J. FAC. SCI., SHINSHU UNIVERSITY, Vol. 25, No. 1, 1990

The Effects of Light on the Formation of Anthocyanin in Petunia Petals.

By SHUICHIRO MATSUBARA* and HITOSHI YASUDA

Department of Biology, Faculty of Science, Shinshu University
(Received Jul. 27, 1990)

Abstract

The formation of anthocyanins in light and dark conditions was investigated using the petals of *Petunia hybrida* Vilm. or their disks. The results obtained were as follows:

1. In the early developmental stages of buds, anthocyanin levels in the petals grown in a dark environment were approximately half to those grown in the light.
2. But in the later stages, the levels in the petals grown in the dark were substantially the same as those grown in the light.
3. The experiments conducted with the disks produced similar results as with the petals.

From these observations, it seems reasonable to suggest that the physiological and/or biochemical systems involved in the response to light in the formation of anthocyanin in petunia petals in the early stages differ from those found in the later developmental stages of the bud.

Introduction

It has long ago been established that light is one of the important factors in initiating or stimulating anthocyanin production in many plant species. Previous information on this subject was reviewed by BLANK (1947). Since THIMANN *et al.* (1949) performed a principal investigation of anthocyanin biogenesis using *Spirodela*, there has been a vast amount of literature written about physiological and biochemical studies in relation to light and anthocyanin biosynthesis. Information concerning more recent research on the anthocyanin pigmentation in plant cells was reviewed by MANCINELLI (1985).

These studies have given the authors of the present paper the notion that seedlings, callus or cultured cells were most often used as experimental materials in the investigations of anthocyanin formation, and other materials were seldom used.

Only from a biochemical standpoint, it can be said that petals are formed by

* Present address: Department of Biochemistry-2, Faculty of Medicine, Kagoshima University, Kagoshima, Japan.

tissues having a simpler metabolism than other organs such as leaves in which photosynthesis extensively proceeds. So it should be emphasized that petals are one of the most suitable materials in studies on flavonoid metabolism.

However, there has been relatively little information about the biochemistry of flavonoid metabolism in petals. There has been a comparatively small number of plant species, including petunias, used in investigations on the physiology and biochemistry of flavonoid in plant.

The works of STEINER (1971, 1972) and of KHO *et al.* (1975) presented a rather limited number of physiological investigations on the effects of light on the anthocyanin formation in petunia petals. But STEINER's and KHO's results opposed each other. That is, STEINER's papers reported that pigmentation from anthocyanins was accelerated in white light, and KHO *et al.* informed that the light did not have any effect on anthocyanin biogenesis.

Here the question arises, regarding the reason why the two investigators mentioned above formed opposing opinions although the same plant species was used as experimental material. However, little effort has been made to answer this question. As a first step in resolving this question, the authors of the present paper will offer some evidences that effects of light produced in anthocyanin formation in petunia petals in their advanced stages are not the same as in the early stages.

Materials and Methods

The plant materials used were flower petals of a garden variety of petunia,*Petunia hybrida* Vilm. The petunia seeds, purchased at a nursery in Matsumoto, Japan, were sown in sand contained in pans. About the time when the plumules became visible between cotyledons in seedlings, they were transplanted into new soil in pots, grown under glass, and fertilized with 'Hyponex' or similar fertilizers.

The flower buds were taken from the branches of mature plants, and utilized in experiments whose procedure will be illustrated later. After the old branches were pruned from the plants, new buds appeared again. In this way the buds were available under glass for the experiments throughout the year.

In order to determine the anthocyanin accumulations in the petals, the following two types of experiments were performed.

Experiment I

Two branches, which were grown to about 15cm and whose flowers were in substantially the same stage of bloom, were selected, cut from a palnt, and all their leaves were removed to counteract any effects of photosynthesis.

About 10cm of the branches from the cut end were sterilized in 1% solution of sodium hypochloride for 5 minutes, after which they were washed with running water to thoroughly remove the sterilizer. The same sections of the branches were sterilized

again with 70% methylalcohol.

Every branch treated in the manner described above, was put in a previously sterilized conical flask containing 5% solution of sucrose. One of every two branches was exposed to continuous fluorescent light (about 3,000 luxes), while the other branches remained in the dark.

Temperature remained at 24 °C throughout the experiments.

Experiment II

Buds were removed from the branches and cut with scissors into two portions along a zone where stamens were shooting up, one being "limb" and the other "tube". Limbs were collected and cut open along the median line of the petals. 5~10 disks, in diameter 7 mm, were prepared by means of pulling out a limb with a cork borer.

The disks were sterilized for 20 seconds with 70% ethylalcohol, and then washed twice with sterilized water. The disks obtained here were incubated with 20ml of phosphate buffer solution (pH5.5) containing 5% sucrose. The incubations were accomplished at 24 °C, and under the continuous illumination of a fluorescent lamp (approximately 3,000 luxes) or in darkness.

After the cultivation or the incubations, the levels of anthocyanin in the petals or disks were estimated using the procedure recommended by SHIBATA *et al.* (1960) which was modified by the present authors.

Briefly, this procedure was as follows: Anthocyanins in the petals or the disks were extracted with 25ml of 0.5% methylalcoholic hydrochloric acid for about 50 hours, or more if needed. Absorbances of the extracts were measured using a spectrophotometer (HITACHI Type-100-10L) at wavelength 539 nm. In the present paper, the anthocyanin levels were expressed as % of the level in light condition based on the calculations of the absorbance figures measured here.

For convenience, six developmental stages (I~V) were defined according to the changes in the lengths of buds, as shown in Table 1.

Results and Discussion

The effects of light on the accumulation of anthocyanin in Experiment I are given

Table 1 The lengths and the colors of petunia buds in their various developmental stages.

Developmental stage	Color of bud	Length of bud (mm)
I	Green	10
II	Yellowish green	12~18
III	Pale yellow	20~28
IVa	Gray	
IVb	Mauve	30~37
V	Purple	40~48

in Fig. 1. This figure shows that the anthocyanin levels in the buds which were grown in darkness were roughly half to those grown in light in the developmental stages I ~IVa, considering them as a whole group. However, the buds grown in darkness had substantially the same pigment levels to those grown in light in developmental stage V.

The effect of light on anthocyanin levels in Experiment II, is illustrated in Fig. 2. From this figure it is clear that the pigment levels in disks incubated in darkness are

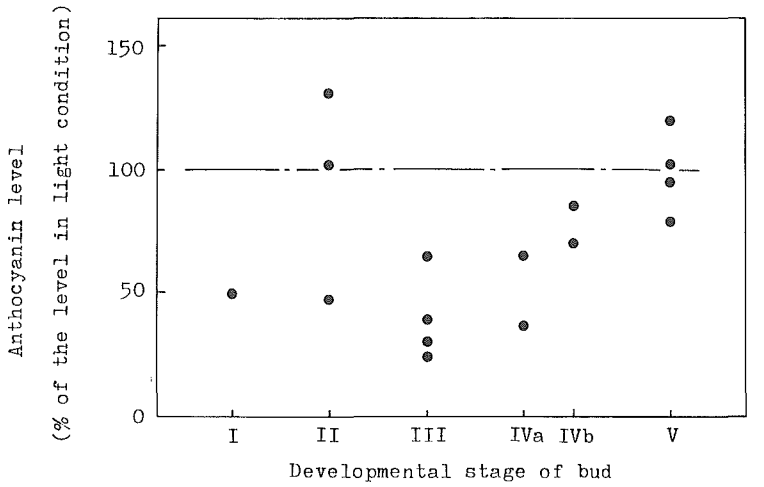

Fig. 1 The effect of light on anthocyanin accumulations in Experiment I at various developmental stages of buds (see Table 1).

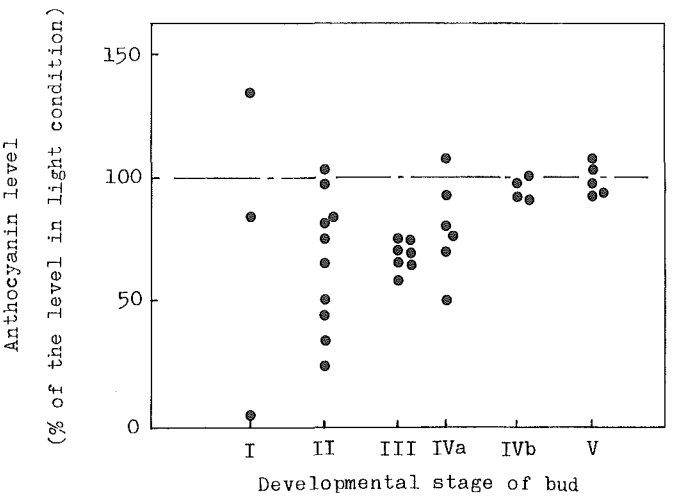

Fig. 2 The offect of light on anthocyanin accumlations in Experiment II at various developmental stages of buds (see Table 1).

less than 100% in the developmental stages I~IVa, but the levels in the disks obtained from stages IVb and V are about 100% as a whole. This situation is similar to the results indicated in Fig. 1.

The results presented in Fig. 1. and 2, strongly suggest that there are two different physiological and/or biochemical systems involved in the responese to light during the biosynthesis of anthocyanins in petunia petals, one being dependent on light and the other independt of it. And it is also clear that anthocyanin metabolism needs light in the first half of the developmental stage of the bud, but does not need it in the latter half.

Thus, the authors of the present paper would like to offer this explanation of the difference between the data of STEINER (1971, 1972) and of KHO *et al.* (1975), which was pointed out in the section titled "**Introduction**" in the present paper.

References

BLANK, F. (1947) The anthocyanin pigments of plant. Bot. Rev. **13**, 251-317.

KHO, K. F. F., BENNIK, G. J. H. and WIERDNG, H. (1975) Anthocyanin synthesis in a white flowering mutant of *Petunia hybrida* by a complementation technique, Planta **127**, 271-279.

MANCINELLI, A. L. (1985) Light-dependent anthocyanin synthesis : A model system for the study of plant photomorphogemesis, Bot. Rev. **51**, 107-157.

STEINER, A. M. (1971) Zum Umstaz von Anthocyan-3-monoglucosiden in Petalen von *Petunia hybrida*, Z. Pflanzenphysiol. **65**, 210-222.

STEINER, A. M. (1972) Der Eingluss der Lichtinsität auf die Akkumulation einzelner Anthocyane in isoliertes Petalen von *Petunia hybrida*, Z. Pflanzenphysiol. **68**, 266-271.

SHIBATA, M. and ISHIKURA, N. (1960) Paper chromatographic survey of anthocyanin in tulip-flowers, I, Jap. Jour. Bot. **17**, 230-238.

THIMANN, K. V. and EDMONDSON, Y. H. (1949) The biogenesis of anthocyanins, I. General nutritional conditions leading to anthocyanin formation, Arch. Biochem. **22**, 33-53.