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Photocontrol of Anthocyanin Synthesis

III. THE ACTION OF STREPTOMYCIN ON THE SYNTHESIS OF CHLOROPHYLL AND ANTHOCYANIN[1]

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ABSTRACT

Streptomycin enhances the synthesis of anthocyanins and inhibits the synthesis of chlorophylls and the development of chloroplasts in dark-grown seedlings of cabbage (*Brassica oleracea*), mustard (*Sinapis alba*), tomato (*Lycopersicon esculentum*), and turnip (*Brassica rapa*) exposed to prolonged periods of irradiation in various spectral regions. These results suggest that the contribution of photosynthesis to light-dependent high irradiance reaction anthocyanin synthesis in seedlings of cabbage, mustard, tomato, and turnip is minimal, if any at all. So far, phytochrome is the only photoreceptor whose action in the control of light-dependent anthocyanin synthesis in seedlings of cabbage, mustard, tomato, and turnip has been satisfactorily demonstrated.

Studies of light-dependent anthocyanin synthesis and of other plant photomorphogenic responses have shown that two photoprocesses are operating (8–10, 15, 17, 18, 24, 27, 35, 36, 39, 40, 44). One is the low energy, R — FR[4] reversible phytochrome system (1, 8, 12, 15, 17, 18, 28, 40, 44) and the other is the high energy reaction, also called high irradiance reaction system of plant photomorphogenesis (4, 9, 10, 14, 15, 28, 44). The nature of the photoreceptor(s) for the HIR responses is a debated question.

The spectral regions of maximum efficiency for HIR responses are also effective in the Pr ⟷ Pfr photoconversion. It has been suggested that the HIR responses, at least in the far red region, can be mediated by phytochrome alone, and are brought about by the low level of Pfr maintained under continuous FR (4, 14, 28, 29, 37, 41, 44). Some results (2, 34) do not support the hypotheses made to explain HIR phenomena in terms of phytochrome alone. Strong evidence in support of phytochrome being the only photoreceptor mediating the response to prolonged periods of FR irradiance has been obtained in studies of the action of light on seed germination (11, 14, 22, 23, 42, 45).

It has also been suggested that HIR responses may involve an interaction between photosynthesis and phytochrome (8, 10, 15, 35, 36). Evidence for the participation of photosynthesis to HIR responses has been obtained in studies of light-dependent anthocyanin synthesis in green systems with a functional photosynthetic apparatus such as apple skin and strawberry leaf disks (6, 8, 10).

The involvement of photosynthesis in the HIR responses of dark-grown seedlings exposed to continuous irradiation is much less evident, and has been considered negligible by some researchers (3, 24, 26, 28, 37), while others have interpreted data relative to the action of various inhibitors on anthocyanin synthesis in turnip seedlings as indicative of a possible contribution of cyclic photophosphorylation to HIR anthocyanin synthesis under continuous FR irradiation (35, 36). It has been reported that some antibiotics can enhance anthocyanin synthesis, while at the same time inhibiting Chl synthesis (28, 38, 40). These reports cast some doubts on the participation of photosynthesis to HIR anthocyanin synthesis of dark-grown seedlings exposed to continuous irradiation.

In this paper, we report data on the action of streptomycin on the synthesis of anthocyanins and Chl in young, dark-grown seedlings exposed to continuous irradiation. For this study we used seedlings of cabbage, mustard, tomato, and turnip. Cabbage seedlings can synthesize considerable amounts of anthocyanins in darkness; the others do not (1, 12, 17, 18, 24, 27, 40). Phytochrome participation in light-dependent anthocyanin synthesis has been shown for all the four species used (1, 12, 17, 18, 24). The peak of action for anthocyanin production under continuous irradiation is in the FR region for cabbage (17), mustard (17, 27), and turnip (13, 35), and in the blue for tomato (unpublished results).

MATERIALS AND METHODS

Seeds of cabbage (*Brassica oleracea* cv. Red Acre) were purchased from the Seed Division of the FMC Corporation; seeds of tomato (*Lycopersicon esculentum*, cv. Beefsteak) and turnip (*Brassica rapa*, cv. Purple Top White Globe) were purchased from W. Atlee Burpee Co.; seeds of mustard (*Sinapis alba*, cv. Fine White) were purchased from Thompson and Morgan Ltd. The seeds were germinated and grown in darkness at 20 C in Petri dishes on two disks of Whatman No. 3MM filter paper, moistened with distilled H_2O or with the solution used. All solutions were prepared in H_2O. The number of seeds per dish was: 30, for cabbage and mustard; 50, for tomato; and 60, for turnip. Ages of the seedlings (from planting) at the beginning of the light treatments were: 96 hr for cabbage and tomato; 72 hr for turnip; and 48 hr for mustard.

Light treatments were given in growth chambers (Percival E-57) equipped with various combination of lamps and filters which have been described previously (17, 21). The irradiances

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[4] Abbreviations: R: Red; FR: far red; B: blue. DMSO: dimethylsulfoxide; DNP: 2,4-dinitrophenol; HER: high energy reaction; HIR: high irradiance reaction.

Table I. *Irradiance of Light Sources Used*

Irradiances were measured with an IL-150 plant photometer. Values reported were obtained by multiplying the measured values (μw cm^{-2} nm^{-1}) by the half bandwidth of the filters used for the separation of the three spectral regions.

Radiation source	Irradiance		
	400–500 nm	600–700 nm	700–800 nm
		$\mu w\ cm^{-2}$	
B	375	ND[1]	ND
White	715	860	430
FR	ND	165	740
R	ND	400	70
R incandescent	ND	295	360
R + FR	ND	625	420
R[2]	ND	110	ND
FR[2]	ND	115	520

[1] ND: not detectable with IL-150 photometer (less than 0.5 μw cm^{-2} nm^{-1}).

[2] R and FR sources used in cyclic light treatments (R/FR/C).

of the light sources used are given in Table I. Temperature during irradiation was 20 C. Dark controls were included in all experiments.

Anthocyanins extraction and measurements. Lots of 30 (cabbage and mustard), 50 (tomato), and 60 (turnip) seedlings each were extracted with 12 ml of 1% (w/v) HCl in methanol for 2 days at 3 to 5 C, with continuous shaking. The extracts were cleared by filtration, and their absorbance at 530 and 657 nm was measured with a Model 300-N Gilford spectrophotometer. In previous experiments, we had noticed that the acidic methanol extracts of anthocyanins from seedlings grown under conditions leading to the formation of high levels of Chl exhibited a brownish coloration and two peaks of absorption at wavelengths above 500 nm, one at 530 nm, and the other at about 657 nm. The brownish tinge and the peak at 657 nm were considerably reduced or absent in the anthocyanin extracts from seedlings grown under FR or in streptomycin solution. Acidic methanol solutions of Chl exhibited a peak of absorption around 657 nm; their absorption at 530 nm was about one-third of that at 657 nm. The addition of purified chlorophylls to an acidic methanol extract of anthocyanin from dark-grown cabbage seedlings resulted in the appearance of the peak at 657 nm and in an increase of the absorbance at 530 nm. The increase of absorbance at 530 nm was again about one-third of the absorbance at 657 nm. Therefore, the absorbance readings at 530 nm were corrected by subtracting one-third of the absorbance at 657 nm. The results reported are the average of at least eight replicates. Standard errors of the means were about 4 to 6% of the average values.

Chlorophyll Extraction and Measurements. Lots of 120 (cabbage and mustard) or 100 (tomato) seedlings each were ground with acetone and a small amount of $CaCO_3$. The homogenate was clarified by centrifugation and filtration and made up to 25 ml with 80% acetone. The absorbance of the clear extracts was measured at 645 and 663 nm with a Gilford 300-N spectrophotometer. Chl content of the extracts was calculated using the McKinney coefficients (20). Values reported are the average of at least six replicates. The standard errors were about 5% of the average values.

Phytochrome Measurements. Phytochrome content was assayed spectrophotometrically *in vivo* on lots of 30 seedlings each with a Ratiospect Model R-2 (ASCO). The wavelengths of the measuring beams were 730 and 800 nm. Values reported are the average of at least eight replicates; standard errors varied considerably from about 10% of the average values for measurements taken at times 0 and 2, to about 40% for some of the very low values obtained in the measurements taken 24 hr after the beginning of the irradiations.

RNA Extraction and Separation. The method used for the extraction of the RNAs was essentially that described by Cherry (5). RNA was extracted from about 100 cotyledon pairs excised from seedlings grown in sterile conditions in H_2O or streptomycin solution. Separation of the RNAs was accomplished by disc electrophoresis in 2.5% acrylamide gels according to Loening (19).

The cotyledons used for the electron microscope observations were fixed in 3% glutaraldehyde, washed in buffer, postfixed in osmium tetroxide, washed again, dehydrated in a graded ethanol series, and embedded in Epon. Sections were cut with a diamond knife, poststained with a lead citrate solution, and examined with a Philips EM 200 electron microscope.

RESULTS

The dosage response curves of Figure 1 show quite clearly the correlation between the inhibition of Chl synthesis and the enhancement of anthocyanin accumulation brought about by streptomycin.

Streptomycin has a strong inhibitory effect on the synthesis of Chl under various light treatments in mustard, tomato, and cabbage seedlings (Tables II, III, IV, and V). Streptomycin has no apparent effect on the light-dependent synthesis of anthocyanins in mustard seedlings (Table II), and a strong stimulatory action on anthocyanin accumulation in tomato, cabbage, and turnip seedlings (Tables III, IV, V, and VI). In cabbage seedlings (Fig. 1, Tables IV and V) streptomycin also enhances anthocyanin accumulation in the dark controls. We do not have exact figures for the levels of Chl formed in turnip (Table VI), but, from the absorbance values of the

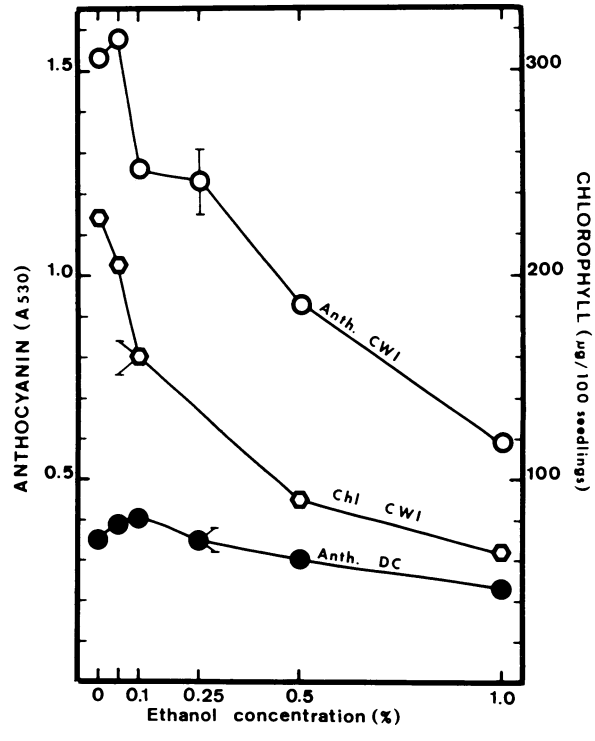

FIG. 1. Action of streptomycin on anthocyanin and Chl synthesis in cabbage seedlings. D: dark control; L: R + FR for 24 hr.

anthocyanin extracts at 657 nm (results not reported), it appears that the inhibition of Chl synthesis caused by streptomycin is of the same order of magnitude as that found in cabbage and mustard. The high values of the Chl *a/b* ratio under continuous FR (Tables II, III, and IV) are in agreement with results reported for the greening of etiolated bean leaves under continuous FR (7). FR is more effective than either B or R for the light-dependent anthocyanin synthesis of cabbage and mustard seedlings (Tables II and IV), but B is more effective than either R or FR for anthocyanin accumulation in tomato seedlings (Table III).

A comparison of the results of Tables IV and V shows that the 8 hr light-40 hr dark (8L-40D) irradiation program results in the production of levels of Chl (seedlings incubated in H_2O)

Table II. *Action of Streptomycin (200 μg/ml) on Synthesis of Anthocyanins and Chl in Mustard Seedlings*

Light Treatment (48 hr)	Incubation Medium	Anthocyanins ($A_{530} - 0.33\ A_{657}$)[1]	Chlorophyll	
			Total	*a/b*
			μg/100 seedlings	
Dark control	W[2]	0.06	7.3	0.6
Dark control	SM[2]	0.05	6.9	0.7
B	W	1.28	514.0	3.4
B	SM	1.26	63.8	3.0
FR	W	1.62	38.4	6.0
FR	SM	1.66	20.4	2.1
R	W	0.85	736.3	2.9
R	SM	0.90	54.5	2.7
R incandescent	W	1.56	609.4	2.8
R incandescent	SM	1.52	56.5	2.5
R/FR/D[3]	W	1.47	382.7	4.7
R/FR/D[3]	SM	1.51	35.4	4.0

[1] Absorbance values of the light treatments were corrected by subtracting the absorbance values of the dark controls. Absorbance values at 530 were corrected by subtracting one-third of the absorbance values at 657 (0.33 A_{657}).

[2] W: water; SM: streptomycin.

[3] R/FR/D = 1 min R − 1 min FR − 8 min D − 1 min R . . . etc.

Table III. *Action of Streptomycin (200 μg/ml) on Synthesis of Anthocyanins and Chl in Tomato Seedlings*

Light Treatments (48 hr)	Medium	Anthocyanins ($A_{530} - 0.3\ A_{657}$)	Chlorophyll	
			Total *a/b*	*a/b*
			μg/100 seedlings	
Dark control	W[1]	0.01	3.6	0.6
Dark control	SM[1]	0.01	3.9	0.6
B	W	0.25	170.5	3.4
B	SM	0.44	84.2	2.9
FR	W	0.04	18.5	9.8
FR	SM	0.05	18.7	2.8
R	W	0.15	235.3	3.2
R	SM	0.35	135.5	3.1
R incandescent	W	0.27	213.9	3.8
R incandescent	SM	0.59	157.9	3.6
R/FR/D[2]	W	0.08	180.1	4.8
R/FR/D[2]	SM	0.17	103.6	3.5

[1] W: water; SM: streptomycin.

[2] R/FR/D = 1 min R − 1 min FR − 8 min D − 1 min R . . . etc.

Table IV. *Action of Streptomycin (200 μg/ml) on the Synthesis of Anthocyanins and Chl in Cabbage Seedlings*

Light Treatment (48 hr)	Incubation Medium	Anthocyanins ($A_{530} - 0.33\ A_{657}$)[1]	Chlorophyll	
			Total	*a/b*
			μg/100 seedlings	
Dark control	W[2]	0.23	2.7	0.4
Dark control	SM[2]	0.38	2.5	0.4
B	W	0.81	270.7	3.2
B	SM	1.57	13.5	1.2
FR	W	1.13	26.9	13.9
FR	SM	1.94	7.6	1.9
R	W	0.57	360.9	2.6
R	SM	0.94	11.5	1.9
R incandescent	W	1.38	348.2	3.1
R incandescent	SM	2.06	15.5	1.9
R/FR/D[3]	W	0.94	170.6	4.3
R/FR/D[3]	SM	1.53	15.3	1.5

[1] Absorbance values for the light treatments were corrected by subtracting the absorbance values of the dark controls.

[2] W: water; SM: streptomycin.

[3] R/FR/D = 1 min R − 1 min FR − 8 min D − 1 min R . . . etc.

Table V. *Action of Streptomycin (200 μg/ml) on Synthesis of Anthocyanins and Chl in Cabbage Seedlings*

Treatment was 8 hr B (or FR, or R, or R incandescent, or R/FR/D) − 5 min R − 40 hr dark.

Treatment	Incubation Medium	Anthocyanins ($A_{530} - 0.33\ A_{657}$)[1]	Chlorophyll	
			Total	*a/b*
			μg/100 seedlings	
Dark control	W[2]	0.23	2.7	0.4
Dark control	SM[2]	0.38	2.5	0.4
B	W	0.60	29.0	3.2
B	SM	1.01	5.8	0.6
FR	W	0.55	8.0	1.6
FR	SM	0.87	4.9	0.4
R	W	0.33	23.0	2.6
R	SM	0.54	4.6	0.8
R incandescent	W	1.01	43.8	3.9
R incandescent	SM	1.68	7.3	1.0
R/FR/D[3]	W	0.77	29.9	4.2
R/FR/D[3]	SM	1.23	5.0	0.6

[1] Absorbance values of the light treatments were corrected by subtracting the values of the dark controls.

[2] W: water; SM: streptomycin.

[3] R/FR/D = 1 min R − 1 min FR − 8 min D − 1 min R . . . etc.

that are no more than 10 to 20% of those produced under a 48 hr light (48L) schedule; but the accumulation of anthocyanins under the 8L-40D program is from 50 to 80% of that under the 48L schedule. Similar results were also obtained with tomato and mustard seedlings. These results are not a consequence of a large accumulation of anthocyanin during the 8-hr light treatment; the level of anthocyanins accumulated at the end of 4- and 12-hr irradiations have been found to be quite low (17, 24).

Streptomycin has very little effect on the R-FR reversibility of anthocyanin synthesis (Table VII). The effects of streptomycin on the decay of phytochrome in mustard (results not

Table VI. *Action of Streptomycin on Synthesis of Anthocyanins in Turnip Seedlings*

Light Treatments (48 hr)	Absorbance ($A_{530} - 0.33\ A_{657}$)		
	Water	SM/100[1]	SM/200[2]
Dark control	0.02	0.02	0.02
R incandescent	0.43	0.56	0.53
FR	0.46	0.63	0.70

[1] SM/100: streptomycin solution, 100 μg/ml.
[2] SM/200: streptomycin solution, 200 μg/ml.

Table VII. *Action of Streptomycin on Red-Far Red Reversibility of Anthocyanin Synthesis in Cabbage in Tomato Seedlings*

The absorbance values for the light treatments were corrected by subtracting the values of the dark controls. Anthocyanins were extracted 24 (cabbage) or 48 (tomato) hr after the beginning of the light treatments.

Light Treatment	Absorbance at 530 nm	
	H_2O	SM (200 μg/ml)
Cabbage		
Dark control	0.30	0.51
5 min R	0.21	0.48
5 min R − 5 min FR	0.12	0.39
Tomato		
Dark control	0.01	0.01
4 hr white	0.08	0.08
4 hr white − 5 min R	0.08	0.09
4 hr white − 5 min R − 5 min FR	0.04	0.04
4 hr white − 5 min FR	0.04	0.04
4 hr white − 5 min FR − 5 min R	0.08	0.08

Table VIII. *Phytochrome Decay in Cabbage Seedlings Exposed to Various Light Treatments in Water or in 200 μg/ml Streptomycin Solution*

Time 0 indicates onset of irradiation. Data for the dark controls are expressed as $\Delta(\Delta O.D.) \times 10^3$; data for the light treatments as per cent of the dark controls.

Light Treatment	Incubation Medium	Phytochrome Content at				
		0	2 hr	4 hr	6 hr	24 hr
Dark control	W[1]	11.1	11.8	11.3	11.7	12.6
Dark control	SM[1]	12.1	12.1	12.4	12.3	12.1
B	W	100	65	34	21	14
B	SM	100	88	41	25	5
FR	W	100	88	72		35
FR	SM	100	92	80	65	40
R	W	100	40	19	8	10
R	SM	100	31	9	7	1
R incandescent	W	100	50	27	17	10
R incandescent	SM	100	50	35	19	8
R/FR/D[2]	W	100		76	53	14
R/FR/D	SM	100	66	55	39	15

[1] W: water; SM: streptomycin.
[2] R/FR/D = 1 min R − 1 min FR − 8 min D − 1 min R . . . etc.

reported) and in cabbage seedlings (Table VIII) were essentially similar. Because of the large standard errors (see "Materials and Methods") obtained in the phytochrome assays *in vivo* and because, with few exceptions the differences in the rate of decay of phytochrome in H_2O and in streptomycin are rather small, we feel inclined to conclude that the action of streptomycin on the rate of decay of spectrophotometrically detectable phytochrome is negligible.

Streptomycin inhibits the synthesis of chloroplast ribosomal RNA (Fig. 2, peaks 2 and 4). Chloroplasts of cotyledons from seedlings grown in streptomycin are much less developed than those of seedlings grown in H_2O (Fig. 3).

Although not directly related to the main purpose of this paper, we would like to introduce here some data relative to the action of ethanol, propanol, DMSO, and Tween 20 on the synthesis of anthocyanins in cabbage seedlings. The above compounds are sometimes used as solvents for the preparation of stock solutions of chemicals poorly soluble in H_2O, or as wetting agents, or for their action on membrane permeability (31). Ethanol and propanol at low concentrations (0.1% or less) have a slightly stimulatory action on anthocyanin synthesis and are inhibitory at higher concentrations (Fig. 4, Table IX). Ethanol inhibits Chl synthesis (Fig. 4). In the range of concentrations tested, DMSO and Tween 20 have very little effect on anthocyanin synthesis in darkness.

DISCUSSION

The interpretation of results obtained in inhibitor studies is not simple and requires a great deal of caution. Even the specific inhibition of a given step in synthesis can affect the steady state levels of other intermediates in the biosynthetic pathway of the product under study, as well as other pathways with common intermediates. *In vivo* inhibitor studies of processes such as anthocyanin synthesis, requiring long periods for completion—several hours to a few days—cannot be expected to have well defined and narrow specificities. The proofs offered in support of a contribution of photosynthesis to HIR anthocyanin synthesis are based mostly on the results of *in vivo* inhibitor studies.

Evidence in support of a contribution of photosynthesis to

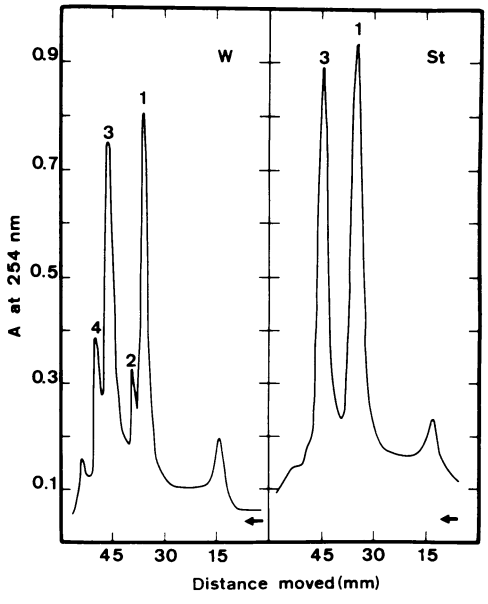

FIG. 2. Action of streptomycin (st, 200 μg/ml) on the synthesis of ribosomal RNA of cabbage seedlings. RNAs were separated by electrophoresis on acrylamide (2.5%) gels, at 3 ma/tube, for 2.5 hr. 1 and 3: heavy and light cytoplasmic ribosomal RNA; 2 and 4: heavy and light chloroplast ribosomal RNA. W: water.

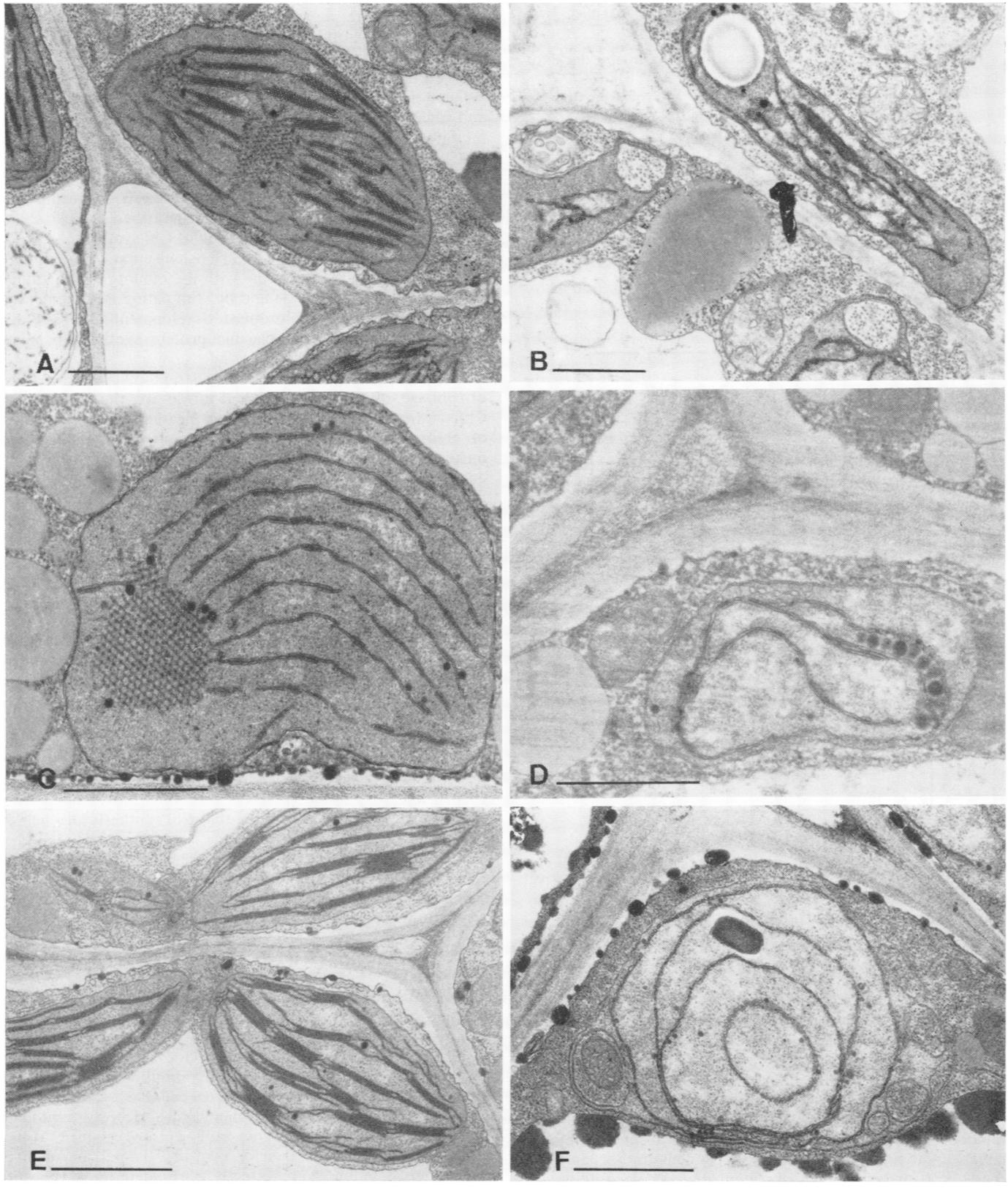

FIG. 3. Chloroplasts of seedlings of tomato (A, B) and cabbage (C, D, E, F), incubated in H_2O (A, C, E) or in streptomycin solution (200 μg/ml) (B, D, F). Light treatments (48 hr) were: blue (A, B), FR(C, D), and R in candescent (E, F). The length of the segments corresponds to 1 μm.

Table IX. *Action of DMSO, 1-Propanol, and Tween 20 on Synthesis of Anthocyanins in Cabbage Seedlings*

The temperature was 20 C. Incubation was in the dark for 6 days.

Concn	Absorbance ($A_{530} - 0.33\ A_{657}$)		
	DMSO	1-Propanol	Tween 20
%			
0.000	0.28	0.28	0.28
0.025	0.26	0.39	0.35
0.050	0.32	0.55	0.34
0.100	0.32	0.28	0.35
0.200	0.29	0.09	0.33

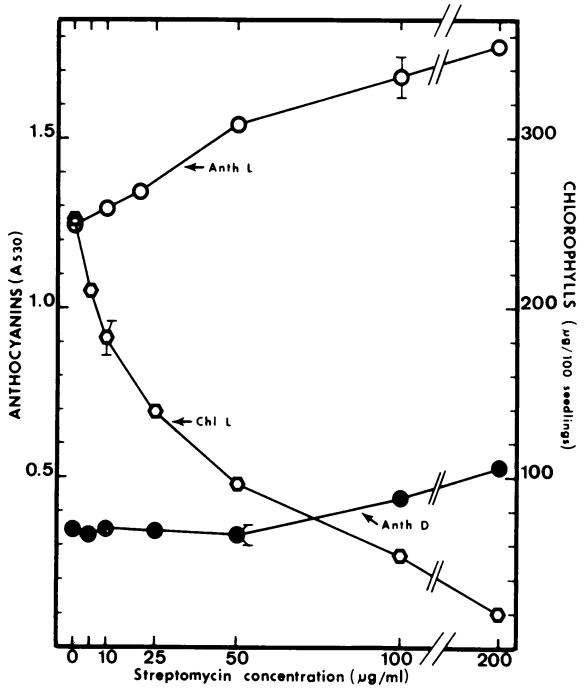

FIG. 4. Action of ethanol on the synthesis of anthocyanins and Chl in cabbage seedlings. Light treatment: 24 hr, white (CWI); DC: dark control.

anthocyanin synthesis has been obtained for some green tissue with a functional photosynthetic apparatus (6, 8, 10). In apple skin sections, the proportional changes in the rates of photosynthesis and anthocyanin accumulation were practically the same for a range of DCMU concentrations causing various degrees of inhibition between 0 to 100% (8, 10). In strawberry leaf disks, DCMU inhibits light-dependent anthocyanin synthesis, and the effect of the DCMU is enormously reduced (from an inhibition of 95% to one of 10%, at a DCMU concentration of 10 μM) when sucrose is added to the medium used for the incubation of the disks (6). Sucrose enhances about 5-fold the light-dependent anthocyanin synthesis of strawberry leaf disks incubated in air containing no CO_2, but has no effects at a CO_2 concentration of 0.03% (6).

The inhibitors used in the studies offered in support of the hypothesis that photosynthesis, and specifically cyclic photophosphorylation, may contribute to HIR anthocyanin synthesis of dark-grown turnip and mustard seedlings exposed to continuous FR (35, 36), have a much wider spectrum of action than DCMU. Antimycin A and DNP act not only as inhibitors of photosynthetic phosphorylation, but also as uncouplers of oxidative phosphorylation. The ammonium ion, which has been used as an uncoupler of photophosphorylation of chloroplast preparations (16), is also known for its effects on enzyme balance and on the level of some nitrogen compounds (43), and for its inhibition of H_2O uptake (33). Levulinic acid is an inhibitor of porphyrin biosynthesis, and inhibits the synthesis of Chl and respiratory pigments. Therefore, we feel that the inhibitory action of the ammonium ion, antimycin A, DNP, and levulinic acid on anthocyanin synthesis in turnip and mustard seedlings (35, 36) cannot be ascribed exclusively to their inhibition of photosynthesis and of Chl synthesis.

Streptomycin binds to the 30S ribosome subunit and inhibits protein synthesis by interfering with the binding of the aminoacyl-tRNA to the ribosome and by stimulating miscoding (29); streptomycin inhibits chloroplast development (32) and has little or no effect on cytoplasmic protein synthesis of plant cells (25).

The final results of the action of streptomycin in seedlings of cabbage, mustard, tomato, and turnip are: the inhibition of Chl synthesis, the lowering of the *a/b* ratio, and the inhibition of chloroplast development, without any appreciable effect on the synthesis of anthocyanin in mustard and with a strong enhancing action on the synthesis of anthocyanins in the other three species. The enhancing action of streptomycin on anthocyanin synthesis is found in darkness (cabbage), under light treatments resulting in widely different levels of Chl formation, and in seedlings differing in the spectral regions of maximum effectiveness for anthocyanin synthesis, FR in cabbage and turnip, B in tomato.

The data obtained with apple skin and strawberry leaves (6, 8, 10) seem to indicate that photosynthesis may contribute photosynthetic products to light-dependent anthocyanin synthesis of green tissues with a functional photosynthetic apparatus. In dark-grown seedlings exposed to prolonged irradiation, it would be reasonable to expect that some degree of development of the photosynthetic apparatus should be attained, before photosynthesis can begin to compensate for the drain of reserve materials used during the initial phase of growth and to provide products for other biosynthetic reactions. Previous results (24) show that Chl synthesis lags behind anthocyanin synthesis in cabbage and mustard seedlings. Results reported here and in previous papers (17, 24) show that considerable amounts of anthocyanins can be synthesized in darkness, after 4 to 12 hr of irradiation. The latter treatment results in the production of levels of Chl that are quite low in comparison, of those formed under continuous, prolonged irradiations (Table IV and V), and there is no photosynthesis in darkness.

The data reported in this paper show that it is possible to inhibit Chl synthesis and the development of the chloroplast without adverse effects on anthocyanin synthesis, and, in most cases, actually enhancing the latter. One possible explanation for the enhancement effect is that the inhibition of the development of the chloroplast may make available a large reserve pool for the biosynthesis of anthocyanins. However, the data reported in this paper are not sufficient to establish if the inhibition of chloroplast development and the enhancement of anthocyanin synthesis are directly correlated or if they are two independent phenomena.

Although we do not have comparative data for the rates of photosynthetic activity of seedlings grown in H_2O and in streptomycin, we believe that the results reported in this paper provide some evidence that the contribution of photosynthesis to HIR anthocyanin synthesis of young, etiolated seedlings exposed to prolonged irradiations is only a minor one. S. O.

Duke (Department of Botany, Duke University) has some preliminary evidence that corn seedlings lacking a photosynthetic apparatus accumulate anthocyanin faster in the nonphotosynthetic portions of the seedlings than green controls do (personal communication).

Phytochrome is the only photoreceptor whose action in the photocontrol of HIR anthocyanin synthesis of young seedlings has been satisfactorily demonstrated (1, 9, 12, 17, 18, 24). Photosynthesis seems to play a role in light-dependent anthocyanin synthesis of some green tissues (6, 8, 10); the participation of photosynthesis to the HIR anthocyanin synthesis of young, etiolated seedlings exposed to prolonged irradiations has not been satisfactorily demonstrated and the results reported in this paper and in a previous one (24) provide some evidence against it.

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