Paper chromatographic studies on flower pigments of *Hibiscus coccinea*, *H. moschata* and F₁, F₂ and F₃ hybrids obtained from the crosses between them

(Studies on interspecific and intergeneric hybridization in the *Malvaceae* VI)¹⁾

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I. Introduction

The author has previously reported on the characters of the hybrids between *Hibiscus coccinea* and *H. moschata*, but did not referred to the flower pigments⁴⁾. In this paper, therefore, the author deals with the manifestation of the anthocyanin pigments of the flowers by paper chromatographic method using the hybrids and its parents.

II. Materials and Methods

Materials used are *H. coccinea*, *H. moschata* and F₁, F₂ and F₃ hybrids obtained from the crosses between them, which were the same as those of the previous report⁴⁾.

The flower colors of *H. coccinea* and *H. moschata* were dark red and exceedingly light red purple respectively. The flower colors of 3 plants in F₁ hybrids were all dark red purple, showing the darker color than both parents. The flower colors of 29 plants in 3 F₂ lines and 80 plants in 8 F₃ lines had various grade colors between dark red purple like F₁ plants and light red or red purple. And such plants as show the same color as *H. moschata* could not be found.

In this paper, then, each one plant of both parents, one plant out of F₁ hybrid, 2 plants of the darkest and the lightest color out of 29 plants of F₂ hybrid, 5 plants of the darkest, the lightest and the various grades between both colors out of 80 plants of F₃ hybrid were used for color analysis.

The individual numbers of plants used and their flower colors are shown in Table 1. The flowers in season were all used.

The extraction and the following treatment of the pigments were as follows :

(1) The extraction of the pigments

Fresh matured petals (5~20 g) were collected and immediately minced in cold 1% methanolic hydrochloric acid (10~15 cc). After centrifugation, the supernatant liquid

Table 1. Flower color of materials used

Materials	Flower color
<i>H. coccinea</i>	Dark red
<i>H. moschata</i>	Very light red purple
F ₁ 1	Dark red purple
F ₂ 2~2	Light red
2~8	Dark red
F ₃ 2~4	Light red
3~1	Dark red
4~1	Light red
7~10	Dark red purple
8~10	Light red

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was transferred to the separating funnel, had a small amount of water and a large excess of ethyl acetate added to it and was shaken. The resultant aqueous layer which contained all anthocyanins was the extraction of the pigments.

(2) The research of aglycon

The extraction (5 cc) of the pigments above mentioned was mixed with an equal volume of 20% aqueous hydrochloric acid and was heated for hydrolysis. After its reaction mixture was diluted with water, the anthocyanidins were drawn with amylalcohol and then filtered. After water and ether were added to it, anthocyanidins were again transferred to aqueous layer. Next, after washing the above aqueous layer with ether, petroleum ether, ethyl acetate and carbon disulfide, its pigments were transferred with proper quantity of amylalcohol, and spotted in duplication on a large sheet of chromatographic paper (Tōyō No. 50; 40×40). Chromatographic separation was effected at room temperature (at 27±2°C) by ascending method using acetic acid-36% hydrochloric acid-water (5 : 1 : 5 v/v).

(3) The research of glycoside

After addition of water to the extraction of the pigments, its solution was washed with ether, petroleum ether, ethyl acetate and carbon disulfide etc., was cleared beforehand of the flavonoid, and was, using the specific solvent, made into the chromatographic belt, which was then cut into several color belt. And the pigments were extracted again with the solution of MeOH-CH₃COOH (5% Acid) from the several belts, and spotted in duplication on a chromatographic paper, using A.A.H. 5:1:5 v/v solvent.

On the details of method, the author referred mainly to three literature.^(1,2,3)

III. Results and Discussions

The researches of aglycons (anthocyanidin) and its glycosides (anthocyanin) were shown in Table 2 and 3 respectively.

As regards aglycons, one spot was obtained in each material, regardless of the kinds and graduation of color.

Table 2. R_f values of Anthocyanidins

Materials	R_f
<i>H. coccinea</i>	0.36
<i>H. moschata</i>	0.36
F ₁ 1	0.35
F ₂ 2~2	0.36
2~8	0.36
F ₃ 2~4	0.34
3~1	0.34
4~1	0.34
7~10	0.34
8~10	0.34
Cyanidin	0.34

Table 3. R_f values of Anthocyanins

Materials	R_f
<i>H. coccinea</i>	0.58 0.76
<i>H. moschata</i>	0.34 0.58
F ₁ 1	0.58 0.75
F ₂ 2~2	0.58 0.75
2~8	0.58 0.74
F ₃ 2~4	0.58 0.73
3~1	0.58 0.73
4~1	0.60 0.74
7~10	0.60 0.75
8~10	0.59 0.74
Chrysanthemin (3-Monoglucoside)	0.61
Idaein (3-Monogalactoside)	0.61
Illicyanin (3-Glucoxyloside)	(0.85)
Keracyanin (3-Glucorhamnoside)	0.74
Cyanin (3.5-Diglucoside)	(0.70)
(Complex glycoside)	0.34

As regards glycoside, two spots appeared in each plant, and one spot of these two was similar, but the other was dissimilar in both parents. The two spots which appeared in eight plants out of F_1 , F_2 and F_3 hybrids were both the same as those of *H. coccinea*.

In consequence, it is revealed that the pigment composition of the flower materials used is as follows: as to the aglycones, cyanidin in each material, and as to the glycosides, either chrysanthein (cyanidin 3-monoglucoside) or idaein (cyanidin 3-monogalactoside) and keracyanin (cyanidin 3-glucorhamnoside) in *H. coccinea* and F_1 , F_2 and F_3 hybrids, and either chrysanthein (cyanidin 3-monoglucoside) or idaein (cyanidin 3-monogalactoside) and unknown one in *H. moschata*. And this unknown spot which appeared in *H. moschata* seems to be complex glycoside, so the author likes to propose that it will be named "*Moschanin*".

The distinction between chrysanthein and idaein will be investigated later.

From the results obtained above, it is shown that *H. coccinea* and F_1 , F_2 and F_3 hybrids contain cyanidin, chrysanthein (or idaein) and keracyanin, *H. moschata* contains cyanidin, chrysanthein (or idaein) and moschanin which was found and named for the first time in this paper.

3 plants in F_1 , 29 plants in F_2 and 80 plants in F_3 were cultivated at the same time as their parents, but no plants were found showing such exceedingly light red purple as *H. moschata*. And the flower pigments of one plant out of F_1 , 2 plants out of F_2 and 5 plants out of F_3 were tested, but could not be found showing the similar R_f values to *H. moschata*, and these all eight hybrid plants showed the same R_f values as *H. coccinea*. This shows that the manifestation of color pigments was controlled by the polygenes. Accordingly, if we cultivate more plants in F_2 , F_3 and later generations, it seems that the plants showing the similar R_f values to *H. moschata* will appear.

Next, the flower pigments of the two plants in F_2 , five plants in F_3 were different on their kind and density of color, but showed the similar R_f values to *H. coccinea*. Accordingly, this shows that many multiple genes are concerned.

IV. Summary

(1) Paper chromatographic experiments were carried out in order to determine the kind of anthocyanins, and to clarify their genetical behaviours using *H. coccinea*, *H. moschata* and F_1 , F_2 and F_3 hybrids between its parents.

(2) The color of *H. coccinea*, *H. moschata* and F_1 hybrids were dark red, exceedingly light red purple and dark red purple. The color of F_2 and F_3 hybrids between its parents were varied between the darkest red purple like F_1 and the lightest color as light red purple excepting such plants as the color of *H. moschata*.

(3) As regards aglycones, the each material used was cyanidin.

(4) As regards cyanidin glycoside, *H. coccinea* and all hybrids used in this experiment were all chrysanthein (or idaein) and keracyanin, but *H. moschata* was chrysanthein (or idaein) and "*moschanin*", which was newly found and named complex glycoside in this paper.

(5) The manifestation of the color pigments of the materials used in this paper seems to be controlled by polygenes and be concerned with many multiple genes.

V. References

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- (2) —; 1957 : Fortschritte der Anthocyaneforschung in Japan mit besondere Berücksichtigung der papierchromatographischen Methoden, *Die Pharmazie* 12 (5) 245~249
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- (4) —; 1962 : Studies on the interspecific hybrids between *Hibiscus coccinea* and *H. moschata* (No. 2) (Studies on interspecific and intergeneric hybridization in the *Malvaceae* III), *Jap. Jour. Breed.*, 12 (2), 69~74

モミジアオイ (*Hibiscus coccinea*), クサフヨウ (*H. moschata*)

ならびにそれらの間の雑種 F_1 , F_2 および F_3 の花色の

ペーパークロマトグラフ法による研究

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花色はモミジアオイでは濃赤色, クサフヨウでは極く淡い赤紫色, F_1 (3個体)では濃赤紫色であり, F_2 (29個体)および F_3 (80個体)では濃赤紫色より淡赤色迄の間の種々の程度の花色を呈し, クサフヨウの如き極く淡い赤紫色の花色の個体は出現しなかつた。これらの材料のうち, 両親, F_1 および F_2 と F_3 の代表花色個体の花色分析の結果, Aglycon としてはいずれも Cyanidin であつた。その配糖体である Anthocyanin についてはモミジアオイと雑種ではいずれも Chrysanthemin (または Idaein) と Keracyanin であつたが, クサフヨウ

では Chrysanthemin (または Idaein) と未知の R_f 値のものであつて, これはいわゆる Complex glycoside と思われ, ここでは "Moschanin" と呼ぶことにする。 F_2 および F_3 において, 各種の色調の代表個体の R_f 値がいずれもモミジアオイのそれと同じで, クサフヨウのそれと同じ R_f 値の個体がなかつたことは, 現実にクサフヨウの如き極く淡い赤紫色の花色の個体が出現しなかつたことと関連があり興味あることであり, 花色の発現は Polygene 系により支配され, また多くの同義因子が関与しているものと推定される。