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Studies on the Spherical Bodies Containing Anthocyanins in Plant Cells, IV. A Survey of the Occurrence of Anthocyanoplasts in the Hypocotyls in some Plant Species.

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Abstract

A survey was conducted on the occurrence of anthocyanoplasts using the hypocotyls of 16 families comprising 35 species, of which the names appear in Table 1. The present survey resulted in the discovery that anthocyanoplasts were recognized in the hypocotyls of 21 species.

The sizes of the anthocyanoplasts, their shapes and their colors found here closely resembled those in the hypocotyls of radish previously reported on by YASUDA *et al.* (1985). However, there were some differences between the anthocyanoplasts found here and those of radish. Especially the morning glory hypocotyl data were strikingly different from those obtained from radish, as is shown below.

1. *The relationship between the pigment level in the hypocotyls and the frequency with which anthocyanoplasts appear in them.*

Anthocyanoplast appeared during whole period of pigment formation of morning glory, while in contrast anthocyanoplasts only appeared in the hypocotyls of radish during the first half of pigment formation.

2. *The relationship between the numbers of anthocyanoplasts per cell and their diameters*

The relationship between them was rather complicated, suggesting that fusion of several smaller anthocyanoplasts and the division of larger anthocyanoplasts occurred alternatively during the developmental course of anthocyanoplasts. This differs very markedly from the hypocotyls of radish which showed fusion only in their developmental stages.

It was also found that the anthocyanoplasts in the hypocotyls of morning glory have the ability to display light reaction in anthocyanin biosynthesis exactly like the hypocotyls of radish.

Introduction

Anthocyanoplast is a kind of organella, discovered by POLITIS (1911) and once more studies by PECKET *et al.* (1980) and SMALL *et al.* (1982). YASUDA and his co-workers (YASUDA and SHINODA, 1985 ; YASUDA and TSUJINO, 1988 ; YASUDA, MITSUI and ONISHI, 1989) also conducted detail investigation of anthocyanoplasts using the hypocotyls of radish, and pointed out several characteristics enumerated below :

1. Anthocyanoplasts have some special properties of cytoplasm.
2. Their appearance may be related to the rapid biosynthesis of anthocyanins in the cells.
3. They have the capacity to display light reaction which is indispensable for the anthocyanin biosynthesis in the cells of this plant species.
4. Two or more anthocyanoplasts fuse with one another to form a larger anthocyanoplast in the course of their developmental stages.

With this information in mind, one can conclude that only a few plant species have been used as material in the studies concerning the appearance of anthocyanoplasts in the hypocotyls of seedlings.The authors of the present paper would like to emphasize that more kinds of plant species should be applied in order to extend a better understanding of anthocyanoplasts. This consideration formed the point of departure for the present survey.

Material and Methods

The plant material used in the present survey consisted of hypocotyls of various plant species which contained anthocyanins in their cells. Names of the plants species dealt with in the present survey are listed in Table 1.

The seeds of these plant species which were purchased from Nakazutaya Nursery in Matsumoto, Japan, were soaked in distilled water for about 24 hours, and then germinated on two disks of filter paper (Toyo filter paper No. 11) which had first been moistened with distilled water in Petri dishes. Germinations were performed at about 25 °C under the continuous illumination of white light (about 7,000 luxes) except when stated otherwise.

The hypocotyls were collected from the seedlings 3～4 days or more after sowing, and the cross sections of fresh hypocotyls were prepared by cutting them with a razor blade. The sections were subjected to anthocyanoplast counts and measurement of their diameters with a microscope.

Anthocyanoplast counts per cell and measurements of diameters of anthocyanoplasts were carried out as described by YASUDA *et al.* (1985, 1989). The levels of anthocyanin in the hypocotyls were estimated following the procedure proposed by MATSUBARA *et al.* (1990).

Table 1 Occurrence of anthocyanins and anthocyanoplasts in the hypocotyls of various plant species.

Plant species		Occurrence	
		Anthocyanin	Anthocyanoplast
Brassica juncea	(Brassicaseae)	+	+
☆*Brassica olerasea*	(〃)	+	+
Brassica rapa	(〃)	+	+
Brassica napus	(〃)	+	+
☆*Raphanus sativus*	(〃)	+	+
Phaseolus angularis	(Papilionaceae)	+	+
Astragalus sinicus	(〃)	+	+
Hibiscus esculentus	(Malvaceae)	+	+
Gossypium indicum	(〃)	+	−
Daucus Carota	(Apiaceae)	+	−
Petroselinum Crispum	(〃)	+	+
Viola × *Wittrockiana*	(Violaceae)	+	+
Lagerstroemia indica	(Lythraceae)	+	−
Pharbitis Nil	(Convolvulaceae)	+	+
☆*Ipomea rubrocaerulea*	(〃)	+	+
Quamoclit pennata	(〃)	+	+
Callicarpa dichotoma	(Verbenaceae)	+	−
Salvia officinalis	(Lamiaceae)	+	−
Salvia splendens	(〃)	+	−
Mentha piperita	(〃)	+	−
Capsicum annuum	(Solanaceae)	+	−
Torenia fournieri	(Rhinanthaceae)	+	−
Patrinia scabiosaefolia	(Valerianaceae)	+	−
Dipsacus Fullonum	(Dipsacaceae)	+	−
Cosmos bipinnatus	(Asteraceae)	+	+
Lactuca Scariola	(〃)	+	−
Arctium Lappa	(〃)	+	+
Zinnia elegans	(〃)	+	+
Chrysanthemum cocoieum	(〃)	+	+
Callistephus chinensis	(〃)	+	+
Tithonia rotindefolia	(〃)	+	+
Liatris spicata	(〃)	+	+
Phleum pratense	(Poaceae)	+	+
Allium Cepa	(Liliaceae)	+	−
Allium schoenoprasum	(〃)	+	−

○Asterisk denotes that authors reexamined data on seedlings by PECKET *et al.* (1980).
○+ : Occurrence ; − : No occurrence

Results and Discussion

The results of the present survey are listed in Table 1. The total number of plant species mentioned in this table is 35 belonging to 16 families. Although the number of plant species, actually used in the present survey, included more than those shown in this table, plant species not containing any anthocyanin in their hypocotyls were omitted in this table. Accordingly, in the column indicating occurrence of anthocyanin in Table 1 only plus signs can be found.

Anthocyanoplasts were recognized in the hypocotyls of 21 in 35 plant species. One thing, however should, in the opinion of the authors of the present paper, not be forgotten. If evidence of anthocyanoplasts failed to be recognized in the present survey, it does not necessarily mean that anthocyanoplasts are entirely absent. There could be two reasons why microscopic examination failed to recognize them as such ; one being that the structure is originally absent in the cells and the other that the structure is too small to be recognized.

The sizes, shapes and colors of the anthocyanoplasts found in the plant material indicated in Table 1, closely resembled those in the hypocotyls previously examined by YASUDA *et al.* (1985). However, there were some differences between the anthocyanoplasts of plant species surveyed here and those of radish reported on

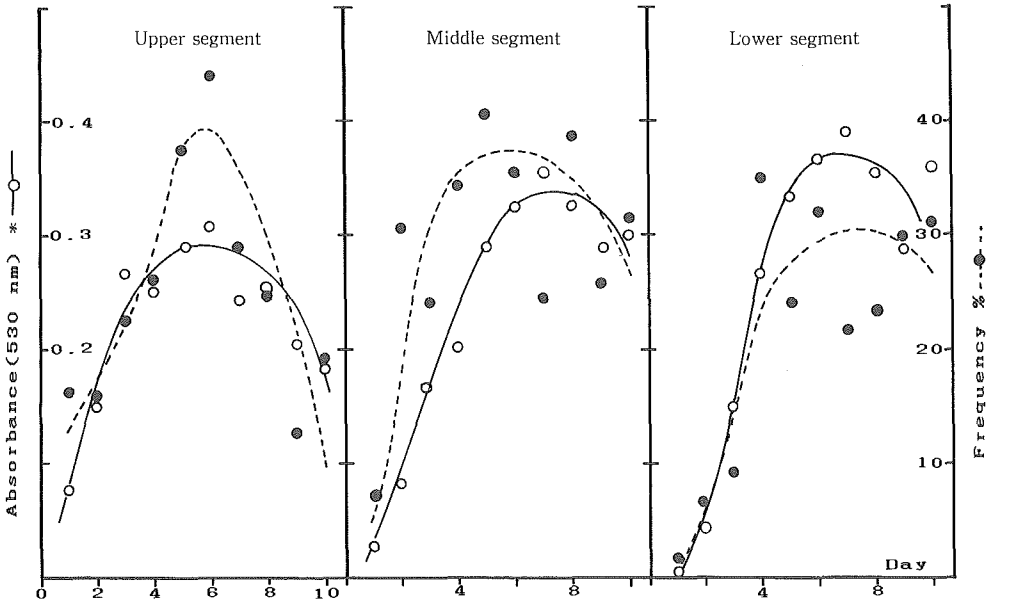

Fig. 1 The relationship between the anthocyanin level and the frequency of appearance of anthocyanoplasts in *Ipomea rubrocaerulea* hypocotyls.

* Absorbance of the extract obtained from three hypocotyls with 5 ml of 0.5 % methanolic HCl.

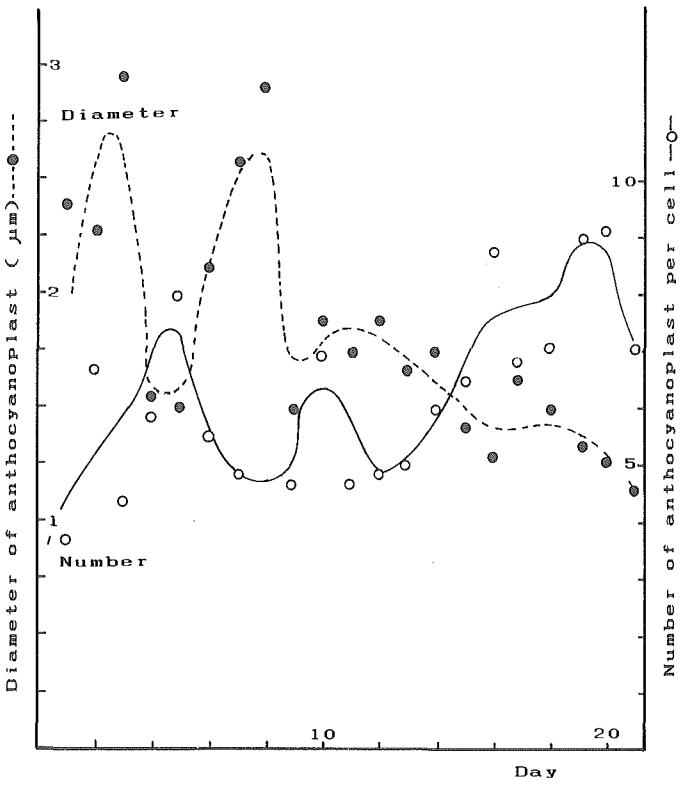

Fig. 2 Time course of the change in numbers and diameters of anthocyanoplast in the middle segment of *Ipomea rubrocaerulea* hypocotyls.

previously, especially with regard to the relationship between their occurrence and anthocyanin levels as well as between their diameter and number in a cell with age of hypocotyls advancing. Data obtained from the hypocotyls of morning glory (*Ipomea rubrocaerulea*), most strikingly different from the results of the radish study, are indicated in Fig. 1 and 2.

Fig. 1 illustrates the relationship between the anthocyanin levels and the frequency of appearance of anthocyanoplasts in the hypocotyls of morning glory. From this figure it is clear that the anthocyanoplasts appeared during the whole period when anthocyanin formed in the hypocotyls of this plant. This result contrasts with the case of the hypocotyls of radish in which anthocyanoplasts appeared during the first half of log-phase of anthocyanin synthesis only (YASUDA *et al.*, 1985).

This figure also shows that both curve peaks, one denoting the anthocyanin levels and the other the frequency of appearance of anthocyanoplasts with age of hypocotyls advancing, approximately correspond with each other. This result is also in contrast with those of the radish hypocotyls.

Table 2 The digest of the results shown in Fig. 2 and the comment given to them.

Day after sowing	Explanation of curve for		Comment
	Number	Diameter	
~2.5	Increase	Increase	Anthocyanoplasts were spontaneously generated and grew up individually.
2.5~4	Increase	Decrease	Division occurs.
4~7	Decrease	Increase	Fusion occurs.
7~9	Increase	Decrease	Division occurs.
9~12	The curve is complicated in shape.		Fusion alternates with division.
12~16	Increase	Decrease	Division occurs.
16~	Decrease	Decrease	Division and disappearance occur.

Table 3 The effect of light on the reddening of cell sap and anthocyanoplast in the cells of *Impomea rubrocaerulea* hypocotyl.

Abbreviation***	Part of observation*	Reddening in post-treatment after 0 hours*	20 hours*	24 hours*	48 hours*
D—L	Cs	−	−	+	+
	Ap	−	+**	+**	+
L—D	Cs	+	+	+	−
	Ap	+	+	+	−
D—D	Cs	−	−	−	−
	Ap	−	−	−	−
L—L	Cs	+	+	+	+
	Ap	+	+	+	+

* : + : Reddening ; − : No reddening ; Cs : Cell sap ; Ap : Anthocyanoplast

** : Reddening of the anthocyanoplast having diameters less than 0.5 μm

*** : See Table 4.

Table 4 Light or dark condition in the pre-and post-treatments during the germination of seeds.

Abbreviation	Distinction between light* and dark**	
	Pre-treatment (48 hourn)	Post-treatment
D—L	Dark	Light
L—D	Light	Dark
D—D	Dark	Dark
L—L	Light	Light

* Light : Dishes containing seeds were put in a place under continuous white light of fluorescent lamp, the average light intensity being about 7,000 luxes.

** Dark : Dishes containing seeds were kept in cases which were wrapped in black paper in order to keep out light completely.

Fig. 2 shows the relationship between the numbers of anthocyanoplasts per cell and their diameters in the middle segment of hypocotyls of morning glory, with the age of hypocotyls advancing. A cursory glance reveals that there are complicated circumstances explaining the relationship between number and diameter. For convenience' sake the authors of the present paper have made up Table 2 according to the number of days after sowing.

From the results summarized in this table, it is clear that the anthocyanoplasts appearing in the hypocotyls of morning glory alternated fusion of several smaller anthocyanoplasts with division of larger anthocyanoplasts in the course of development. This differs very markedly from the radish hypocotyls which showed fusion only in their developmental stages. The same results were obtained from the upper and lower segments.

Table 3 clearly demonstrates the effects of light on the reddening of anthocyanoplasts in the hypocotyls of morning glory as follows :

1. In the dark anthocyanin did not appear and anthocyanoplasts did not redden.
2. In the light anthocyanin appeared and anthocyanoplasts also reddened.

These facts make it probable that in the hypocotyls of morning glory anthocyanoplasts have the ability to display light reaction in the anthocyanin biosynthesis, very much like in the hypocotyls of radish on which YASUDA *et al.* (1988) previously reported

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