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PHOTOCONTROL OF BETACYANIN SYNTHESIS IN *AMARANTHUS CAUDATUS* SEEDLINGS IN THE PRESENCE OF KINETIN

K.-H KOEHLER

Sektion Biologie der Ernst-Moritz-Arndt-Universitat, Greifswald, DDR-22 Greifswald, L -Jahn-Straße 15a

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Abstract—In *Amaranthus caudatus* seedlings, phytochrome activity in controlling amaranthin biosynthesis is dependent on the presence of kinetin Besides the phytochrome system, a blue light dependent high-energy reaction, which is controlled partly by phytochrome, influences betacyanin synthesis Both the light- and the kinetin-dependent amaranthin production show a characteristic lag phase of about 6–8 hr

INTRODUCTION

THE INFLUENCE of light on photomorphogenic reactions, and especially on betacyanin[1] synthesis, the red–violet cell sap soluble pigments of Centrospermae, is different in several species of this Order so far investigated.[2] Even within the genus *Amaranthus* quite different types of reaction have been observed Hypocotyls and cotyledons of some species are able to synthesize pigments in the dark, while for other species light is compulsory. The presence of phytochrome was demonstrated in *A tricolor*,[3] whereas in *A. salicifolius* no phytochrome activity in pigment synthesis could be demonstrated.[4] Further, it is thought that irradiation with light is possibly realized by different photoreceptor systems.[5] The properties of *Amaranthus* phytochrome depend to a large degree on the quality and intensity of light.[6] Kinetin also promotes betacyanin synthesis in *A. caudatus* seedlings.[7] In particular, various light sources lead to large and complex changes in the photomorphogenesis and different environmental conditions may be possible reasons for the inconsistency of various reports The following experiments were undertaken to study some more detailed conditions of irradiation with light and correlations between light-influenced and kinetin-promoted betacyanin synthesis

RESULTS AND DISCUSSION

High-voltage electrophoresis showed that *A caudatus* seedlings contain only one betacyanin, amaranthin The influence of light and of kinetin on pigment production in *Amaranthus* seedlings of different physiological age is shown in Table 1 The seedlings (germination started after 20 hr) were grown for 24, 48, 72 or 96 hr respectively on water-agar and then transferred to kinetin/tyrosine solution (10^{-5} mol/l ; 0 01 %). After floating on this solution for 24 hr it was replaced by water-agar for a further time to make 120 hr total Irradiation

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[5] J W MCCLURE and K. G. WILSON, *Phytochem* **9**, 763 (1970)
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[7] K -H KOEHLER, *Naturwissenschaften* **52**, 561 (1965)

TABLE 1 EFFECT OF LIGHT AND KINETIN ON AMARANTHIN BIOSYNTHESIS DEPENDING ON THE SEEDLING AGE

Time (hr)		Extinction (extract of 50 seedlings)		
Water–agar	Kinetin-tyrosine solution	Water–agar	Dark	Irradiated (white light)
24	24	72	0 167	0 210
48	24	48	0 229	0 370
72	24	24	0 455	0 713
96	24	0	0 271	0 591
120	0	0	0 074	0 108

was performed immediately after incubation with the kinetin/tyrosine solution (700 lux cool white fluorescent light) The maximal susceptibility of the *A caudatus* seedlings for light occurs in the period between 72–96 hr This is in accordance with the measurements of phytochrome in *A caudatus* seedlings[6] which show a net production of phytochrome up to 72 hr. In earlier experiments, kinetin was found to promote amaranthin synthesis in tyrosine-fed seedlings.[7] The data in Table 1 demonstrate that the application of kinetin does not alter the period of highest susceptibility to light treatment and that the time is the same for both light and kinetin

If *Amaranthus* seedlings are irradiated with light or incubated in kinetin/tyrosine solutions, pigment production starts after a lag period of about 6–8 hr from the onset of the treatment The maximum was attained at 24 hr, thereafter the amount of pigment slowed down somewhat Both the light-influenced and the kinetin-dependent pigment synthesis had the same time course (Fig 1)

The effect of an irradiation with red light becomes clearly visible only in the presence of kinetin (Table 2) whereas seedlings submersed in tyrosine solution alone produced only small amounts of betacyanin after irradiation In water controls the effect of red light (R) was not measurable under the conditions used The action of low-intensity R light could be

TABLE 2 AMPLIFICATION OF LIGHT ACTION ON AMARANTHIN BIOSYNTHESIS BY KINETIN*

Light used	Extinction (extract of 50 seedlings) Incubation solution	
	Tyrosine	Kinetin/tyrosine
Control (dark)	0 050	0 410
White	0 200	0 770
Red	0 100	0 530
Far-red	0 075	0 420
Blue	0 180	0 730
Blue + red	0 190	0 800

* Seedling age 96 hr, irradiation 4 hr

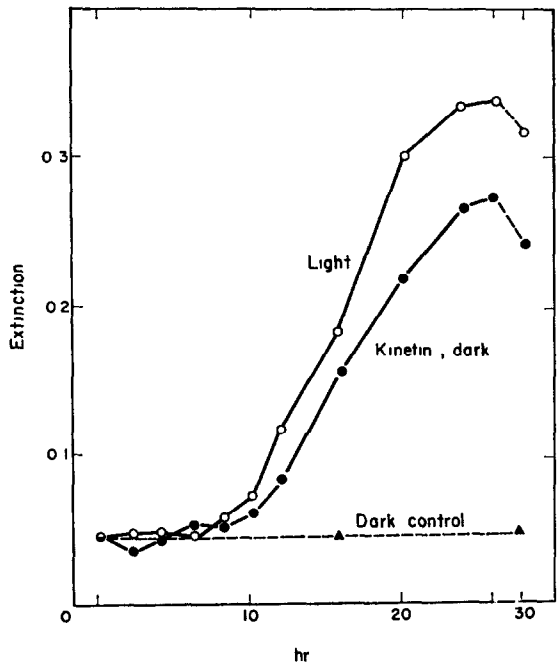

FIG 1 TIME COURSE OF AMARANTHIN FORMATION AFTER 4 hr EXPOSURE TO LIGHT OF FLUORESCENT LAMPS OR IN THE DARK IN THE PRESENCE OF KINETIN
Control, dark —▲— Light —○—Kinetin, dark —●—

partly reversed by a subsequent exposure to FR (in agreement with results for *A tricolor*[3]). This effect was repeatedly reversible, although not completely, and is due to phytochrome action The influence of kinetin on phytochrome activity seems to be similar to other findings in which enhanced phytochrome action was caused by different cofactors [8,9]

Further, it is remarkable that an irradiation with white fluorescent light yields even higher amounts of pigment than a saturating illumination with red light. This points to the additional influence of light of different wavelength than red The extra pigment produced (over the control) after red irradiation amounts to only one third of that after an irradiation by white light. The amount of pigment produced after irradiation with white can almost be obtained after an irradiation with blue light Samples subsequently illuminated with R light produce more pigment than after a blue irradiation alone [5,10] This signifies that the long-term illumination with blue leads to a high-energy photoreaction (HER) which usually requires a corresponding phytochrome level for their effects to be fully apparent This additional pigment production can only partly be reversed by FR After 4 hr of blue light either the necessary processes were irreversibly started or a part of the amaranthin synthesis is controlled by systems other than phytochrome

The action spectrum for amaranthin biosynthesis (Fig 2) demonstrates that the incident energy required is lowest in the R (640 nm), in the FR (753 nm), or in the blue region (495

[8] W F BERTSCH and W S HILLMAN, *Am J Bot* **48**, 504 (1961)
[9] W S HILLMAN and W K PURVES, *Planta* **70**, 275 (1967)
[10] H SCHERF and M H ZENK, *Z Pflanzenphysiol* **57**, 401 (1967)

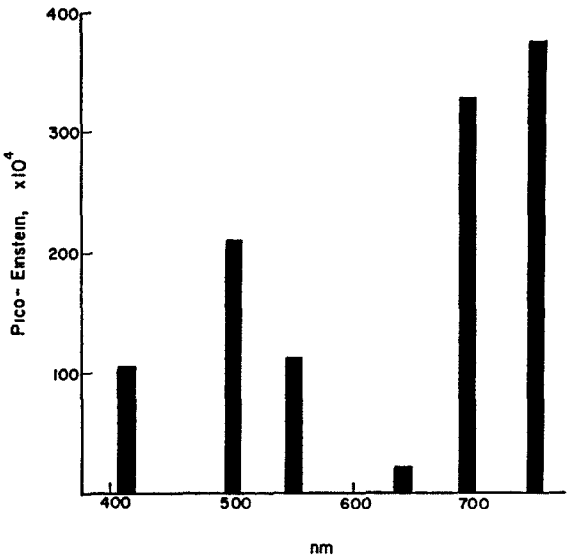

FIG 2 QUANTA DENSITY (IN PICO-EINSTEIN) FOR INDUCTION OF AMARANTHIN SYNTHESIS IN RELATION TO THE WAVELENGTH OF THE LIGHT

and 405 nm) much higher quanta densities are used At 640 nm a quanta density of 6×10^5 pE gives significant results (see also[6]) Even an irradiation of 1 min with 700 erg cm^{-2} sec^{-1} at 640 nm leads to pigment production in the seedlings The efficient intensity in the FR is much higher (3000 erg cm^{-2} sec^{-1}) although considerable pigment production is possible under continuous FR where the total amount of light is equivalent to a short-term red irradiation

CONCLUSIONS

Amaranthin biosynthesis in *A caudatus* seedlings is controlled by phytochrome in the presence of kinetin, by a blue light HER reaction, and by kinetin alone. All light reactions are similar to those required in anthocyanin synthesis [10] As the precursor for betacyanin biosynthesis is DOPA (or tyrosine),[1] and acetyl coenzyme A is not involved in these processes, it is a good model for further investigation to clarify mechanisms of light action. Such results are important because different opinions exist about the mode of action of the light effects

EXPERIMENTAL

Plant material Sterilized (1% Br water) seeds of *A caudatus* L var viridis (Fa F C. Heinemann, Erfurt) were germinated and grown on 1% water-agar in darkness at constant temperature (20°) After 96 hr the seedlings were transferred under dim green safety light into different solutions (water, 1% tyrosine solution, 1% tyrosine + 10^{-5} mol/l kinetin solution) During this time—if not otherwise stated—the irradiation of the seedlings was carried out for 4 hr Then the seedlings were put for a further 24 hr in the dark, after which the pigments were extracted

Extraction and estimation of the amaranthin The seedlings were dried with filter paper After weighing, the plant material was placed in centrifuge tubes in 5 ml of H_2O and frozen at $-20°$ Thawing was carried out at room temp (20°) This procedure was repeated to guarantee a complete extraction of the pigment, especially of the more slowly extractable amaranthin from the hypocotyls The decanted clear extracts

were measured at 542 nm and corrected for insignificant turbidity by a further measurement at 620 nm (extinction < 0·20) [11]

Light sources Irradiations with white light were performed with cool white fluorescent lamps (VEB BGW, Berlin) with an intensity of the unfiltered light of 700 lux at the seedling level

Far-red, red and blue light were produced by a system of a projector with a tungsten filament lamp in conjunction with different interference filters (405, 495, 554, 640, 692 and 753 nm) (VEB Carl Zeiß, Jena) For cooling, the light was passed through a water cuvette Determination of the light intensities was made with a thermopile (E 20, Kipp & Zonen, Delft, Netherlands) or in other cases with a vaccuum-thermo-element ("V-Th-5," VEB Carl Zeiss, Jena), and expressed in erg cm^{-2} sec^{-1} (for details see[12])

[11] K -H KOEHLER and K. CONRAD, *Flora* **159A**, 293 (1968)
[12] K -H KOEHLER, *Biolog Zentralblatt* (in press)

Key Word Index—*Amaranthus caudatus*, Amaranthaceae, betacyanin biosynthesis, kinetin; photocontrol