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Maekawa, Susumu

Nakamura, Naohiko

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STUDIES ON THE COLORATION OF CARNATION FLOWERS

X. Uptake of Sugars and Malonic Acid to Detached Petals in Relation to Petal Growth and Anthocyanin Formation

Susumu MAEKAWA* and Naohiko NAKAMURA*

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Abstract

To know the role of sugar in the anthocyanin formation and in the growth of carnation flowers, the detached petals of cultivar 'Coral' were cultured in the media which contained different kinds or concentrations of sugar and different concentrations of malonic acid.

The petal width did not significantly differ among the kinds of sugar, but the petal length increased remarkably in glucose medium.

The most production of anthocyanins was obtained in sucrose followed by the order of maltose, glucose and fructose.

As to the response of the growth and pigmentation of petals to the concentration of sucrose, the petal width increased with the increase in concentration of sucrose up to 40%, and the petal length and anthocyanin content reached the peak in 10% and 20% sucrose media respectively. These responses were higher under light irradiation than in darkness.

The sugar content in detached petals increased with the increase of concentration of sugar in medium and when cultured in 10% sucrose medium, the sugar content in the petals was the same as that in intact blooming flowers.

When malonic acid was given to the medium together with sugar, the anthocyanin formation was promoted as compared with sucrose only.

Introduction

As reported in the previous papers[10,11], the fact that sugar content in petals was noticeably high as compared with that in leaves is specially interested in relation to anthocyanin synthesis in carnation flowers.

Several investigators have shown already that the anthocyanin synthesis was promoted by sugar uptake in plant tissues[1,3,5,17,22,24] and the ^{14}C-labelled sugar was incorporated into the flavonoids[6].

In biosynthetic researches of flavonoids, the influence of chemicals has been examined with some plants[12,16,18,19,20] and FAUST[4] found that malonic acid known as metabolic inhibitor enhanced anthocyanin production in apple skin.

Such feeding experiments are effective to analyze further the relationship between the environmental factors and the coloration or pigmentation in flowers. However, few experimental results have so far been reported on the flowering plants.

In view of the above, this experiment was conducted *in vitro* to make clear the effect of kinds or concentrations of sugar along with the effect of malonic acid on the anthocyanin formation in detached carnation petals.

Materials and Methods

The material used is cultivar 'Coral' of carnations, *Dianthus caryophyllus* L.. Five uncolored petals detached were cultured in the sterilized medium consisting of 1 mm glass balls (about 8 mm in depth) soaked

* Laboratory of Floriculture and Olericulture

with each test solution in petri-dish (5×6 cm) for 18 days. The procedures of petal culture have been described in detail in the previous paper[9].

Temperature was regulated at 20°C during the culture, and light intensity was at 3,500 lux of white fluoresent light.

After the culture of 18 days, anthocyanin in 5 petals in each petri-dish was extracted immediately with 10 ml of methanolic 0.2% HCl. Optical density of crude extract was measured at 510 nm.

Somogyi-Nelson method[23] was used to determine reducing and total sugars. Sugar concentration was indicated with percentage per dry weight of samples.

Details of treatments will be described in each of items in the results and discussion.

Results and Discussion

1. Kind of sugar in medium

Ten kinds of sugar were used in this experiment and the sugar concentration in each medium was 0 M (distilled water only), 0.12 M in monosaccharide, 0.06 M in disaccharide and 0.04 M in trisaccharide. After the detached petals were planted in each medium, they were incubated in darkness for 10 days and then were irradiated with fluorescent light for 8 days. The results in the growth and anthocyanin content of petals obtained are shown in Table 1.

No significant differences in the width of petals were recognized among various kinds of sugar. However, the growth in length of the petals cultured in glucose medium was promoted remarkably as compared with that in other sugar media. The petal elongation in sucrose, maltose and fructose media followed glucose.

The amount of anthocyanin in the petals was the largest in sucrose (0.503 in OD) and decreased gradually in order of maltose (0.344), glucose (0.317) and fructose (0.246), but these latter sugars were still stimulative for anthocyanin formation as compared with the remainder sugars.

ISHIKURA *et al.*[7] pointed out that among the kinds of sugar in roots of radish and turnip, glucose was a major component throughout the growing stage, and sucrose and fructose were present in larger quantity in the red root than in the white.

THIMANN *et al.*[24] examined the effects of various kinds of sugar on anthocyanin formation of *Spirodela* and pointed out that sucrose permitted the greater pigment synthesis, glucose was the least effective and fructose was intermediate in effectiveness. STRAUS[22] obtained a similar result by corn endosperm-tissue-culture.

Thus, it was confirmed in most of the experiments that sucrose was the most suitable sugar for anthocyanin formation, but it could not be understood from this experiment, why did sucrose stimulate.

From the fact that glucose and fructose (monosaccharide), and sucrose and maltose (disaccharide) which are hydrolyzed to glucose or fructose, brought good results for anthocyanin formation of carnation flowers, it may be considered that glucose and fructose are utilized favorably as the material of anthocyanins and/or act as a stimulant for anthocyanin synthesis.

Table 1. The effects of kinds of sugar on the petal size and the anthocyanin content of detached carnation petals.

Kind of sugar		Petal size (mm)		OD
		Width	Length	
1. Water		14.1	16.9	0.037
2. Xylose		14.5	17.9	0.144
3. Arabinose		14.9	17.9	0.110
4. Rhamnose		14.1	17.9	0.044
5. Glucose		14.4	27.2	0.317
6. Fructose		15.2	18.7	0.246
7. Galactose		15.0	17.8	0.184
8. Maltose		15.2	18.8	0.344
9. Lactose		14.7	17.6	0.091
10. Sucrose		15.3	19.9	0.503
11. Raffinose		14.8	17.9	0.145
L.S.D.	5%	N.S.	1.53	.0981
	1%		2.06	.1289

Sugar concentration in medium : 2–7 ·····0.12 M
8–10·····0.06 M
11·····0.04 M

2. Concentration of sugar in medium

The detached petals were cultured in medium containing 0, 5, 10, 20 or 40% sucrose, then they were maintained immediately under light irradiation or in darkness for 18 days. The results obtained are summarized in Figure 1.

The response of the petal growth and anthocyanin formation to sugar concentration was higher under light irradiation (L) than in darkness (D).

The petal width was enhanced with the increase of sugar concentration up to 40 %, while the petal length reached the peak at 10 % sucrose and declined gradually with the increase of concentration above 10 %.

On the other hand, the anthocyanin formation was most stimulated in 20 % sucrose, but decreased with further increased concentrations.

The amount of anthocyanin produced in 20 % sucrose in darkness was equal to that in 5 % sucrose under light irradiation.

NAITO *et al.*[13] confirmed that the content of anthocyanin in Muscat Bailey A grapes correlated highly with the color degree and with the content of reducing sugars of berries.

STICKLAND[21] reported that anthocyanin in cultured chrysanthemum florets was highest with 4 % sucrose and carotenoid reached a maximum at 0.6 % sucrose, while chlorophyll concentration declined continuously as the sucrose concentration was raised.

KLEIN *et al.*[8] showed that the anthocyanin formation in detached petals of *Impatiens balsamina* increased with the increasing concentration of sucrose up to 2.5 %, and SMOCK[17] recognized that the anthocyanin content in skin disk of apple increased still in high concentration of 0.3 M (ca. 10 %).

CREASY *et al.*[2] manifested with the leaves of strawberry that anthocyanin formation reached the maximum in 0.05-0.10 M (ca. 1.7-3.4 %) sucrose media under light irradiation, but in darkness, the increase of anthocyanin was proportional to the increase of sucrose concentration within 0.3 M.

The stimulative sugar concentration for anthocyanin formation in detached carnation petals was remarkably high as compared with those obtained by KLEIN *et al.* and others.

Such differences among various crops may be due to the differences in absorbing ability of sugar and/or in sensitivity to sugar concentration.

3. Sugar contents in detached and intact petals

In order to clarify the relationship between anthocyanin formation and sugar content in the petals, the sugar contents in the detached petals cultured in various sucrose concentrations (0, 2, 5, 10, 20 and 40 %) and in the intact petals at four growing stages of flower buds were measured. The results obtained are shown in Figure 2.

As to the detached petals, the sugars in the petals increased with the increase of sucrose concentration up to 40 % in media.

Total sugar in the petals cultured in 40 % sucrose was 46 % per dry weight of samples (petals) and 80 % of total sugar was reducing sugar.

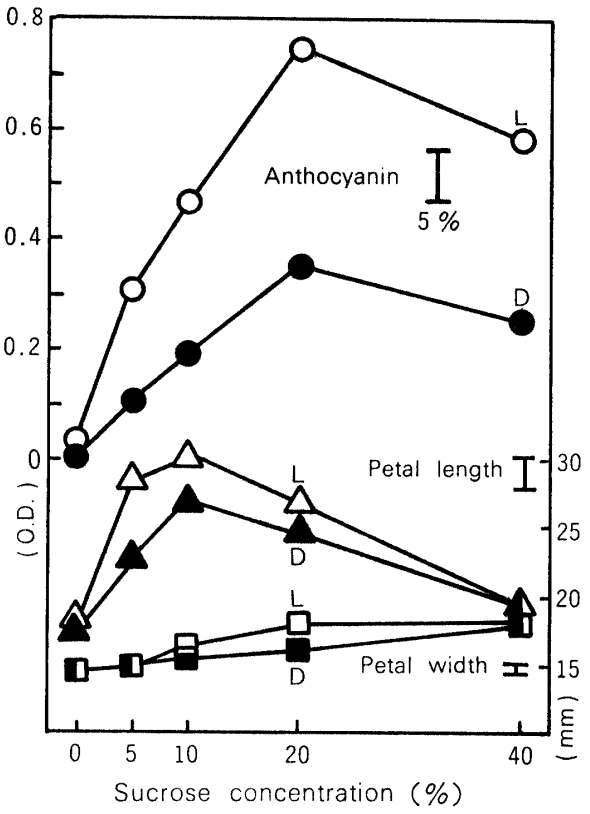

Fig. 1. The effects of sucrose concentration on the petal size and anthocyanin content of detached carnation petals.
L: under light irradiation, D: in darkness.

On the other hand, the sugar content in the intact petals increased rapidly just after the beginning of blooming (Stage 3) and the content in blooming flowers (Stage 4) was almost equal to that in the detached petals cultured in 10 % sucrose medium.

Based on the facts that firstly, a large amount of sugars was accumulated in the intact petals and secondly, the growth and anthocyanin formation of the detached petals were promoted by relatively high concentrations (10-20 %) of sugar as described at section 2, it was suggested that the sugar in petals related closely with the growth and anthocyanin formation of petals.

It was ascertained by the observation that the period (Stage 3-4) when the sugar content in the intact petals increased rapidly corresponded with the period when the nectar was secreted actively from the nectary in the flower buds.

NICHOLS *et al.*[14,15] confirmed by the feeding with ^{14}C-sucrose that the sugar in flowers had an important role for the senescence of carnation cut flowers and it was transferred easily to the nectary, gynaecium and stem.

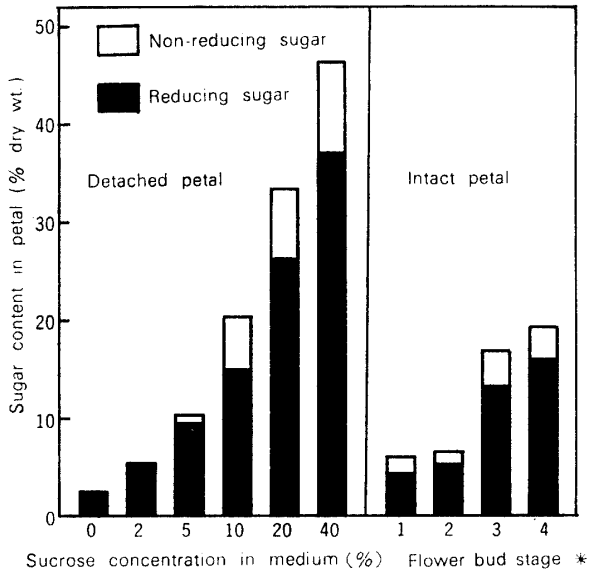

Fig. 2. The comparison of sugar contents in detached petals cultured in the media containing various sucrose concentrations (left) and in intact petals at various flower stages (right).
* 1 : closed flower bud (2.0 cm length),
2 : closed flower bud (2.5 cm),
3 : beginning of blooming,
4 : blooming.

Such a fact would suggest a close relationship between the sugar in petals and nectar.

The kinds of sugar in the petals and nectar were tentatively examined by paper chromatography. Glucose, fructose and sucrose were detected in both the petal and nectar.

It is interesting that these sugars were effective for the growth and anthocyanin formation of detached petals as shown in Table 1.

4. Uptake of malonic acid

Detached petals were cultured in the medium containing 0, 0.001, 0.005, 0.01 or 0.02 M malonic acid only and in the medium containing malonic acid of each concentration together with 0.06 M sucrose.

They were continuously irradiated for 8 days after 10 days of culture in darkness. All media were adjusted to pH 6.8 with sodium hydroxide.

When the petals were cultured in malonic

Table 2. The effect of malonic acid on the anthoyanin content of detached carnation petals.

Malonic acid (M)	OD
0	0.097[a]*
0.001	0.101[a]
0 005	0.098[a]
0.01	0.088[a]
0.02	0.085[a]

* Mean separation by Duncan's multiple range test, 5% level.

Table 3. The effect of malonic acid and sugar on the anthocyanin content of detached carnation petals.

Malonic acid (M)	Sucrose (M)	OD
0	0	0.110[a]*
0	0.06	0.620[b]
0.001	0.06	0.677[bc]
0.005	0.06	0.789[cd]
0.01	0.06	0.856[d]
0.02	0.06	0.788[cd]

* Mean separation by Duncan's multiple range test, 5% level.

acid media without sucrose, as shown in Table 2, no significant differences in anthocyanin formation were recognized among various concentrations of malonic acid.

However, in the media containing both malonic acid and sucrose, as shown in Table 3, the anthocyanin formation in petals was promoted as compared with the medium of sucrose only, and was stimulated most in the medium of 0.01 M malonic acid.

FAUST[4] examined the effect of malonic acid on anthocyanin formation in apple skin and concluded that by the malonic acid treatment, the Embden-Meyerhof-Parnas (EMP) pathway was inhibited more than the pentose phosphate (PP) pathway and glucose metabolism preceded through the PP pathway, resulting in an increased amount of anthocyanins.

From the fact that malonic acid was effective on anthocyanin formation in carnation petals when given together with sucrose, it was assumed that anthocyanin formation was promoted because malonic acid inhibited peculiarly the reactions in glucose metabolism as concluded by FAUST.

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カーネーションの花色発現に関する研究（第10報）

単離花弁の生育とアントシアン生成に及ぼす糖及びマロン酸の影響

前川　進・中村直彦

要　約

カーネーション花弁のアントシアン生成に対する糖の役割を明らかにするため，'コーラル'の色素生成前の単離花弁を用いて，糖の種類や濃度を異にした培地で培養を行なった。なお，糖の代謝とも関係をもつマロン酸の影響についても合わせ検討した。

まず，糖の種類については，花弁巾に差は見られなかったが，花弁長は特にGlucose培地での伸長が著しかった。

花弁内のアントシアンはSucrose培地で最も多く生成され，Maltose, Glucose, Fructose培地がこれに次いだ。一方，Sucroseの濃度については，暗黒下及び光照射下ともに，花弁巾は40%培地で，花弁長は10%培地で，そして，アントシアン含量は20%培地で最高値を示した。しかしながら，暗黒下より光照射のもとで，花弁の生育及び色素の生成はより促進された。

Sucrose 濃度が高い培地ほど培養された単離花弁の糖含量は増加した。また，10% Sucrose培地での培養花弁の糖含量は開花時における花弁の糖含量とほぼ等しかった。

マロン酸（0.001～0.02M）が糖（0.06M）とともに培地に与えられたとき，糖のみの培地よりアントシアンの増加が見られ，マロン酸濃度が0.01Mのとき，アントシアン生成量は最も多かった。しかしながら，マロン酸が単独で培地に与えられたときはアントシアン生成に対してほとんど効果がなかった。