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The Studies on the Spherical Bodies Containing Anthocyanins in Plant Cells, II.

The Effects of Light on the Pigmentation of Spherical Bodies in the Seedling Hypocotyls of the Radish Plant.

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Abstract

In order to answer the question whether anthocyanoplasts have the capacity of anthocyanin biosynthesis or not, the effects of light on their pigmentation were investigated using the seedling hypocotyls of the radish plant, *Raphanus sativus L.*

Microscopic observations were carried out on slices which were prepared by cutting with a razor from the seedling hypocotyls of radish plants germinated under light or dark conditions.

The cells of hypocotyls germinated under dark conditions bore some colorless spherical bodies, whereas those germinated under continuously light conditions had entirely red-colored bodies. The colorless bodies found in the cells of hypocotyls germinated under the dark condition turned red after they had transferred to light conditions.

From these facts it was concluded that the anthocyanoplasts in radish hypocotyls have the capacity of light reaction indispensable for the anthocyanin biosynthesis.

Introduction

In the previous paper (YASUDA *et al.*, 1985) it was demonstrated that the cells of the first layer adjacent to the epidermis of the hypocotyls of radish seedlings bore one or more red-colored spherical bodies with anthocyanins. At the same time, it was noted that the appearances of these bodies might be closely related to the rapid biogenesis of anthocyanins in the cells. PEKET *et al.* (1980) and SMALL *et al.* (1982) also investigated the anthocyanoplasts, which are analogous to our red spherical bodies, using various kinds of plants including red cabbage, and implied that they display the biochemical activities of the anthocyanin formation, especially of the latter stage of their metabolism. However, in the reports mentioned here the suggestion proposed as to the correlation between the appearance of the bodies and the production of the pigments was not extracted from difinite experimental evidence.

It has long been established that light is one of the important factors in initiating or stimulating the anthocyanin productions in many plant species (BLANK, 1947: MANCINELLI, 1985). The radish seedling belongs to the group of plants whose initiation of pigment formation is light dependent (BELLINI *et al.*, 1970, 1973).

This paper deals with the effects of light on the pigmentation of spherical bodies. From clear changes in the bodies to red from colorless, the present authors attempted to resolve the question whether anthocyanoplasts have the capacity anthocyanin formation or not.

Material and Methods

The hypocotyls of radish (*Raphanus sativus L.* cultivar Kohaku) were used as an experimental material in the present investigation. The seeds, purchased from Nakazutaya Nursery at Matsumoto, Japan, were soaked in distilled water for about 24 hours, and then germinated on two discs of filter paper (Toyo filter paper No. 1) moistened with distilled water in Petri dishes. The temperature throughout the experimental period was 25°C.

Two types of treatments were set up in the germination of seeds, in order to subject the seedlings to light or dark conditions. One was "pre-treatment", which was for 48 hours after soaking the seeds, the other being "post-treatment" which immediately followed pre-treatment. An outline of the distinctions between light and dark conditions in pre- and post-treatments is laid down in Table 1.

Hypocotyls were separated from the seedlings and cut into slices with a razor every four hours after being transferred to post-treatment from pre-treatment.

For the microscopic observation of the slices, Olympus, BH-2 were employed. The current observations were made only upon the cells of the first layers of cortex in the hypocotyls.

Two histochemical tests were applied to establish the nature of anthocyanins in red spherical bodies:

(1) 1% of hydrochloric acid

(2) 1% of sodium hydroxide solution

Table 1 Light or dark conditions in the pre- and post-treatments during the germination of seeds.

Abbreviation	Distinction between light* and dark**	
	Pre-treatment (48 hours)	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 supplied with continuous white light through a fluorescent lamp, the mean light intensity being about 7,000 luxes.

** Dark : Dishes containing seeds were kept in cases which were wrapped in black paper in order to shield the light perfectly.

Results

The results obtained are summarized in Table 2 and some representative color micrographs are indicated in Fig. 1~4.

In D-D, which was subject to perfectly dark conditions during germination, the hypocotyl cells had no red-colored bodies, although some faint greenish spherical bodies were recognized in the cells (Fig. 1 a and b). This situation contrasts greatly with that of the hypocotyl cells obtained from the seedlings germinated under L-L, which was subject to continuously light conditions. The hypocotyls germinated under light conditions in both pre- and post-treatments bore entirely red-colored bodies (Table 2, Fig. 2).

In D-L, only colorless bodies were recognized in the hypocotyl cells which were subject to dark conditions in the pre-treatment, even though remarkably red bodies were found in those which were subject to light conditions during post-treatment (Table 2, Fig. 3). In L-D, the hypocotyl cells contained red bodies during pre-treatment, which was light, but when they were transferred to the post-treatment, which was dark, the bodies became partially decolored (Table 2, Fig. 4).

As a result of the application of 1% hydrochloric acid, the bodies were observed in red color at first, and after that the bodies broke down. In the case of application of 1% sodium hydroxide solution, too, the bodies broke down after a change of color from red to greenish blue. While not shown in the color micrographs, these results are suggestive of the presence of anthocyanins involving reddening of the bodies.

Table 2 The effects of light on the reddening of anthocyanoplasts

Abbreviation	Reddening*	
	Pre-treatment	Post-treatment
D — L	−	+
L — D	+	**
D — D	−	−
L — L	+	+

* + : Reddening; − : No reddening
** Partially decolored

Discussion

It seems reasonable to expect that the reddening of the spherical bodies may be a light dependent phenomenon, from the evidence that the hypocotyl cells grown under completely dark conditions (D-D treatment) bear colorless spherical bodies, while those hypocotyl cells germinated under continuously light conditions (L-L treatment) contain bodies clearly colored in red.

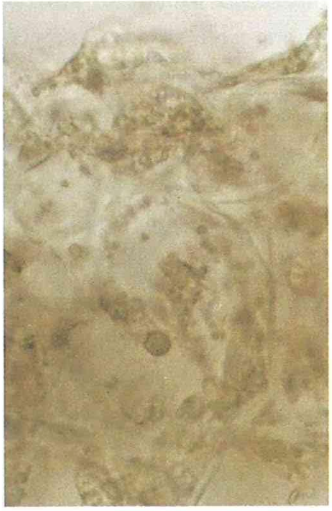
Furthermore, the fact that the colorless bodies found in the cells germinated under the dark condition turned into red ones by lighting (D-L treatment), adds strong probability to this assumption. From the positive results of the histochemical tests for anthocyanins, it is quite clear that the production of anthocyanins is responsible for producing the red color in the spherical bodies.

It has been well known for a long time that anthocyanin accumulations in many kinds of plant stand in need of light. The information published formerly on this subject has been reviewed by BLANK (1947). Since THIMANN *et al.* (1949) made the principal investigation as to the anthocyanin biogenesis, there has been a vast amount of literature about physiological and biochemical studies on the relationship between light and anthocyanin formation. Available information concerning more recent research on the anthocyanin pigmentations in plants has been reviewed by MANCINELLI (1985).

According to these descriptions, further scientific investigations ensured that a lot of plant species have a light dependent nature in the initiation or stimulation of anthocyanin biogenesis. Radish seedlings, the plant material in the present investigation, belong to the group in which light is needed to initiate the pigment formations (BELLINI *et al.*, 1970, 1973). Thus, the conclusion to be drawn from the results indicated in the present paper is that the anthocyanoplasts appearing in the cells of the radish hypocotyls have the capacity of light reactions involved in anthocyanin biogenesis.

FRITSCH *et al.* (1975) showed that cytoplasm is the site of some steps of reaction in the anthocyanin biogenesis, the membrane being the site of others

1 a



1 b

2

3

4

Fig. 1～4 Representative color micrographs of anthocyanoplasts in hypocotyl cells from the seedlings germinated under the light or dark conditions. ├──┤ 30μ

1 a : Pre-treatment in D-D
1 b : Post-treatment in D-D
2 : Post-treatment in L-L
3 : Post-treatment in D-L
4 : Post-treatment in L-D

in the cell cultures of *Happlopapus gracillis*. The facts reported in the present paper concerning radish hypocotyls offer a new example about the intracellular localizations of pigment formation.

STEINER (1971, 1973), MARGNA *et al.* (1981) and ZENNER *et al.* (1987) demonstrated that in several plants a phenomenon termed "turnover" occurs in the anthocyanin metabolism. The word was used by these authors as meaning that in plant cells anthocyanin forms on one side and degrades on the other. On the basis of this explanation, the partial decoloration of anthocyanoplasts in the hypocotyls germinated in L-D treatment (Fig. 4) can be interpreted as reflecting an occurrence of the turnover, that is, anthocyanins undergo both formation and degradation in the pre-treatment (light condition), but in the post-treatment (dark condition) only the degradation of anthocyanins proceeds.

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