Zeitschrift: Berichte der Schweizerischen Botanischen Gesellschaft = Bulletin de la Société Botanique Suisse

Herausgeber: Schweizerische Botanische Gesellschaft

Band: 82 (1972)

Heft: 3

Artikel: Betalain synthesis in Centrospermae seedlings : the action of light on Betacyanin Formation

Autor: Rast, Dora / Skivanová, Romana / Wohlpart, A.

DOI: https://doi.org/10.5169/seals-57666

Ber. Schweiz. Bot. Ges. *82* (3), 213–222 (1972)

Betalain Synthesis in *Centrospermae* Seedlings: The Action of Light on Betacyanin Formation

Dora Rast, Romana Skřivanová, and A. Wohlpart

Institute of General Botany, University of Zürich, Switzerland
Department of Biology, Kenyon College, Gambier, Ohio, USA

Received May 3, 1972

Introduction

Following the elucidation of the structure of betanin (the major betacyanin pigment of the red beet, *Beta vulgaris)* by the Dreiding group (Mabry *et al.*, 1962) a number of investigators showed that radioactive phenylalanine, tyrosine, and dopa were incorporated into betacyanins (Hörhammer *et al.*, 1963; Minale *et al.*, 1965; Garay and Towers, 1966; Miller *et al.*, 1968; Liebisch *et al.*, 1969). Moreover, Garay and Towers reported that betacyanin synthesis was increased by feeding tyrosine to *Amaranthus* seedlings grown in the light, whereas application of this compound to dark-grown seedlings had no effect on pigment formation. One of the first reports concerning the light-dependency of betacyanin biogenesis was that of Gimesi *et al.* (1952). They demonstrated that light was necessary for the synthesis of water-soluble red pigments in *Amaranthus caudatus* seedlings and that red light was the most efficient in its formation.

In contrast to the latter two reports, formation of betanin and other betacyanins has been shown to occur even in complete darkness in seedlings of *Beta vulgaris, Gomphrena globosa* and some other species, albeit synthesis was very much reduced as compared to that in light (Wohlpart and Mabry, 1968). Since the enhancement of pigment production could have been brought about by stimulation of photosynthesis (for example by increasing the supply of potential betacyanin precursors), a general light requirement for the genesis of this chromophore was not thought to exist. Light-dependency of betacyanin synthesis is, therefore, still a matter of controversy. It is the aim of the present paper to clarify this question, to give quantitative data on the time-course of pigment formation in seedlings grown in the dark and in light, to increase the number of species or varieties studied with respect to the light requirement for betacyanin synthesis, and to show that the phytochrome system mediates the genesis of this chromophore. These analyses are also considered a first step in the elucidation of the hitherto unanswered question of why betacyanins and anthocyanins do not occur together.

Materials and Methods

Plant Material. Seeds of *Amaranthus caudatus* L., *Amaranthus paniculatus* L., *Kochia trichophylla* Roth, *Gomphrena globosa* L., and *Beta vulgaris* „Fire Globe" were purchased from Samen Mauser AG, Zürich. Switzerland.

Preparation of Seeds and Growth of Seedlings. For the time-course studies of pigment formation the seeds, which had been stored in the dark, were counted and placed in Petri dishes on filter paper in a dark room illuminated by a dim green safelight. (Seeds of *Kochia* and *Beta* were thoroughly washed with water to remove yellowish-brown pigments which inhibited germination). After watering the seeds, one set of dishes was placed in the laboratory under fluorescent lights (giving an illumination of approximately 1200 ft-c) near a window; the second was kept in complete darkness. Although light-conditions were not absolutely controlled and not necessarily uniform for all species examined, this does not affect our results concerning the light-dependence of betacyanin synthesis. The seedlings were grown for 7 to 9 days after germination and watered whenever necessary. In the experiments designed to study the photoreceptor involved in betacyanin formation. irradiation was effected in the darkroom with incandescent filament lamps (25 and 75 W for continuous and short-time irradiation, respectively) used together with interference filters (50 cm^2, type DIL, Schott & Gen., Mainz, Germany). These were mounted in a hole on the top of black boxes containing the seeds which had been placed on dishes as described above.

Extraction Procedures and Spectrophotometric Measurements. In the time-course studies on betacyanin formation, the seedlings (100 to 300, depending on the species; see legend to figures 1 through 5) were ground in a mortar with about 40 ml of water until no more pigment was removed. The extract was filtered through Celite Analytical Filter Aid and reduced to 2 ml on a rotary evaporator at 30^0. Absolute alcohol was then added to a final concentration of 80%. In the case of *Kochia* and *Beta* a brown precipitate formed which was removed by filtration. Further purification was achieved by adsorption on aluminum oxide (column size 1x3 cm) and washing successively with 80% EtOH (40 ml), 50% EtOH (30 ml), and water until the eluate was colorless. The betacyanins were finally eluted with 1 N pyridine formate (100 ml).

Because of the low amounts of betacyanins formed in some of the experiments described under heading 2 of the following section, a more efficient procedure had to be adopted. The method of Piattelli and Imperato (1970), slightly modified in several respects, proved to be suitable. All manipulations were carried out in the cold room (4^0). After preparation as described above, the extract was freeze-dried, the residue taken up in approximately 1 ml of water and chromatographed on a polyamide column (1.2 cm x 2.5 cm) with MeOH/10% Na acetate (3:1). The betacyanin pigment travelled as a very narrow band and was collected in a small volume.

The optical densities of the extracts were measured on a Beckman-DB at 536 nm.

Results

1. Kinetics of Betacyanin Formation in Continuous White Light and in the Dark

Seedlings of *A. paniculatus, A. caudatus,* and *K. trichophylla* formed betalains only in the light (Figures 1, 2, and 3, respectively). The pigment content of these seedlings increased for about six days and then leveled off. These species thus display an absolute light requirement for betacyanin

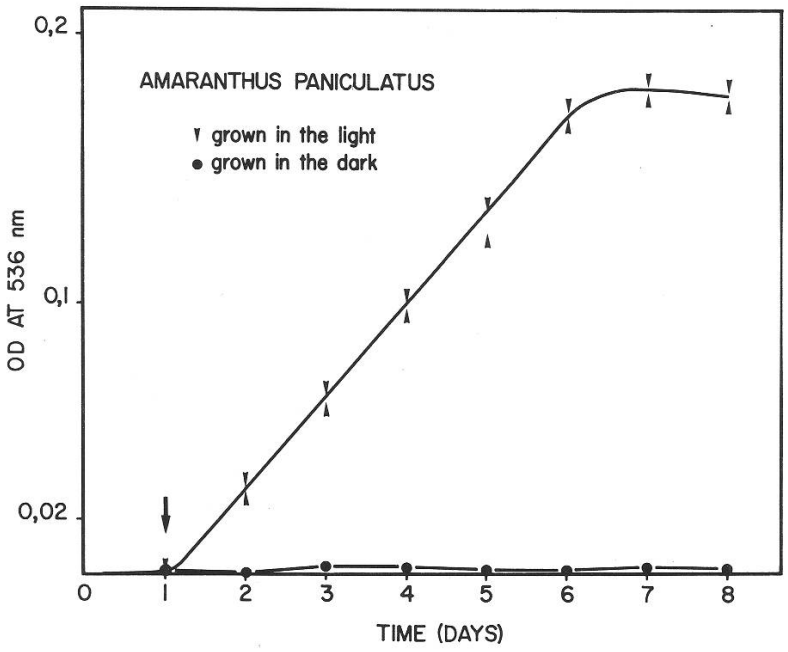

Fig. 1. RAST et al., Betalain Synthesis in Seedlings

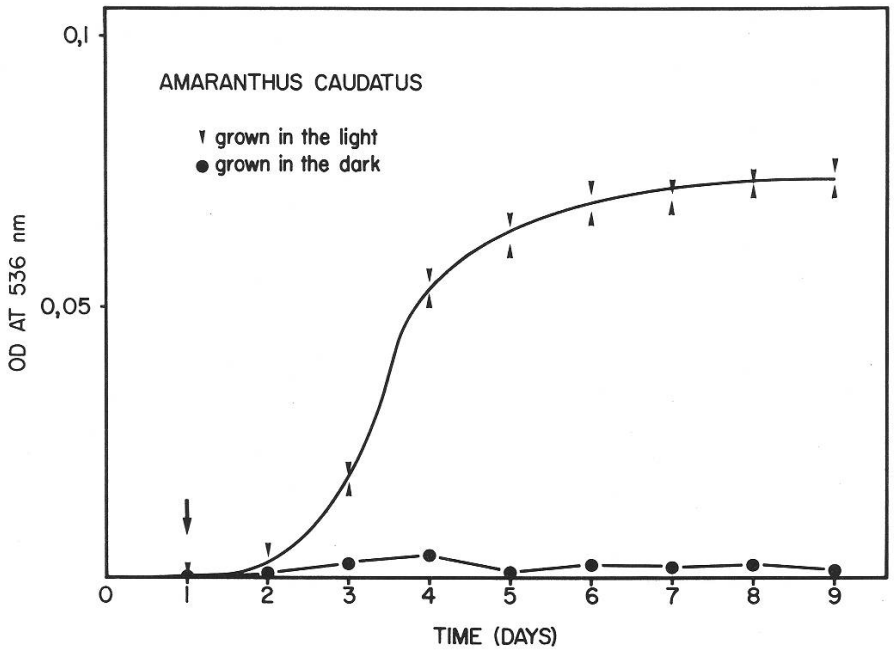

Fig. 2. RAST et al., Betalain Synthesis in Seedlings

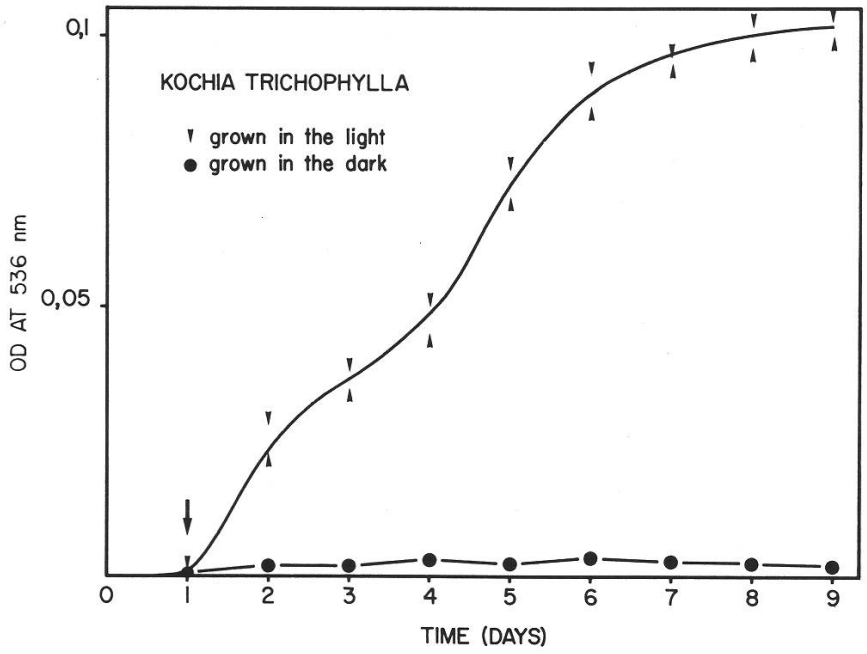

Fig. 3. RAST et al., Betalain Synthesis in Seedlings

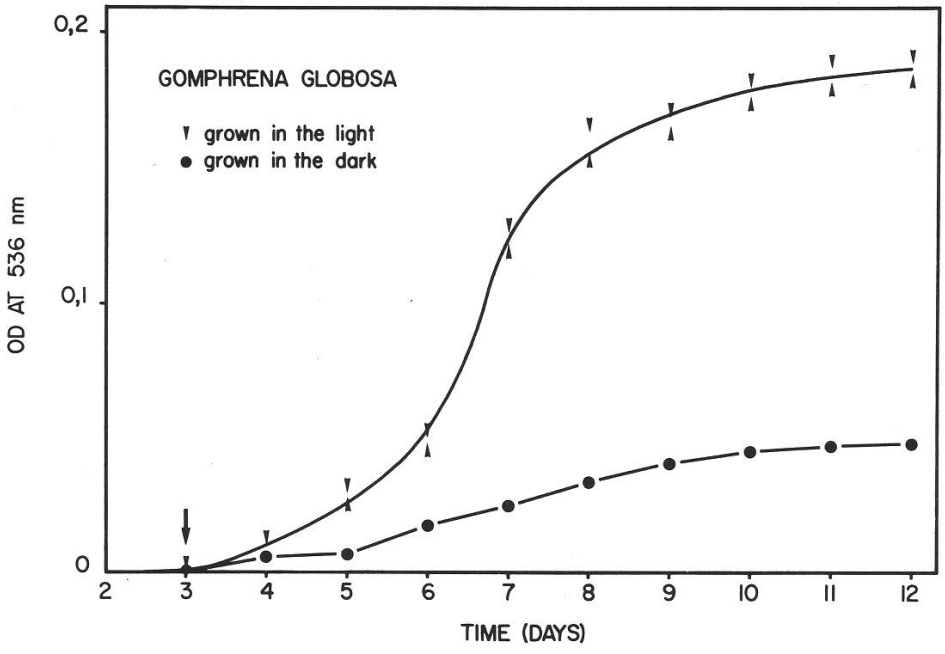

Fig. 4. RAST et al., Betalain Synthesis in Seedlings

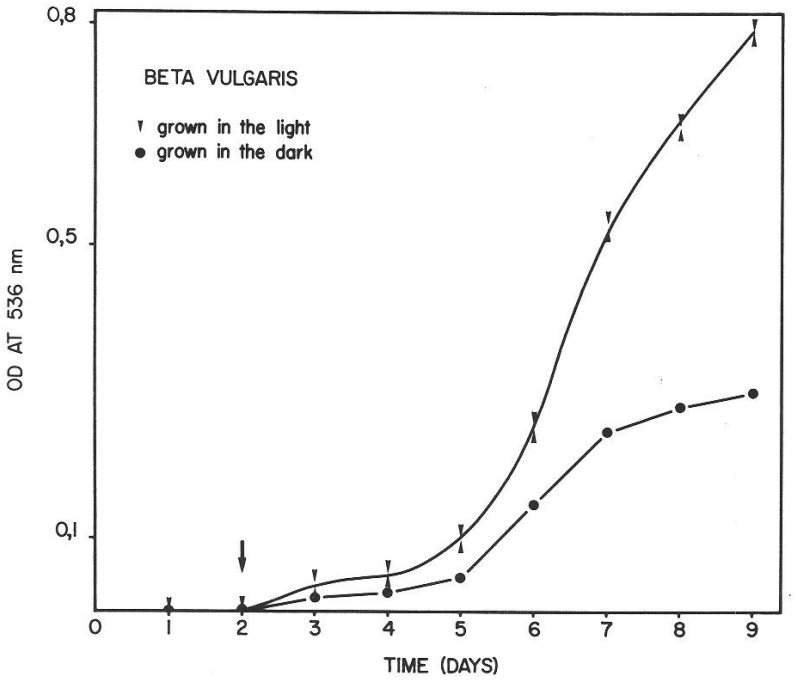

Fig. 5. RAST et al., Betalain Synthesis in Seedlings

Fig. 1–5: Time-course studies of betacyanin synthesis in *Centrospermae* seedlings. Each experiment was run in duplicate. The OD values were obtained by measuring the purified extract of 300 *(A. paniculatus)*, 300 *(A. caudatus)*, 200 *(K. trichophylla)*, 100 *(G. globosa)*, and 100 *(B. vulgaris)* seeds or seedlings. The points of the isosceles triangles represent the actual OD values. Time of seed germination is indicated by an arrow. Seeds of all species examined were found to be free of betacyanins at zero incubation time, and did not synthesize pigments before germination.

genesis. *G. globosa* and *B. vulgaris,* on the other hand, do not have an absolute light requirement for betacyanin formation. Seedlings grown in the dark formed some pigment, although the amounts are well below the pigment level obtained by light-grown seedlings (Figures 4 and 5).

The maximum rate of betacyanin biogenesis was attained on the fourth day after germination in *B. vulgaris* and *G. globosa,* on the third in *A. caudatus,* and the second in *A. paniculatus.* It is interesting to note that seedlings of the latter species maintained their maximum rate of pigment formation for several days. *K. trichophylla* showed relatively high intensities of betacyanin synthesis from the second to the fourth day of growth.

2. The Nature of the Photoreceptor Involved in Betacyanin Formation

Since *A. paniculatus* displays an absolute light requirement for the generation of the betacyanin chromophore and since it has a very high rate of pigment synthesis in the light (see Fig. 1), it was selected for experiments designed to

investigate the phytochrome-dependence of betacyanin formation. Exposure of *A. paniculatus* seedlings to red light induced the synthesis of betacyanins in dark-grown seedlings. This effect was partially reversed by subsequent irradiation with far-red (Table 1). Pigment synthesis (mostly amaranthin) under continuous far-red was inhibited by actinomycin D applied prior to the light treatment (Table 2). These results show that betacyanin synthesis is phytochrome controlled. This conclusion is not impaired by the facts that short-time far-red (Table 1) and prolonged red (Table 3) are quite effective in promoting the formation of the betacyanin chromophore in etiolated seedlings. For more details see Discussion.

Table 1:

Effect of short periods of irradiation with red or far-red on betacyanin formation in dark-grown seedlings of A. paniculatus

Treatment[a]	OD_{536}
control (darkness)	0.02
far-red	0.28
red	0.39
red + far-red	0.30
far-red + red	0.40
far-red + red + far-red	0.24
far-red + far-red	0.26

[a] 60 hours after sowing the seedlings (200) were exposed to red and/or far-red light for 1 min and then brought into darkness again. Analysis was made when they were 84 hours old.

Table 2:

Inhibition by actinomycin D of light-induced betacyanin synthesis in A. paniculatus

Sample	Treatment[a]	OD_{536}
1	water, darkness	0.02
2	actinomycin D, darkness	0.02
3	water, far-red	0.66
4	actinomycin D, far-red	0.22

[a] 200 dark-grown seedlings (60 hours old) were suspended in a solution of the antibiotic (60 µg/ml; sample 2 and 4) or in water (sample 1 and 3) during 4 hours and brought back to a moist cellulose-pad prior to the onset of illumination (2 hours; sample 3 and 4). The young plants were analyzed 88 hours after sowing.

Table 3:

Variation in the betacyanin content of A. paniculatus seedlings (dark-grown for 52 hours) after prolonged exposure to red or far-red light

exposure time (hours) to red	far-red	darkness	OD_{536}	OD(far-red)/OD(red)
1	–	23	0.28	0.64
–	1	23	0.18	
4	–	20	0.45	0.69
–	4	20	0.31	
8	–	16	0.62	1.05
–	8	16	0.65	
12	–	12	0.77	1.12
–	12	12	0.86	
24	–	–	0.88	1.30
–	24	0	1.14	
–	–	24	0.03	

Number of seedlings per treatment: 200

Discussion

The observation that some *Centrospermae* seedlings display an absolute light requirement for betacyanin synthesis while others do not, is analogous to the situation found in anthocyanin genesis. Another similarity between betacyanin and anthocyanin formation is the observed decrease in the rate of pigment formation or the cessation of synthesis, in seedlings maintained in continuous white light for several days (Fig. 1 through 5) (for anthocyanins see Eddy and Mapson, 1951).

Anthocyanin synthesis in white mustard and several other species is known to be phytochrome-dependent (Mohr 1957; 1969). The demonstrations that amaranthin synthesis in *A. paniculatus* seedlings can be induced by red light, that this effect can be reversed by far-red, and the fact that the light-induced betacyanin synthesis can be inhibited by actinomycin D (Tables 1 and 2, respectively), indicate that the formation of the betacyanin chromophore in *A. paniculatus* is phytochrome-dependent. Our results thus extend and confirm those of Piattelli *et al.* (1969; 1970) and Wagner and Cumming (1970), who showed similar red and far-red effects in seedlings of *A. tricolor* and *Chenopodium rubrum,* respectively. These observations oppose the recent suggestion that betacyanins and anthocyanins differ not only by virtue of their unique structural features and biosynthetic pathways, but also in the mode of action of light in their formation (Mabry and Dreiding 1968).

A further parallelism between anthocyanin and betacyanin synthesis in seedlings is revealed by the fact that prolonged red is quite effective in promoting pigment formation in both cases (Table 3; Wagner and Cumming 1970; Mohr 1969). For young mustard plants this phenomenon has been explained by the hypothesis that these contain two different types of P_{730}, both involved in pigment formation, the one, however, being resistant to irreversible destruction (Wagner and Mohr, 1966). Our experimental results suggest the same for *A. paniculatus* seedlings.

The synthesis of betacyanins in dark-grown seedlings of *A. paniculatus* (known to have an absolute light requirement for amaranthin synthesis; see above) after a short exposure to far-red (Table 1), points to the presence of P_{730} in these seedlings. This may indicate that pigment formation is controlled by the P_{730}/P_{total} ratio, rather than the absolute concentration of P_{730}, as is the case for germination in *A. caudatus* (Kendrick and Frankland 1969). Whether this pool of P_{730} which exists in the seedlings prior to illumination originates from P_{660} by the process of ‚Inverse dark reversion' (Kendrick et al., 1969) is not known.

Garay and Towers (1966) showed that tyrosine and dopa were incorporated into amaranthin in light-grown seedlings of *Amaranthus* ‚Molten Fire', but that dark-grown ones could not carry out this biotransformation. These results and the now generally accepted view of the mode of action of P_{730} in positive photoresponses, i.e. differential gene activation (Mohr 1969), suggest that the synthesis of at least one of the enzymes of the metabolic pathway leading from dopa to the betacyanins is light-dependent, possibly that of the „betacyanin-synthesizing" enzyme itself.

The betacyanin pigments are of special interest to the botanist as well as the molecular biologist and the biochemist because of (1) their restricted distribution in the plant kingdom – they occur only in the *Centrospermae* –, (2) the mutual exclusion of anthocyanins and betacyanins and (3) the fact that the members of the *Caryophyllaceae* family are anthocyanin-synthesizing plants although, by morphological evidence, they do form an integral part of the *Centrospermae* class (for more details see Mabry, 1966). The situation becomes even more intriguing when taking into consideration that the function of these two pigment classes (involving in part floral coloration to attract animal vectors) and the action of light in the genesis of their chromophores in seedlings (this work), are identical. As regards their respective pathway of synthesis, there exists at first sight no reason why they could not occur together since the modes of their formation seem to be largely independent and since the betacyanin plants can synthesize the flavonoid nucleus.

The situation may perhaps be clarified by studying the formation of betacyanins in the presence of anthocyanin-generating systems and *vice versa.* Isolation of a betacyanin-synthesizing cell-free system would represent a first step in this direction. Synthesis of betanin *in vitro* has been shown to occur in very crude homogenates from cactus fruits (Minale *et al.* 1965). Recent attempts to isolate the corresponding enzyme system from the more easily available red beet have, however, been unsuccessful, due to the high phenolase activities shown by such extracts (Wohlpart, unpublished).

A somewhat related approach has been adopted by Liebisch *et al.* (1969), who studied the effect of betanin on the synthesis of anthocyanins *in vivo*.

The present study indicates that seedlings of *A.paniculatus*, grown in a far-red field on a medium which supplies only water, represent a system particularly well suited for enzyme work on betacyanin genesis since the synthesis of the enzyme(s) involved can easily be controlled and since the enzyme inventory of seedlings is without doubt less complex than that of mature plants. Photosynthesis being prevented in the far-red adds to the relative simplicity of the system.

Summary

Some *Centrospermae* seedlings display an absolute light requirement for the synthesis of betacyanins, while others do not. In all species examined so far, pigment formation decreases or comes to a complete halt after several days of continuous illumination with white light. The effects of red and/or far-red light and of actinomycin D on pigment formation in *Amaranthus paniculatus* show that betacyanin synthesis is mediated by phytochrome. Light, therefore, plays a similar role in the synthesis of anthocyanins and betacyanins. The experimental results are discussed in relation to the biochemistry of betacyanin genesis and to their usefulness for future causal analysis of the well-known fact that these two classes of water-soluble red pigments are mutually exclusive.

Zusammenfassung

Es wird der Einfluss des Lichtes auf die Betacyanbildung in Centrospermenkeimlingen untersucht. In einigen Arten ist diese absolut lichtabhängig; in andern erfolgt sie prinzipiell auch im Dunkeln. Bei Dauerbehandlung der Sämlinge mit weissem Licht nimmt die Syntheserate der betreffenden Pigmente in allen untersuchten Species sehr rasch ab. Die Effekte von hellrotem und/oder dunkelrotem Licht sowie von Actinomycin D auf die Betacyangenese zeigen, dass diese durch das Phytochromsystem kontrolliert wird. Es besteht demnach bei der Betacyan- und der Anthocyanbildung in allen erwähnten Situationen Parallelität der Wirkung des Lichtes.

Die experimentellen Resultate werden diskutiert in bezug auf ihre Bedeutung für die Suche nach einem zellfreien Betacyane synthetisierenden System und für eine künftige Kausalanalyse des allgemein bekannten Faktums, dass Betacyanpflanzen keine Anthocyane bilden und *vice versa*.

Financial support from the Swiss National Foundation for the Advancement of the Sciences (Grant No. 3.264.69) and the Foundation for Scientific Research at the University of Zürich is gratefully acknowledged. It is also a pleasure to thank Miss *Eszter Szönyi* and Miss *Lotti Brugger* for capable technical assistance.

References

Eddy, B.P., Mapson, L.W.: Some factors affecting anthocyanin synthesis in cress seedlings. Biochem. J. *49,* 694–699 (1951).

Garay, A.S., Towers, G.H.N.: Studies on the biosynthesis of amaranthin. Can. J. Bot. *44,* 231–236 (1966).

Gimesi, N., Garay, A., Farkas, G., Pozsár, B.: On the physiology of pigment formation in plants. Acta Biol. Acad. Sci. Hung. *3,* 247–259 (1952).

Hörhammer, L., Wagner, H., Fritzsche, W.: Zur Biosynthese der Betacyane. I. Biochem. Zeitschr. *339,* 398–400 (1963).

Kendrick, R.E., Frankland, B.: Photocontrol of germination in *Amaranthus caudatus.* Planta (Berl.) *85,* 326–339 (1969).

– Spruit, C.J.P., Frankland, B.: Phytochrome in seeds of *Amaranthus caudatus.* Planta (Berl.) *88,* 293–302 (1969).

Liebisch, H.-W., Matschiner, B., Schütte, H.R.: Beiträge zur Physiologie und Biosynthese des Betanins. Z. Pflanzenphysiol. *61,* 269–278 (1969).

Mabry, T.J.: The betacyanins and betaxanthins. In: Comparative phytochemistry, p. 231, Swain, T., ed., London and New York: Academic Press 1966.

– Dreiding, A.S.: The Betalains. Rec. Adv. Phytochem. *1,* 145–160 (1968).

– Wyler, H., Sassu, G., Mercier, M., Parikh, I., Dreiding, A.S.: Die Struktur des Neobetanidins. Über die Konstitution des Randenfarbstoffes Betanin. Helv. Chim. Acta *45,* 640–647 (1962).

Miller, H.E., Rösler, H., Wohlpart, A., Wyler, H., Wilcox, M.E., Frohofer, H., Mabry, T.J., Dreiding, A.S.: Biogenese der Betalaine. Biotransformation von Dopa und Tyrosin in den Betalaminsäureteil des Betanins. Helv. Chim. Acta *51,* 1470–1474 (1968).

Minale, L., Piattelli, M., Nicolaus, R.A.: Pigments of *Centrospermae* – IV. On the biogenesis of indicaxanthin and betanin in *Opuntia ficus-indica* Mill. Phytochemistry *4,* 593–597 (1965).

Mohr, H.: Der Einfluss monochromatischer Strahlung auf das Längenwachstum des Hypokotyls und auf die Anthocyanbildung bei Keimlingen von *Sinapis alba* L. *(Brassica alba* Boiss.). Planta *49,* 389–405 (1957).

– Photomorphogenesis. In: The physiology of plant growth and development, pp. 507, Wilkins, M.B., ed., London: McGraw-Hill 1969.

Piattelli, M., Imperato, F.: Betacyanins from *Bougainvillea.* Phytochemistry *9,* 455–458 (1970).

– Guidici de Nicola, M., Castrogiovanni, V.: Photocontrol of amaranthin synthesis in *Amaranthus tricolor.* Phytochemistry *8,* 731–736 (1969).

– – – : The inhibition by actinomycin D and puromycin of light-stimulated amaranthin synthesis. Phytochemistry *9,* 785–789 (1970).

Wagner, E., Cumming, B.G.: Betacyanin accumulation, chlorophyll content, and flower initiation in *Chenopodium rubrum* as related to endogenous rhythmicity and phytochrome action. Can. J. Bot. *48,* 1–18 (1970).

– Mohr, H.: Kinetic studies to interpret ‚high energy phenomena' of photomorphogenesis on the basis of phytochrome. Photochem. Photobiol. *5,* 397–406 (1966).

Wohlpart, A., Mabry, T.J.: On the light requirement for betalain genesis. Plant. Phys. *43,* 457–459 (1968).

Prof. Dr. Dora Rast and Romana Skřivanová, M.S.
Institute of General Botany
University of Zurich
CH-8006 Zürich, Switzerland

Prof. Dr. Alfred Wohlpart
Department of Biology
Kenyon College
Gambier, Ohio 43022, U.S.A.