the synthesis of pigment was consequently much less; the chromatographic conclusions, however, were unequivocal.

3. Results and conclusions

Imbibition of dihydroquercetin by the acyanic and aflavonic *niv* flower allowed synthesis of cyanidin. The *niv* gene blocks the pigment pathway at an early stage (fig. 1) and the artificial insertion of the flavanonol overrides the block and the pathway can be completed. The *inc* gene blocks the pathway at a later stage, *i.e.* after flavanone production; aurone is formed on the face of the corolla but no anthocyanidin can be formed. The insertion of dihydroquercetin circumvents this block and considerable synthesis of cyanidin is possible. If *niv* and *inc* are both homozygously present cyanidin is synthesised, after addition of dihydroquercetin, in similar amounts to those obtained in *niv* flowers. However, the *pal* block, exemplified in unmutated *pal-rec* plants (Harrison and Fincham, 1964), remains unaffected by dihydroquercetin and no cyanidin is formed; the *pal* block is therefore after flavanonol synthesis and before anthocyanidin formation. Cyanidin is produced by dihydroquercetin in *niv*:*inc*:*Pal* plants irrespective of the plant being *eos* or *Eos*. If the plant is *Niv*:*Inc*:*Pal*:*eos* (*i.e.* naturally producing pelargonidin as its anthocyanidin and giving the pink flowered type) then the naturally produced pelargonidin is augmented by artificially synthesised cyanidin. No normal *Antirrhinum* produces both anthocyanidins in the same flower but the insertion of dihydroquercetin can produce cyanidin in a flower that is producing large quantities of pelargonidin. A duality in

anthocyanidin synthesis has been identified as occurring naturally in some species *e.g. Papaver rhoeas* (Scott-Moncrieff, 1937).

The incorporation of the precursor dihydrokaempferol into *niv*, *inc* and *niv*:*inc* plants that are also *Eos* enables cyanidin to be produced but if the plants are *eos* then pelargonidin is synthesised (table 2). Thus dihydrokaempferol can initiate the synthesis of cyanidin in a cyanidin blocked flower (*e.g. niv*:*Eos*) and pelargonidin in a pelargonidin blocked flower (*e.g. niv*:*eos*); both anthocyanidins can be synthesised from one precursor depending on the genotype of the naturally acyanic plant. In the case of dihydroquercetin, however, only the one anthocyanidin, namely cyanidin, can be synthesised irrespective of the *Eos*/*eos* constitution. The incorporation of either dihydroquercetin or dihydrokaempferol into *niv* plants only initiates synthesis of anthocyanidin; the treated flowers are still aflavonic although pigmented.

TABLE 2

	Block A		Block B	Block C	
Precursor	*nivea*:*eosinea*	*nivea*:*Eosinea*	*incolorata*:*Eosinea*	*pallida*	*Pallida*:*eosinea*
Dihydroquercetin	Cy	Cy	Cy	0	Pg+Cy
Dihydrokaempferol	Pg	Cy	Cy	0	—
Dihydromyricetin	—	Dp+[Cy]	Dp+[Cy]	—	—

[Cy = Cyanidin; Pg = pelargonidin; Dp = delphinidin; 0 = no anthocyanidin; (−) = not tested.]

The pigment delphinidin occurs in some species of *Antirrhinum* (*A. nutallianum*) but no hybrids between delphinidin producing species and *A. majus* have been reported and no success has been achieved at this Institute. However, artificial incorporation of the precursor dihydromyricetin has enabled delphinidin to be synthesised in *A. majus* flowers of the genetic constitution *niv*:*Inc*:*Eos* and *Niv*:*inc*:*Eos*, the only two genotypes we have been able to test with the small amount of dihydromyricetin currently available. In addition to the delphinidin, some cyanidin was also identified chromatographically: this may be due to impurities in the sample but a possible duality in pigment pathway cannot at this stage be excluded. In this case removal of one hydroxyl group would be implied.

Although only the anthocyanidin aglycones are mentioned above they were all found mainly as the 3-rutinoside, although a small amount of 3-glucoside was also produced.

These observations show that anthocyanidins can be produced from introduced flavanonols, but this does not prove that these flavanonols are on the normal biosynthetic pathway. Flavones were not synthesised from flavononols by any reversal of the scheme shown in fig. 1. However, it is possible for *Antirrhinum majus* flowers both to add a 3′-hydroxyl group since cyanidin was formed from dihydrokaempferol, and to attach sugars to the aglycones at this late point in the pathway.

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PRECURSORS AND GENETIC CONTROL OF PIGMENTATION

2. GENOTYPE ANALYSIS OF PIGMENT CONTROLLING GENES IN ACYANIC PHENOTYPES IN *ANTIRRHINUM MAJUS*

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SUMMARY

Imbibition of dihydroquercetin, dihydrokaempferol and naringenin by the flowers of certain genotypes of *Antirrhinum majus* enables rapid identification of hypostatic genes involved in pigment synthesis. Induced synthesis of anthocyanidin in acyanic flowers has been shown to occur after imbibition of particular acyanic flower homogenates.

1. INTRODUCTION

THE pigment pathway in *Antirrhinum majus* culminating in anthocyanidin can be blocked in several places by known genes. It has been found possible to synthesise anthocyanidins in acyanic flowers by administering the flavanonol precursors dihydroquercetin, dihydrokaempferol and dihydromyricetin (Stickland and Harrison, 1974). The presence of homozygous *nivea* (*niv*) in the genotype precludes the identification by visual inspection of the hypostatic genes *incolorata* (*inc*), *delila* (*del*), *pallida* (*pal*) alleles and *eosinea* (*eos*) except by breeding techniques; however, the use of these precursors and the flavanone, naringenin, has enabled many genotypes containing these genes to be readily identified.

2. MATERIALS AND METHODS

With the exception of naringenin (5,7,4'-trihydroxy flavanone) obtained from Koch-Light Laboratories Ltd. and used as a saturated aqueous solution all other materials and methods are as described by Stickland and Harrison (1974). The homogenates were prepared from macerated and ground