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Anthocyanins of *Impatiens holstii*

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Antokyany u *Impatiens holstii*

1. Byly chromatografovány antokyany tří typů *Impatiens holstii.* Bylo zjištěno, že

typ A (*f. nana*, sorta Záře, s šarlatovými květy) obsahuje ve stonku — glukosidy malvidinu, v petalech — glukosidy pelargonidinu a peonidinu, v sepalech — glukosid malvidinu a cyanidinu, v pylu — glukosidy a snad i xylosidy malvidinu;

typ B (*f. nana*, sorta Záře, s červenofialovými květy) obsahuje ve stonku — malvidin, v petalech — peonidin, v sepalech — malvidin a cyanidin, v pylu — malvidin;

typ C (vzpřímený typ, s červenofialovými květy) obsahuje v petalech — malvidin a peonidin, v sepalech — malvidin a cyanidin, v pylu — malvidin.

U typů B a C cukry nebyly sledovány.

2. Jsou uvedeny úvahy o metabolismu těchto pigmentů v ontogenesi a fylogenesi některých druhů rodu *Impatiens.*

Summary

1. Chromatographic analysis of anthocyanins of three types of *Impatiens holstii* was carried out. It was found that

the type A (*f. nana*, variety Záře, with scarlet blossoms) contains in the stem — glucosides of malvidin, in the petals — glucosides of pelargonidin and paeonidin, in the sepals — glucosides of malvidin and cyanidin, in the pollen — glucosides and possibly also xylosides of malvidin;

the type B (*f. nana*, variety Záře, with red-violet blossoms) contains in the stem — malvidin, in the petals — paeonidin, in the sepals — malvidin and cyanidin, in the pollen — malvidin;

the type C (erect type, with red-violet blossoms) contains in the petals — malvidin and paeonidin, in the sepals — malvidin and cyanidin, in the pollen — malvidin.

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In the types B and C, sugars were not analyzed.

2. The metabolism of these pigments in the ontogeny and in the phylogeny of some species of the genus *Impatiens* is discussed.

Introduction

It has been known that the anthocyanins occur in the most varied plant organs. They are most abundant in the blossoms but are present in other parts of the plant as well (stems, leaves, sepals); they were found even in the root tips (e.g. Molisch 1928) and in pollen grains (e.g. Lubliner-Manowska 1955).

The anthocyanins were identified in the blossoms and leaves of a variety of species, by different methods: colour reactions, distribution coefficients, (e.g. Robinson and Robinson 1931, 1932), spectral analysis and most of all paper chromatography (e. g. Hayashi and Yukihide, 1956, and others). On the other hand, the data on the occurrence of anthocyanins in other parts of the plant are rather scarce.

The physiological role of anthocyanins has not been solved so far. In order to elucidate the problem conclusively the maximum amount of experimental material is required, concerning the distribution of anthocyanins in the plants not only from the point of view of phylogeny, ontogeny and ecology, but also that of organ distribution. It is the aim of the present communication to identify the anthocyanins as genetic charactes of *Impatiens holstii.*

Most attention was devoted here to the type with scarlet blossoms, anthocyanized stem (particularly in the nodes), blue-red pollen and coloured sepals (*Impatiens holstii*, *f. nana*, variety Záře, designated as type A in the sequel). For the sake of comparison, an orientation analysis of two other types of this species with somewhat bluer blossoms was carried out (the type B belongs to the same variety as the type A, the type C differs from the two by its erect growth, by a slightly different red-violet tinge of the blossoms and by the fact that it does not have an anthocyanized stem). The origin of the type C could not be ascertained. The plants were reproduced vegetatively.

The anthocyanins analyzed here were derived from the petals, sepals, stem and pollen. According to Robinson and Robinson (1932) the blossoms of *Impatiens holstii* should contain pelargonidin (the orange-red variety) and paeonidin (the blue-pink variety): the authors, however, used only distribution coefficients to carry out their analyses. No more recent data could be found in the literature although some species of the genus *Impatiens* have received considerable attention of late (e.g. Alston and Hagen 1957; Hagen 1958; Alston 1958).

No data on the type of pigments in other parts of the plants could be found. The same holds for the analysis of the sugars present.

Methods

The anthocyanins were extracted from fresh material (blossoms, stem, pollen and sepals) with methanolic hydrochloric acid (1% solution). The extracts were purified by chromatography, by developing in two mixtures: 1st — butanol: 2N-HCl (1 : 1), 2nd — butanol: glacial acetic acid : water (4 : 1 : 5).

The individual spots were cut out, eluted with 50% methanol and hydrolyzed by 10-min. boiling with 20% HCl (with an equal or a two-fold volume). After cooling, the anthocyanidins were extracted with amylalcohol, the free sugars of the glucosides remaining in the aqueous phase.

The aglucones were identified chromatographically, using pure standards, in the descending direction: 1. glacial acetic acid : conc. HCl : water (30 : 3 : 10) — the Forestall mixture; 2. acetone : 5% HCl (1 : 1); 3. m-cresol : glacial acetic acid : water (25 : 1 : 24). As no synthetic standards or crystalline samples were available, anthocyanidins from plants, repeatedly identified previously, were used as standards* (their R_F values being compared with the data in the literature at the same time): malvidin from *Malva mauretanica* and *Impatiens balsamina*, paeonidin from *Paeonia officinalis*, cyanidin from *Rosa* sp., *Paeonia officinalis*, and pelargonidin from *Pelargonium zonale*.

The sugars were also analyzed chromatographically, the aqueous phase remaining after extracting the aglucone with amylalcohol being used here. At first, the solutions were evaporated to dryness over NaOH, the residue dissolved in water, desalted with a mixture of the cation exchange resin Zerolite 225 and of the anion exchange resin Zerolite E. The de-ionized sugars were again evaporated, dissolved in 50% ethanol and placed on Whatman paper No. 1. The samples were developed, together with the standards, in the following systems: 1. isobutanol : glacial acetic acid : water (4 : 1 : 1), in the descending direction, three or four times (MÁLEK et al. 1957); 2. butanol : pyridine : benzene : water (5 : 3 : 1 : 3), in the ascending direction, five times; 3. acetone : butanol : water (7 : 2 : 1), circular chromatography, twice.

The detection was carried out by the very sensitive but less specific method with $AgNO_3$; further with anilin phthalate and a mixture of dinitrophenylamine, anilin, ethylacetate and phosphoric acid (modification according to VÍTEK 1958).

Results and Discussion

1. Type A (with scarlet blossoms):

Petals: it was found with the aid of the above methods that the petals contain 2 types of aglucones: pelargonidin and paeonidin. The first and the third glucoside spots (counting from the start to the solvent front) correspond to paeonidin, the second and the fourth spots to pelargonidin. Glucose was found to be their sugar moiety.

Stem: malvidin and glucose were identified here.

Sepals: glucosides of malvidin and cyanidin were found here.

Pollen: malvidin could be detected, clearly accompanied by glucose, but in addition, the second and the fourth glycoside spots seemed to be accompanied by another corresponding to xylose.

2. Type B (with blue-violet blossoms):

This type contains only malvidin in the pollen and in the stem, while only paeonidin could be found in the petals. The sepals contained malvidin and cyanidin. No analysis of sugars was carried out.

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* Other authors have worked along this line some time ago (WERCKMEISTER 1954).

3. Type C (erect, no anthocyanins in the stem):

The pollen of this type contains only malvidin, while the petals were found to contain malvidin and paeonidin. The anthocyanins of the sepals are of the same type as in the preceding types. No analysis of sugars was carried out. For the sake of clarity, the results are summarized in Table 1.

Thus it follows that the anthocyanins of the stem, pollen and sepals are of the same type (the sepals containing cyanidin in addition to the much greater amount of malvidin). The petals, however, contain completely different types of anthocyanins, namely pelargonidin and paeonidin. The general synthetic process of anthocyanins appears thus to differ in the petals in one of the last phases where the degree of hydroxylation and methoxylation is determined. A certain independence of the petals in the biological synthesis of the anthocyanins was demonstrated by KLEIN and HAGEN (1961) for petals of *Impatiens balsamina* grown *in vitro*; there the buds weighing 25 mg. possessed a finished apparatus for the formation of anthocyanins.

If the types of anthocyanidins of the analyzed types of *Impations holstii* are compared with those of different *Impatiens* species it may be seen that

Table 1

	Petals	Pollen	Stem	Sepals
Type A	Paeo, Pel, Glu	Mal, Glu, (Xyl)	Mal, Glu	Mal, Cya, Glu
Type B	Paeo	Mal	Mal	Mal, Cya
Type C	Paeo, Mal	Mal	—	Mal, Cya

Paeo — paeonidin, Pel — pelargonidin, Mal — malvidin, Cya — cyanidin, Glu — glucose, Xyl — xylose.

malvidin occurs here rather frequently (Table 1). ALSTON and HAGEN (1957) found it in 6 out of 12 genotypes tested, to occur in the petals of *Impatiens balsamina* and quite frequently also in the sepals (in 5 genotypes). Pelargonidin is also rather frequent (in 8 genotypes). On the other hand, cyanidin is rather rare in this species (in the sepals of 2 genotypes) but it was found e.g. in *Impatiens capensis* and *Impatiens pallida*. Paeonidin was found only in the sepals of four genotypes of *Impatiens balsamina*. According to the participation of the individual anthocyanin types in the species and types of *Impatiens* examined heretofore it appears probable that malvidin represents the fundamental type of anthocyanins, being the most complicated of them (it has two methoxy and one hydroxy groups in the side ring B). This assumption is supported by the fact that a more complex type of anthocyanin is always dominant with respect to a simpler one, as is known from genetic work. For a confirmation of this assumption, however, further investigation, especially of the genetic character, is necessary.

As concerns the sugar moieties of the anthocyanins, glucose could be demonstrated quite unequivocally (HAGEN 1960, found it also in the species

Impatiens balsamina). In some cases, a spot corresponding to arabinose could be found on the chromatograms but this appears to be an artifact. The occurrence of xylose in the pollen anthocyanins is rather probable (xylose being known to be frequently bound in the anthocyanins). It is not likely that we should be dealing here with freely occurring xylose as it could not be found in the samples where the anthocyanins were adsorbed on an ion exchange resin during desalting and where only the free sugars were chromatographed.

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Антоцианы у *Impatiens holstii*

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1. Хроматографированы антоцианы трех типов *Impatiens holstii*:

тип «А» (форма nana сорт «Záře» s шарлаховыми цветками) содержит: в стебле глюкозид мальвидина, в лепестках глюкозиды пеларгонидина и пеонидина, в чашелистиках глюкозид мальвидина и цианидина, в пыльце глюкозиды, может быть, даже ксилозиды мальвидина.

тип «Б» (форма nana сорт «Záře» с краснофиолетовыми цветками): в стебле мальвидин, в лепестках пеонидин, в чашелистиках мальвидин и цианидин, в пыльце мальвидин.

тип «В» (прямостебельный тип с краснофиолетовыми цветками): в лепестках мальвидин и пеонидин, в чашелистиках мальвидин и цианидин в пыльце мальвидин.

Сахара типов «Б» и «В» не изучались.

2. Обсуждается вопрос метаболизма этих пигментов в онтогенезисе и филогенезисе некоторых видов рода *Impatiens*.