Anthocyanin Carrying Structures in Specific Genotypes of *Matthiola incana* R. Br.

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Several lines of the genetically well defined *Matthiola incana* (Brassicaceae) concerning the anthocyanin biosynthesis (line 02, 06, 10, and 14) store the anthocyanin pigment as crystals (line 06) or in anthocyanoblasts ("balls") in the epidermal cells (line 02, 10, and 14) of the flower petals. The genetic constitution of these genotypes is in addition to the basic factors f^+, g^+, and e^+, b^+b^+ or bb (cyanidin or pelargonidin type), ll, u^+u^+, and v^+v^+ or vv, demonstrating that the ll, u^+u^+ constitution is responsible for precipitation of anthocyanins in a certain structure independent of the other modificationally acting genes b and v. The ll, u^+u^+ types accumulate preferentially acylated 3-biosides.

Introduction

Anthocyanins are usually water soluble pigments localized in the vacuole of the upper and/or lower epidermal or subepidermal cells of plants [1]. However, it is known for several plant species that they accumulate the pigment in crystal-like structures or specific organelles called anthocyanoblasts [2, 3]. We investigated the localization of anthocyanins in *Matthiola incana* since in cell nuclei preparations of line 06 [4] crystal-like structures were observed. 16 different genotypes were analyzed which differ only in their genetic constitution with respect to anthocyanin biosynthesis [4, 5].

Materials and Methods

Plant material

Matthiola incana R. Br. (Brassicaceae), lines 1–16 (Table I) were cultivated under field conditions or in the greenhouse [4, 6]. Squash preparations of the flower petals were used for light microscopic studies.

Isolation of anthocyanin crystals

Flower petals of line 02, 06, 10, and 14 were homogenized in 0.06 M phosphate buffer pH 7.4 or in cell nuclei isolation medium [7]. The homogenate was filtrated through one layer of vlieseline and centrifuged at 6000 rpm (Minifuge, Heräus-Christ) for 10 min. The pellet was analyzed by light microscope. Sometimes Triton X-100 (0.1–1%) or sodium docecylsulfate (1%) was added to study the solubilization of the crystals.

Results and Discussion

The genetically very well defined lines of *Matthiola incana* offer a good system to study the effect of different gene combinations on the biosynthesis and localization of anthocyanin [4–6]. 16 differently coloured genotypes are available which contain the three complementary acting factors responsible for the construction of the basic flavonoid compound, genes f^+, e^+, and g^+ in a homozygous stage and in addition different combinations of the modifying genes b^+, l^+, u^+, and v^+ (Table I [5, 8]). Gene b^+ is coding for the 3′-hydroxylase [8–10] which adds an hydroxy group in 3′-position of the B-ring (cyanidin type, lines 1–8); in the case of the recessive stage bb only pelargonidins are formed (lines 9–16). The allel l^+ glycosylates the 5-position of the A-ring giving rise to 3,5-diglucosides. u^+ has a pleiotropic effect: 3-monosides are changed to 3-biosides, 3,5-diglucosides to 3,5-triglucosides by adding a xylose residue; biosides and triglucosides are further modified by acylation with different cinnamic acids. v^+ is only responsible for acylation [8]. The effects of the modifying genes are summarized in Fig. 1.

Light microscopic studies on squash preparations of flower petals from fully developed flowers revealed that in some mutants the anthocyanin pig-

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Table I. Genotypes of *Matthiola incana*, lines 1–16, and localization and type of anthocyanin accumulation

Line	Complementary factors for colour formation			Modificationally acting genes				Site and form of anthocyanin accumulation
	e	*g*	*f*	*b*	*l*	*u*	*v*	
01	+	+	+	+	+	+	+	w. s.[a]
02	+	+	+	+	*l*	+	+	balls in epidermis cells
03	+	+	+	+	+	*u*	+	w. s.
04	+	+	+	+	+	+	*v*	w. s.
05	+	+	+	+	*l*	*u*	+	w. s.
06	+	+	+	+	*l*	+	*v*	crystals in epidermis cells
07	+	+	+	+	+	*u*	*v*	w. s.
08	+	+	+	+	*l*	*u*	*v*	w. s.
09	+	+	+	*b*	+	+	+	w. s.
10	+	+	+	*b*	*l*	+	+	balls in epidermis cells
11	+	+	+	*b*	+	*u*	+	few balls in subepid. cells
12	+	+	+	*b*	*l*	*u*	+	w. s.
13	+	+	+	*b*	*l*	+	*v*	few balls in subepid. cells
14	+	+	+	*b*	*l*	+	*v*	balls in epidermis cells
15	+	+	+	*b*	+	*u*	*v*	few balls in subepid. cells
16	+	+	+	*b*	*l*	*u*	*v*	few balls in subepid. cells

[a] w. s. = water soluble.

ment is stored in "ball"-like structures in every epidermis cell or even as crystals (Table I). Lines 02, 10, and 14 show anthocyanin "balls" starting as an amorphic structure; line 06 contains a prominent rhombic crystal in every epidermal cell (Fig. 2). Lines 11, 13, 15, and 16 produce anthocyanin accumulations in some subepidermal cells as shown for red cabbage [3] called anthocyanoblasts, whereas the epidermal cells are filled with water soluble anthocyanin compounds in the vacuole. All the other lines contain only water soluble pigments in the vacuoles of the epidermal cells.

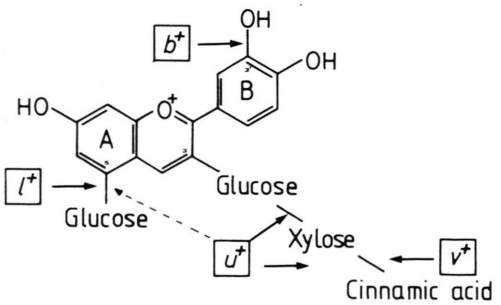

Fig. 1. Effects of the alleles b^+, l^+, u^+, and v^+ of *Matthiola incana* on the flavonoid molecule, modified from Seyffert [8].

Fig. 2. Anthocyanin crystals of *Matthiola incana*, line 06. Fully developed flower petals were homogenized in cell nuclei isolation medium, filtrated and centrifuged at 6000 rpm for 10 min. × 6000.

The attempt to isolate and collect these anthocyanin carrying structures succeeded only in line 06 where the rhombic crystals can be sedimented from a cell homogenate together with the cell nuclei (Fig. 2). The anthocyanin accumulations of the lines 02, 10, and 14 are immediately solubilized in 0.06 M phosphate buffer. The crystals of line 06 are soluble in about 1% Triton X-100 or 1% sodium dodecylsulfate assuming that an organic membrane is surrounding the anthocyanin pigment. This membranous structure has been discussed to be the site of anthocyanin biosynthesis [3].

The common genotype of the lines 02, 06, 10, and 14 is *ll*, u^+u^+ (Table I) suggesting that this genetic constitution is responsible for anthocyanin precipitation. With the allel u^+ in the absence of a functioning *l* allel the plants produce mainly 3-biosides which are acylated with various cinnamic acids [8]. The assumption, therefore, is that the acylated 3-biosides accumulate in the epidermal cells as antho-

cyanin containing specific structures (lines 02, 10, and 14) or in the cyanidin type line 06 as rhombic crystals. The precipitation of anthocyanin pigment may influence quantitative studies on enzyme activities involved in the anthocyanin biosynthesis during flower development.

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