ANALYTICAL STUDY OF PETAL COLOUR OF MATTHIOLA INCANA

INADA, ASAJI
Biological Laboratory, General Education Department, Kyushu University

YOSHITAKE, TETSUO
Horticultural Laboratory, Faculty of Agriculture, Kyushu University

https://doi.org/10.15017/7340413

出版情報：Sieboldia : acta biologica . 2 (3), pp.187-195, 1960-10-20. 九州大学教養部生物学教室
バージョン：
権利関係：

ANALYTICAL STUDY OF PETAL COLOUR OF *MATTHIOLA INCANA*

ASAJI INADA

(Biological Laboratory, General Education Department, Kyushu University)

and

TETSUO YOSHITAKE

(Horticultural Laboratory, Faculty of Agriculture, Kyushu University)

There has been many investigations of plant pigments, but there has been few data concerning with pigment production and genetical factors. Since paper partition chromatography and spectrophotometoric method has been widely used to identify the pigment constituentes, petal colour analysis has become very convenient. In the present work the relations between genetical factors and anthocyanin productions are given.

Experiments and results

Material: Next 12 horticultural varieties were cultivated in a green house since 1956: Golden rose, Balls rubby, American beauty, Balls pink, Deep apricot, Balls blue, Balls white, Hansens all double rose, Paciffic pink, Butter scotch. Each two of these were crossed, and F_1 were grown next year. When these P and F_1 plants were grown, petals were picked up, and were used at once, or dried at 40°C in an oven for overnight and then stocked in dessicators. Extraction procedures: [I], 10 g. of fresh petals or 1 g of dried petals were macerated in a mortar with 30 ml of methanolic HCl (97 : 3 v/v). After 1–2 hrs, extracts were filtered through Buechner's funnel. One more extraction was repeated by 30 ml of methanolic HCl. Extracts were combined. These pigment extracts were concentrated to 2–3 ml at 40°–50°C under reduced pressure, then used as material for analysis. [II], The other procedure which was often used was as follows. Extraction procedures were as same as the above. The combined extracts were poured into a separatory funnel, added 10 ml of 1% HCl and 200 ml of benzen, vibrated gently, stood still for 2–3 hrs untill two layers were separated. Poured off the pigment layer, added small volume of 1% HCl

and 30 ml of ethyl ether. Mixed, stood still untill two layers were separated, then decanted ethylether layer. Repeated this procedure twice. Added 30 ml of ethyl acetate into this red pigment layer. Decanted ethyl acetate layer. Added 30 ml of ethyl acetate. Repeated twice. Then red pigment layer poured off, and were concentrated at 40°–50°C under reduced pressure, and then used as material for analysis. This procedure was used most frequently.

Paper partition chromatography and spectrophotometory: Above mentioned samples were smeared on Toyo filter paper no. 50, 30×30 cm, as a stripe from 4 cm distance from the edge. The solvents were as follows: BuOH*: AcOH*: H_2O, 4 : 1 : 2 (v/v), EtAc*: AcOH : H_2O, 3 : 1 : 1 (v/v), BuOH : 1N HCl, 1 : 1 (v/v) (upper layer). Of these Et · Ac : AcOH : H_2O solvent had very rapid developing ability, and it gave fairly clear separation of anthocyanins, but it gave tailing of the spots. Both BuOH : AcOH : H_2O and BuOH : 1N HCl gave clear spots but by those sovents each spots were situated too closely. BuOH : AcOH : H_2O solvent was used most frequently. Fig. 1 was the

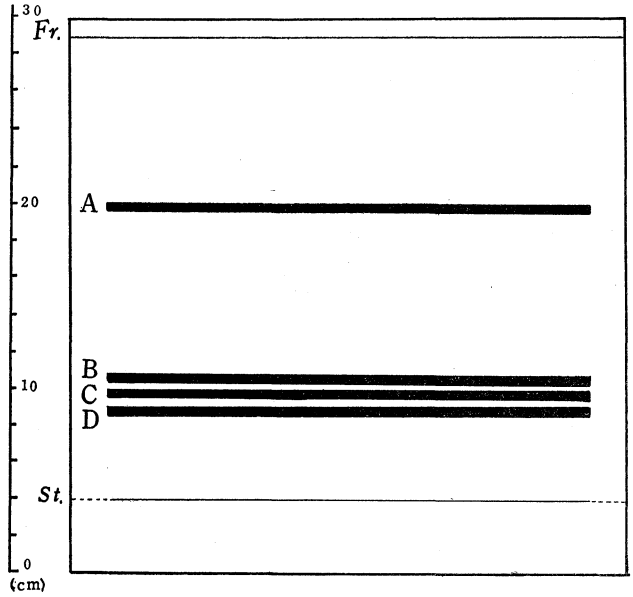

Fig. 1. Paper-partition-chromatogram of the refined anthocyanin from variety Fire bird. Solvent: BuOH : AcOH : H_2O, 4 : 1 : 2 (v/v). Explanation of A, B, C and D bands see the text. Fr. (Solvent front), St. (starting line)

paper-chromatogram of Fire bird. Of these A, B, C and D, the first two band A and B had purplish red color and the mirgin of those bands were seen dimly. The C band was pink and the mirgin was seen fairly clear, but the volume was very small, and it gave orange fluorescence irradiated with U.V.-ray. The D band was dirty purple, and was seen to be

* BuOH : =butanol, AcOH=acetic acid, EtAc=ethylacetic acid

destructed product. These bands were cut free each other, and the pigment were effluented from the paper pieces. Effluences were concentrated at 40°–50°C under reduced pressure. Each concentrated effluence was spotted on Toyo filter paper no. 50, dried, and then rechromatographed. Each effluence gave different Rf. value, and those Rf. value were the same order of Fig. 1. (Fig. 2). When mixed of those effluences, it gave 4 spots. So these 4 spots were considered having different structures.

The mirgins of A and B bands of Fig. 1 being dimly, it was considered that those pigments were acylated. Refind anthocyanin prepared by the above mentioned process [II] was mixed with 10% KOH, and stood still for 70 minutes. Then added 1–3 drops of concentrated HCl untill pinkish orange color reappeared. Concentrated to small volume at 40°–50°C under reduced pressure. Rechromatographed. It gave two spots. Upper spot was correspond to A and lower spot was correspond to C band of Fig. 1.

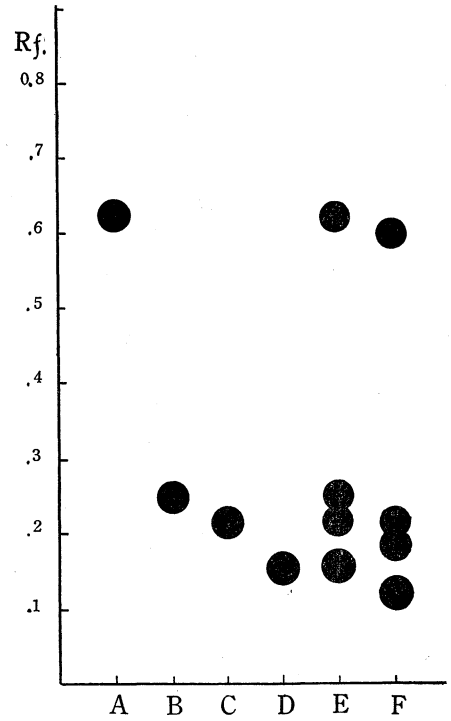

Fig. 2. Paper-partition-chromatogram of the effluences from the A, B, C and D bands of Fig. 1. Solvent: BuOH : AcOH : H_2O 4 : 1 : 2 (v/v). A (effluence from A band) B (effluences from B band) C (effluences from C band) D (effluence from C band) E (mixed of all effluences) F (mixed of all effluence from A, B, C, and D band of Balls blue).

The same was smeared as a stripeon Toyo filter paper no. 50, and was developed. A and C bands were obtained. The A band was cut free and effluented by methanolic HCl. Added 10% KOH, stood still 70 min, then added 2 or 3 drops of concentrated HCl untill orange color reappeared. Concentrated at 40°–50°C under reduced pressure. Spotted on and developed. Only C spot was obtained. The 3 bands, A, B, and C which were obtained from the chromatogram of refined anthocyanin were cut free each other, effluented by methanolic HCl, added 10% KOH to each effluences, stood still for 70 minutes, then added 2 or 3 drops of concentrated HCl untill orange colour reappeared. Aerated was continued for 2–3 hrs to expel HCl, then concentrated at 40°–50°C under reduced pressure and then rechromatographed. They gave only C spot. The effluence from B band and the hydrolysed effluence by 10% KOH from B band were mixed, then rech-

romatographed. A, B and C spots were obtained. But this effluence and the hydrolysed product were developed parallel, A spot did not appeared.

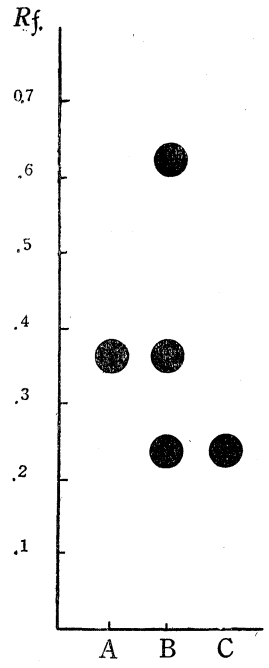

Fig. 3. Paper-partition-chromatogram of the effluence and of the hydrolysis product from the B band of variety Fire bird. (A): the effluence, (C): the hydrolysis products, (B): mixed of them. Solvent: BuOH : AcOH : H_2O 4 : 1 : 2 (v/v).

Spectrophotometery was made by Hitachi spectrophotometor UV type. Samples were made by paper - partition - chromatography, namely row anthocyanin extracts or refined anthocyanin were smeared on filter paper as a stripe, dried, and then developed by BuOH : AcOH : H_2O, 4 : 1 : 2 (v/v). After chromatography was accomplished, dried, and each bands were cut free from each other, then the pigments were effluented by methanolic HCl. Effluences concentrated at 40°–50°C under reduced pressure, resmeared on filter paper, and paper-partition-chromatography were repeated. Accomplished pigment bands were cut free, effluences were made. These effluences were used as a sample for spectrophotometery. Such a sample of Fire bird as of Fig. 1, effluences from A and B bands gave similar absorption spectra (Fig. 4), and the samples which was obtained from the chromatogram of the hydrolysis products of effluence of A, and B bands by 10% KOH gave absorption spectra similar to pelargonidin-diglucoside. Parallel experiment was made with the crystalline specimen of pelargonidin diglucoside obtained from *Pelargonium* sp. The C band of Fig. 1, and the C spot of Fig. 2 were the same as the crystalline pelargonidin-diglucoside, and the absorption spectra were the same.

The samples obtained from other strains were analysed by the similar procedure, and the results were summarised in Table 1. In this table the results from Balls blue was different from those of Fire bird, and the F_1 which were produced by crossing with Balls blue were different from the F_1 crossed with Fire bird. Balls blue had acylated cyanidin derivatives, and the F_1s which were produced by crossing with Balls blue had the same acylated cyanidin derivatives.

Identification of glycosidic sugars were made by paper-partition-chromatography (Fig. 5). Effluence from the C band of the above mentioned procedure

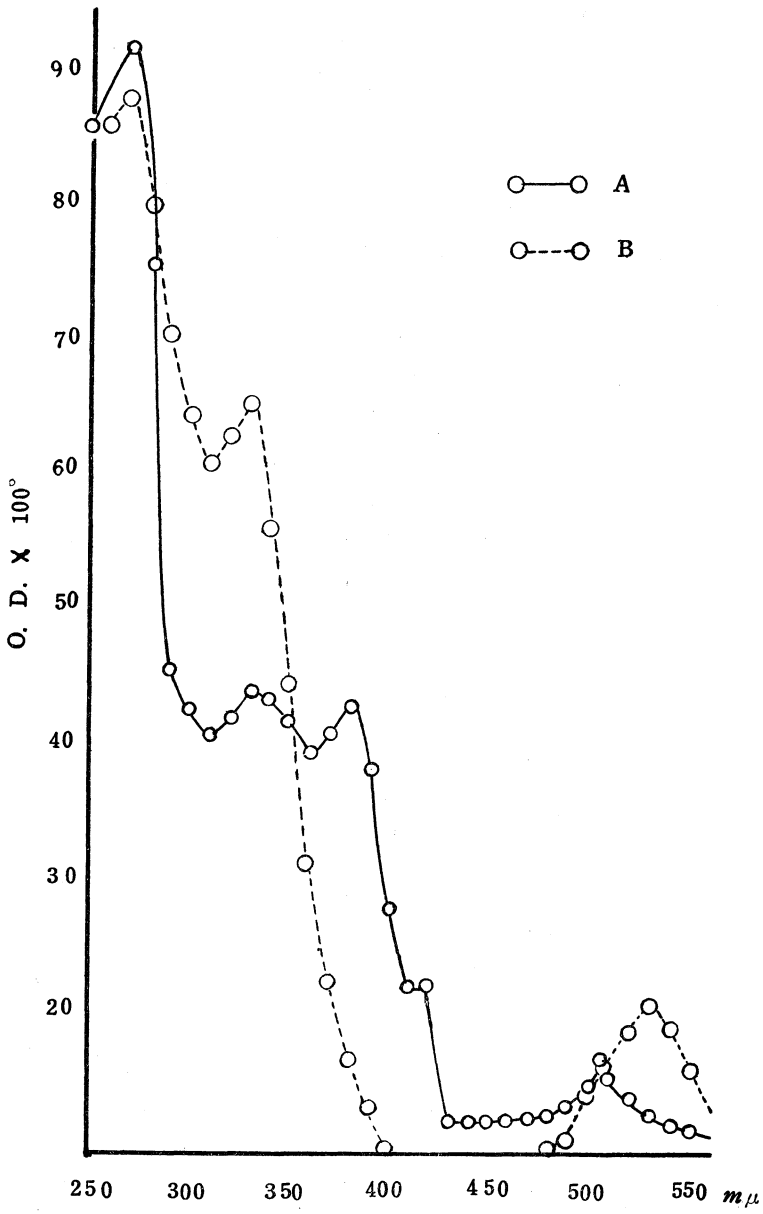

Fig. 4. Absorption spectra of the pigments. A. acylated pelargonidin diglucoside (Fire bird). B. acylated cyanidin diglucoside (Balls blue).

was collected, added a few drops of concentrated HCl, boiled for 3 minutes, and cooled at once. Added iso-amylalcohol about 1/3 volume of the sample. Vibrated gently in a separatory funnel. When two layers were separated,

Table 1. Anthocyanin compositions of horticultural varieties of *Matthiola incana*.

Strain no.	Varieties	Petal colors	Cyanidin derivatives	Pelargonidin derivatives
2.	Golden rose	Pink	− − − − − −	+ + +
21.	Balls rubby	Crimson	− − − − − −	+ + + + +
31.	American beauty	Crimson	− − − − − −	+ +
41.	Balls pink	Pink	− − − − − −	+ +
51.	Deep aplicot	Pinkish yellow	+	− − − − − −
61.	Balls blue	Purple	+	− − − − − −
71.	Balls white	White	− − − − − −	− − − − − −
81.	Hansens all double rose	Purple	+	− − − − − −
91.	Pacific pink	Pink	− − − − − −	+
121.	Butter scotch	Cream	− − − − − −	− − − − − −
141.	Minoret	White	− − − − − −	− − − − − −
131.	Malmeson pink	Crimson	− − − − − −	+ +
203.	Fire bird	Crimson	− − − − − −	+ + +
2 ×61.		Purple	− − − − − −	+ + +
42×61.		Purple	− − − − − −	+ +
91×71.		Purple	− − − − − −	+
51×61.		Purple	+ +	− − − − − −
21×61.		Purple	+ + +	− − − − − −
293×61.		Purple	+ +	− − − − − −
31×61.		Purple	+ + +	− − − − − −
21×61.		Purple	+ + +	− − − − − −
51×21.		Pink	+ +	− − − − − −
21×203.		Dark red	− − − − − −	+ +

water layer was poured off. Added small volume of active charcoal and warmed for few minutes. Through filtration clear sample was obtained. Aeration was continued in order to eliminate HCl for 4–5 hrs, then concentrated at 80°C under reduced pressure. Concentrated sample was spotted on Toyo filter paper no. 50. Dried and developed by following solvents. BuOH : AcOH : H_2O, 4 : 1 : 2 (v/v), or water saturated phenol. From the result of this paper-partition-chromatogram, the glycosidic sugar of this sample was correspond to glucose.

Polyphenol oxidase activity: When petals were dried in an oven, colorless petals were decayed earlier than coloured petals. As it was thought that necrosis were caused by polyphenol oxidase activity in petals, the polyphenol oxidase activity was estimated following the next procedure. 1.4 g of fresh petals or leaves were macerated in 10 ml of M/15 phosphate buffer pH 7.0 in a mortar. Filtered off residues, and took 5 ml of this samples. Added 1 ml of 3% pyrogallol, stood still in 35°C water bath for 15 minutes and then added 1 ml of concentrated H_2SO_4 to stop the reaction. Added 5 ml

of ethyl ether, vibrated gently, then the yellow pigment formed in water layer were transferred to ethyl ether layer. Poured off ethyl ether layer and the absorption spectrum of it was measured by Hitachi spectrophotometor. Absorption peak was observed at 426 mμ. If the 10% transmitant change at 426 mμ should make one unit, the polyphenol oxidase activity could be shown as Table 2.

Table 2. Polyphenol oxidase activities of petals of *Matthiola incana*.

Variety no.	Petal color	Activities
141×22	White	3.5
81×71	Pink	5.2
131×203	Pink	3.4
51×21	Pink	4.4
21×203	Pink	3.4
21×31	Pink	4.1
21×203	Red	4.8
61×71	Purple	5.3
42×61	Purple	5.2
203×61	Purple	4.0
91×71	Purple	3.2
21×61	Purple	4.4
61×203	Purple	4.0
31×203	Red	3.4

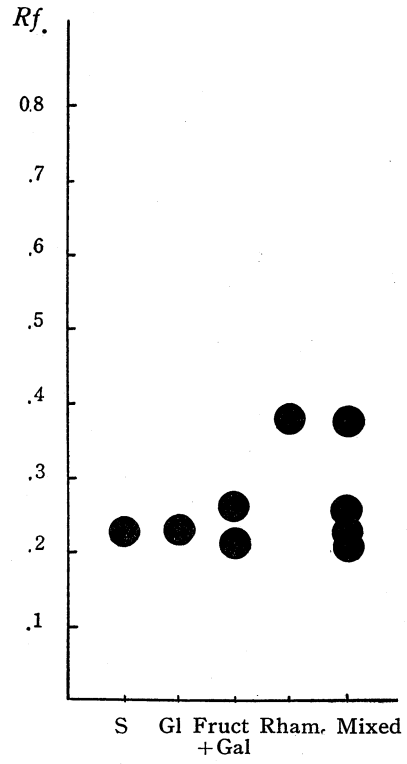

Fig. 5. Paper-partition-chromatogram of the glucosidic sugars of effluences. S. (effluence from C band) Gl. (Glucose), Fruct.+Gal. (Fructose+Galactose), Rham. (Rhamnose), Mixed (Mixed the glucosidic sugars of the effluence with all of these authentic sugars) Solvent: BuOH : AcOH : H_2O, 4 : 1 : 2 (v/v).

Discussion

It has been well known that anthocyanin production of *Matthiola incana* is controlled by compensatory genes C and R. Further, the petal colours are controlled by other two genes B and V. The B gene has an ability to change red colour to purple and V gene has a relation of petal colour purity and dullness. In present study, it has become clear that the petal

anthocyanin has two aglycon types. For example, variety Fire bird has pelargonidin derivatives and Balls blue has cyanidin derivatives respectively. So it must be remembered that the coloured petals which has both dominant C and R genes must have another gene which controls the production of pelargonidin derivatives or of cyanidin derivatives. On this study variety Fire bird had 3 anthocyanins, of which 2 were acylated pelargonidin. According to spectrophotometric study of the pigment (Haborne '58) acylated anthocyanidin which has its absorption peak at 269 mμ, 505 mμ is pelargonidin 3-5 diglycoside acylated with cinnamic acid. In this study, the B band of Fire bird had its absorption peak at 270 mμ, 330 mμ, 505 mμ. So the anthocynin of Fire bird was thought to have similar constitution. Anthocyanins of Balls blue were acylated cyanidin derivatives from its spectra and paper-partition-chromatograms.

That the gene which controlls the cyanidin derivative production is dominant to the gene controlling pelargonidin derivetive production was considered from the petal colour of F_1 and its analytical study.

Variety Butter scotch had cream color and had no anthocyanin. Of these colourless petalls had flavones and plastid. Other colourless varieties were the same compositions.

Polyphenol oxidase activity seemed to have no concrete relation to colour production. But the colourless petals were decayed earlier than coloured petals, so it seemed probable that the colourless petals had more substrate for polyphenol oxidase than coloured petals.

The authers wish to express their thanks to honoured Prof. H. Ito and to Prof. E. Fukushima for helpful suggestions and valuable advices.

Summary

1) Anthocyanin composition of *Matthiola incana* were determined. One groups have acylated cyanidin derivatives and the other groups have acylated pelargonidin derivatives.
2) The F_1 plants which were produced by crossing between the cyanidin and pelargonidin groups have acylated cyanidin derivatives. The gene controlling production of cyanidin derivatives are dominant to the gene controlling production of pelargonidin derivatives.
3) Polyphenol oxidase activities were determined. The activities are not so different between the different groups.

References

Bate-Smith, E. C. (1948). Paper chromatography of anthocyanin and related substances in petal extracts. Nature, London., **161**: 835.

Bate-Smith, E. C. (1954). Leuco-anthocyanins. 1. Detection and identification of anthocyanins formed from leuco-anthocyanins in plant tissues. Biochem. J. **58**: 122.

Bateson, W., E. R. Saunders and R. C. Punnet. (1906). Experimental studies in the physiology of heredity. Rept. Evol. Com. Roy. Soc., Rept. **III**: 53.

Dodds, K. S. and Long, D. H. (1955). The inheritance of color in diploid potatoes I. Types of anthocyanidins and their genetic loci. J. Genetics **53**: 136.

Geissman, T. A. and Lurd, L. (1955). The anthocyanin of *Spirodela oligorhiza*. Arch. Biochem. Biophys. **56**: 259.

Harbone, J. B. (1956). Biochemical genetics of the acylated anthocyanins of tuberous solanums. Biochem. J. **63**: 330.

Harborne, J. B. (1958a). Late stages in the biosynthesis of flavonoids. Biochem. J. **68**: 128.

Harborne, J. B. (1958b). Anthocyanidins of the *Primulaceae*. Nature, Lond., **181**: 26-27.

Harborne, J. B. (1958c). Spectralsmethods of a characterizing anthocyanins. Biochem. J. **70**: 22.

Harborne, T. B. and Sherratt, H. S. A. (1957). Variations in the glycosidic pattern of anthocyanins. Part. II. Experientia **13**: 486.

Hayashi, K. (1936). Spectrophotographische Untersuchungen ueber die Farbstoff vom Benzopyryliumtypus. V. Ueber die Beziehungen zwischen Lichtabsorption und Hydroxylsowiezuckersubstitution beiden 2-Phenylbenzopyrylium-Farbstoffen. Acta Phytochim. Tokyo, **9**: 1.

Matsuura, H. (1933). A bibliographical monograph on plant genetics. second edition; Sapporo. 200-205.

Sounders, E. R. (1911). Further experiments on the inheritance of 'doubleness' and other characters in stocks. J. Genetics **1**: 303.