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INFLUENCE OF VISIBLE AND NEAR INFRARED RADIANT ENERGY ON ORGAN DEVELOPMENT AND PIGMENT SYNTHESIS IN BEAN AND CORN[1]

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The principal morphogenic responses of dicotyledonous seedlings to red radiant energy have been reported to be an inhibition in growth of hypocotyl when present, an accelerated straightening of the plumular hook, an accelerated leaf expansion, an increased rate of epicotyl development, and a generally increased rate of tissue maturation (**7, 10, 13, 15**). In the monocotyledons, the most conspicuous response is a reduced growth rate of the first internode (**11**). The longer wave lengths of the visible spectrum also cause chlorophyll synthesis and photosynthesis. Due principally to the overlapping of the action spectra of these three photochemical reactions, it has been impossible to separate photomorphogenesis from the other two reactions. Consequently, when red radiant energy is used, the biochemical investigation of photomorphogenesis is complicated by the products of three photochemical reactions occurring simultaneously. It, therefore, would be advantageous to be able to separate photomorphogenesis from chlorophyll synthesis and photosynthesis. Attempts to accomplish this separation by the use of albino or chlorophyll-deficient plants have not been successful since all plants so far tested in this laboratory have contained significant quantities of chlorophyll.

The curve for chlorophyll synthesis in barley seedlings as reported by KOSKI *et al.* (**9**) shows a long wave length limit of ca. 680 mμ. Action spectra curves for photosynthesis as determined by EMERSON and LEWIS (**4**) for Chlorella, and CHEN (**2**) for the Hill reaction in Swiss chard chloroplasts indicate limits of 700 to 710 mμ. On the other hand, PARKER *et al.* (**10**) found evidence of a limit of 720 to 730 mμ for leaf expansion in pea seedlings, and data on the inhibition of the first internode of Avena by GOODWIN and OWENS (**7**), and WEINTRAUB and PRICE (**13**) likewise indicate that the longer wave length limit for the growth reactions of the seedling occurs somewhat further toward the infrared than the limits for either chlorophyll synthesis or photosynthesis. Using a long wave pass dyed gelatin filter

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which began to transmit at ca. 720 mμ, WITHROW (**15**) reported that bean seedlings expanded their leaves, lost the plumular hooks, and developed shortened hypocotyls without any visible green coloration. Similar results were obtained with other seedlings and with shoots of potato tubers. However, bean leaves which had been exposed to the far red contained appreciable quantities of chlorophyll as shown by subsequent acetone extraction. These observations indicated that it might be possible to separate photomorphogenesis from chlorophyll synthesis and photosynthesis by the use of more refined filter techniques.

In addition to inducing various growth responses in young shoots, converting protochlorophyll to chlorophyll, and supporting photosynthesis, visible radiant energy has been shown to cause an increased rate of synthesis of such diverse pigments as the carotenoids and the anthocyanins. These pigments usually are synthesized in the dark, but irradiation greatly increases the rate of synthesis in some tissues (**1, 5, 12**) and the evidence points to photochemically accelerated synthesis of both groups of pigments by other than photosynthetic products, although the question is by no means definitely resolved.

The present report represents the results of experiments designed to achieve photochemical separation of the photomorphogenic reactions from chlorophyll synthesis and photosynthesis. In the course of examining acetone and aqueous extracts of the plant material, it was observed that the spectral regions which were most effective in inducing the growth reactions also altered the pigment picture as regards the chloroplast pigments and anthocyanin. Data on these pigment changes are presented, although no direct relationship to photomorphogenesis is as yet clear.

Materials and methods

The plant material was grown in subirrigation nutrient culture (**18**) in eight growth chambers placed in a room where the temperature and humidity were controlled. These chambers were constructed of marine plywood as shown in figure 1 and were enameled white inside and black outside. A small blower in the space below the culture pan of each compartment forced air from the room into the compartment and out through baffles near the top of the chamber. Warm air from the irradiation system in the top of the compartment was discharged into the room before reaching the plants. A small amount of filtered fresh air from outside the building was forced into the room continuously.

The complete irradiation system for each compartment consisted of internal reflector lamps mounted in a water-cooled glass-bottomed tank, a 10-cm. aqueous primary filter, and a dyed gelatin secondary filter. The irradiation system used is described elsewhere (**16**). The dyed gelatin secondary filters were prepared in this laboratory by a technique also presented in detail elsewhere (**17**). The composition of the filters used and their per cent. transmittances are given in table I and figure 2, respectively. These filters are stable and have withstood months of intense irradiation with little

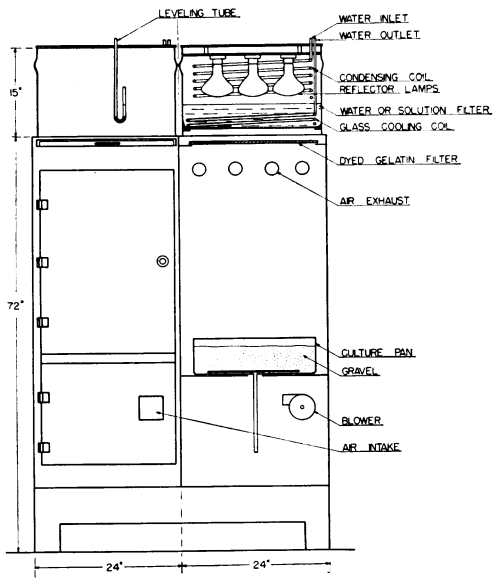

FIG. 1. Details of two growth chambers and two water-cooled lamp systems. On the left is an external view of a growth chamber and lamp tank; on the right are constructional details of a chamber and lamp system.

measurable change. The wave length designation for each far red filter was arbitrarily taken as that point, to the nearest 5 mμ, where the transmittance was ca. 0.5%.

All irradiances were measured with a fast spectrometer (Perkin-Elmer) type thermocouple operating into a contact modulation amplifier (Liston-Becker) and a one milliampere strip chart recorder for direct deflection (Esterline-Angus). The thermocouple was mounted in a thermally insu-

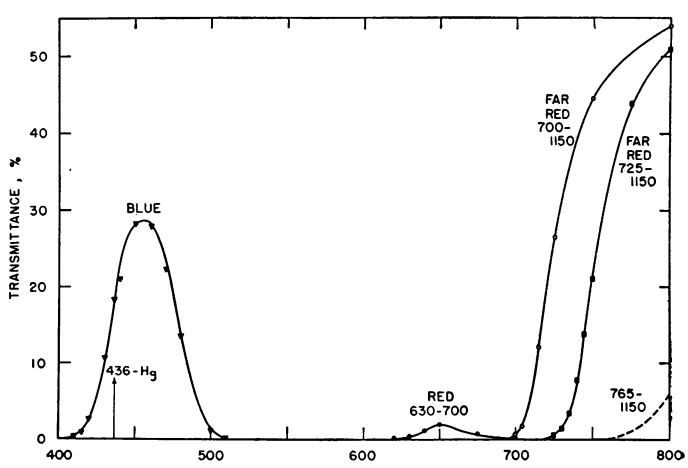

FIG. 2. Transmission curves of the radiation filters. Ordinate is per cent. transmittance (balanced against air) of the complete system of primary and secondary filters.

TABLE I

PROPERTIES OF THE IRRADIATION SYSTEMS.

Wave length limits	Irradiance*	Radiation source	Primary filter aqueous	Secondary filter dyed gelatin
Far red 765–1150 mμ	300 μw./cm.^{2}	3 × 150-watt reflector flood, incandescent	10 cm. water	Pontamine diazo blue, 1.0 mg./cm.^{2} Pontamine fast red 8 BL, 0.5 mg./cm.^{2}
Far red 725–1150 mμ	150 μw./cm.^{2}	150-watt reflector flood, incandescent	10 cm. water	Acid green no. 1854, 0.8 mg./cm.^{2} Pontamine fast red 8 BL, 0.7 mg./cm.^{2}
Far red 725–1150 mμ	450 μw./cm.^{2}	300-watt reflector flood, incandescent	10 cm. water	Acid green no. 1854, 0.8 mg./cm.^{2} Pontamine fast red 8 BL, 0.7 mg./cm.^{2}
Far red 725–1150 mμ	1500 μw./cm.^{2}	3 × 300-watt reflector flood, incandescent	10 cm. water	Acid green no. 1854, 0.8 mg./cm.^{2} Pontamine fast red 8 BL, 0.7 mg./cm.^{2}
Far red 700–1150 mμ	450 μw./cm.^{2}	300-watt reflector flood, incandescent	10 cm. water	Pontacyl brilliant blue E, 1.5 mg./cm.^{2} Pontamine fast red 8 BL, 0.5 mg./cm.^{2}
Red 630–700 mμ	2 μw./cm.^{2}	150-watt reflector flood, incandescent	10 cm. 1% $CuSO_4 \cdot 5\,H_2O$	Pontamine fast red 8 BL, 2.0 mg./cm.^{2}
Red 630–700 mμ	0.05 μw./cm.^{2}	150-watt reflector flood, incandescent	10 cm. 1% $CuSO_4 \cdot 5\,H_2O$	Pontamine fast red 8 BL, 2.0 mg./cm.^{2}
Blue 436 mμ	2 μw./cm.^{2}	100-watt reflector flood, mercury arc	10 cm. 5% $CuSO_4 \cdot 5\,H_2O$	Victoria pure blue BO, 0.2 mg./cm.^{2} 8-Hydroxyquinoline sulphate, 1.5 mg./cm.^{2}
Blue 436 mμ	0.05 μw./cm.^{2}	100-watt reflector flood, mercury arc	10 cm. 5% $CuSO_4 \cdot 5\,H_2O$	Victoria pure blue BO, 0.2 mg./cm.^{2} 8-Hydroxyquinoline sulphate, 1.5 mg./cm.^{2}

*Neutral filters were employed to obtain the indicated values.

lated wood case with a metal shutter. A water cell 3 cm. thick was placed between the receiver and shutter to remove energy of long wave lengths from warm filters and other objects at slight thermal disequilibrium with the thermocouple. The thermocouple was calibrated, without the water cell, against a standard lamp from the National Bureau of Standards, with a factor applied to correct for losses by the cell. For the visible spectrum, this factor amounted to about 10%.

Since the far red filters transmitted all wave lengths from the wave length designation of the filter to 1150 mμ and the nonselective thermocouple measured total transmitted energy, no indication of the level of the effective far red energy is given by such thermocouple measurements. Therefore, in order to avoid uncertainty as to the relative irradiance at the short wave length limit of the filters for the far red series (725 mμ), a photocell detector (Welch Densichron) with an S-1 photosurface sensitive to the near infrared was used. The photocell was covered with an interference filter two inches by two inches (Bausch and Lomb) with a peak transmission at 720 mμ, limiting the detector sensitivity to a narrow region from ca. 710 to 730 mμ. The energy values in this region then were adjusted until the desired ratios were obtained; in most cases, the total irradiance values were in the same ratios. Serious deviations occurred only when there was a difference in lamp color temperature sufficient to produce a difference in spectral energy distribution of the sources.

Bean (*Phaseolus vulgaris*), variety Black Valentine, and a hybrid field corn (*Zea mays*) variety U.S. 13, were used in all experiments. The seedlings were grown in gravel culture beds two feet square and were subirrigated automatically every six hours. During germination the beds were subirrigated with tap water and the temperature maintained at 25° C and 70% relative humidity. When the seedlings had just emerged from the gravel, the temperature was reduced to 20° C and a complete nutrient solution (**18**) was used. For the remainder of the experimental period, irradiation was continuous with no intervening dark periods.

Seedlings of bean and corn were exposed for eight and five days, respectively, to five spectral regions: the 436 mμ line of the mercruy arc; a red band between ca. 630 and 700 mμ; and three far red bands from ca. 700 mμ, ca. 725 mμ, and ca. 765 mμ. In all the far red treatments, the long wave length was limited at ca. 1150 mμ by a 10-cm. water filter. The 436 mμ line was selected for the blue treatment because the action spectra of leaf growth in pea (**10**) and first internode shortening in Avena (**7**) both show a minimum effect in the region of 450 to 500 mμ. The 436 mμ line is close enough to this region to anticipate that comparable results would be secured. The red band from ca. 630 to 700 mμ coincides with the region of maximum effectiveness for the photomorphogenic response and includes the red maximum for chlorophyll synthesis. This region at 2 μw./cm.^{2} or 0.05 μw./cm.^{2} therefore served as a reference point with which to compare the relative photomorphogenic effectiveness of the blue and far red regions.

In bean, the leaf blade alone was used for chloroplast pigment analyses, but in corn, the entire excised shoot was used. The portions to be extracted were removed from the plants in complete darkness and weighed in tight aluminum cans. They were immediately macerated in the dark in 50 ml. of acetone in a Waring Blendor for five minutes at 2° C and the slurry was filtered with suction. The acetone filtrate was added to 40 ml. of ethyl ether and washed with water three times in a 1000-ml. separatory funnel by the method used by KOSKI *et al.* (**9**). The ether solutions were made up to equal volumes per unit fresh weight of tissue and the absorbancy (**6**) or density ($\log I_o/I$) was determined in a Beckman spectrophotometer at selected wave lengths. For protochlorophyll and chlorophyll, absorbancies were measured at 625, 645, 665, and 690 mμ in 10-cm. cells. The following equations were used for determining the concentrations in micrograms per gram of fresh weight of tissue and were adapted from those developed by KOSKI *et al.* (**8**) as follows:

$$C_p = (-3.0\,A_{665} - 4.1\,A_{645} + 25\,A_{625})\,V/Wb$$
$$C_{ch} = (7.9\,A_{665} + 17\,A_{645} - 0.56\,A_{625})\,V/Wb$$

where C_p = protochlorophyll in μg./gm. fresh weight, C_{ch} = chlorophyll (a + b) in μg./gm. fresh weight, A = absorbancy or density at indicated wave length in millimicrons, V = volume of solution in milliliters, W = fresh weight of tissue in grams, b = length of absorption cell in centimeters.

The absorbancy value at 690 mμ was used as a measure of the scattering resulting from colloidally dispersed material which sometimes appeared due to incomplete filtering or separation of the ether solution into two phases. No pigments with appreciable absorption at 690 mμ are present, and an appreciable absorbancy at this wave length indicates energy loss by scattering. It therefore serves as an index of the error entering the determinations of absorbancy.

TABLE II

STEM LENGTH AND DISTRIBUTION OF DRY MATTER IN BEAN SEEDLINGS UNDER VARIOUS IRRADIATION CONDITIONS FOR 11 DAYS. VALUES ARE AVERAGES FOR 100 PLANTS

Irradiation treatment	Average stem length			Average dry weight					Dry weight
	Hypocotyl	Epicotyl	Total	Leaves	Epicotyl	Cotyledons	Hypocotyl	Total shoot	
	cm.	*cm.*	*cm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	%
Dark	25	11	36	14	31	38	85	168	5.1
Far red, 725 mμ 450 μw./cm.^{2}	18	17	35	26	40	26	69	161	5.6
Far red, 700 mμ 450 μw./cm.^{2}	13	14	27	33	33	25	65	156	6.1
Red, 630–700 mμ 2 μw./cm.^{2}	14	12	26	39	26	27	59	151	6.0
Blue, 436 mμ 2 μw./cm.^{2}	20	14	34	21	36	27	78	162	5.1

The carotene absorbancy values were determined at 440 mμ in ethyl ether, using 1-cm. cells. The quantities in micrograms per gram of fresh weight were calculated by using the value of 200 gm./l./cm. for the specific absorbancy of carotene (**19**), which was taken as an approximation for crude mixtures.

Anthocyanin was extracted separately from the hypocotyl and cotyledon portions of bean with 0.1 *N* HCl by the method of THIMANN and EDMONDSON (**12**). The extraction period was usually 48 hours at about 25° C. Bean hypocotyls were cut into sections 3 to 5 cm. long for extraction, whereas the cotyledons were extracted without sectioning. It was found that macerated tissues produced solutions too opalescent for precise photometric assay. The relative anthocyanin values were determined by measuring the absorbancy at 520 mμ for solutions containing the same fresh weight of tissue per liter. The values given in tables III and V are therefore proportional to the anthocyanin content per unit of fresh weight.

Results and discussion

GROWTH RESPONSES

The results on stem lengths and dry weight distribution in bean obtained with the various irradiation treatments are presented in table II. All irradiation treatments produced some inhibition of the length and of dry weight of the hypocotyl, an increase in fresh and dry weight of the leaves, and a decrease in weight of the cotyledons, as compared to plants grown in complete darkness. The maximum photomorphogenic response was induced by 2 μw./cm.^{2} red (630 to 700 mμ) irradiance and the minimum by the blue (436 mμ) at the same irradiance. The treatment with the 700 mμ irradiance at 450 μw./cm.^{2} was next to the red in effectiveness.

TABLE III

FRESH WEIGHT AND PIGMENT CONTENT OF BEAN SEEDLINGS UNDER VARIOUS IRRADIATION CONDITIONS FOR 11 DAYS.

Irradiation treatment	Average fresh weight of leaves	Leaf pigment per gram fresh weight				Relative concentration of anthocyanin	
		Carotene	Protochlorophyll (P)	Chlorophyll (C)	P/C	Hypocotyl	Cotyledons
	mg.	*μg.*	*μg.*	*μg.*			
Dark	90	120	12	0.0	.	5	12,000
Far red, 725 mμ 450 μw./cm.^{2}	130	180	16	1.2	13	65	17,000
Far red, 700 mμ 450 μw./cm.^{2}	240	130	3	4.8	0.6	120	17,000
Red, 630–700 mμ 2 μw./cm.^{2}	270	....	....	420	..	90	15,000
Blue, 436 mμ 2 μw./cm.^{2}	115	....	....	260	....	45	14,000

The morphological responses to varying irradiances of far red (725 mμ) are graphed in figure 3. Cotyledon weight decreased and length and weight of the epicotyl and weight of the leaves increased with irradiance, although nearly the maximum response occurred at 150 or 450 μw./cm.^{2} This result indicates saturation for this wave band at these irradiances, excepting for the hypocotyl, which decreased progressively in length and weight with irradiance to the highest value used. The fresh weights of the bean leaves used in the pigment assays are given in table III and figure 4. The increase in fresh leaf weight with irradiance follows the same general pattern as the dry leaf weight increase. It was observed qualitatively that those treatments which were most effective in causing leaf development, inhibition of

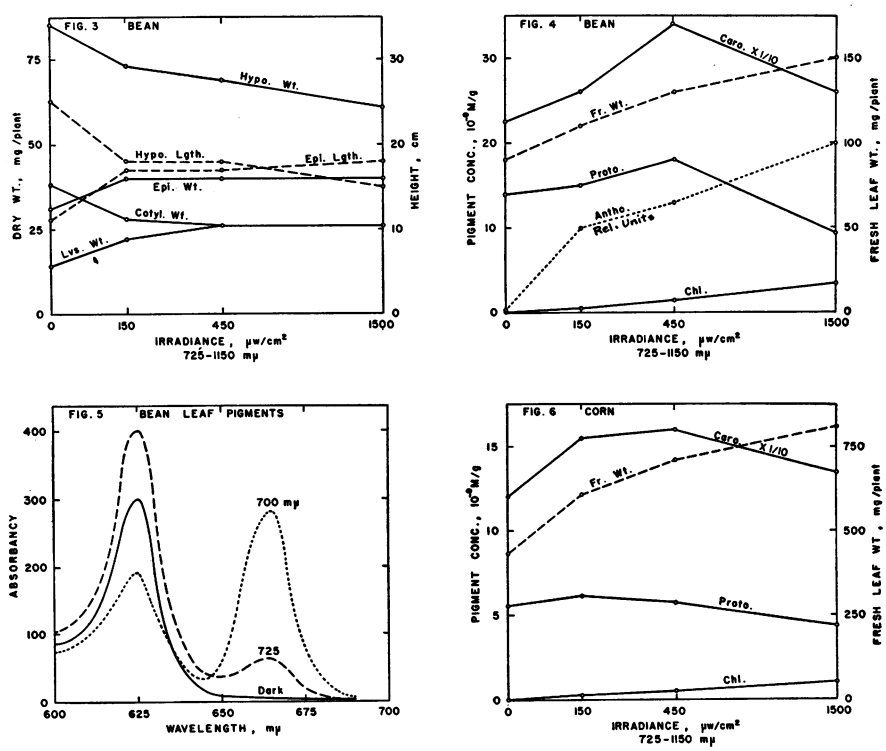

FIG. 3. Influence of irradiance of far red (725 mμ) on the distribution of dry weights and stem lengths of Black Valentine bean after eight days of continuous irradiation. Each determination is for 100 plants.

FIG. 4. Effect of irradiance of far red (725 mμ) on the molar concentrations of bean leaf pigments per gram of fresh weight, hypocotyl anthocyanin, and average leaf fresh weight of bean after eight days of irradiation. Carotene values are 10 times the ordinate scale indicated.

FIG. 5. Transmission curves of ethyl ether extracts of bean leaves. The protochlorophyll maximum appears at 625 mμ and the chlorophyll maximum at 665 mμ. The short wave length limit of the filter used for irradiation is indicated on each curve.

FIG. 6. Effect of irradiance of far red (725 mμ) on the molar concentration of corn leaf pigments per gram of fresh weight and average fresh weight of leaves. Carotene values are 10 times the ordinate scale indicated.

hypocotyl growth, and epicotyl elongation in bean were also the treatments most effective in causing the straightening of the plumular hook. In general, the hook disappeared most rapidly in the red and least rapidly in the blue. At harvest, there was always some evidence of a hook present in the plants in blue irradiance.

Corn (table IV, fig. 6) exhibited an increase in leaf fresh weight, in response to irradiance of 725 mμ, similar to that shown by bean. The red and blue treatments produced the smallest leaves of any of the irradiation treatments in corn as contrasted with bean, although in both treatments the leaves were about 25% heavier than those of the dark controls. In two preliminary experiments with corn, for which the data are not reported, similar results were obtained.

None of the responses reported here can be explained in terms of the thermal effects of the radiant energy. Noon sunlight at 1.4 cal./min./cm.^{2} is equivalent to ca. 100,000 μw./cm.^{2} and the highest far red irradiance used in these experiments, 1500 μw./cm.^{2}, was only 1.5% of this value. A small additional infrared irradiance was produced by the heating of the secondary filters at the highest irradiance, but this did not more than double the irradiance as measured by the water cell and thermocouple detector. Thermometers placed in the plots indicated that there was never more than a 1° C difference in air temperature between compartments.

These results indicate that the central feature of photomorphogenesis in bean is the photochemical initiation of active growth in the epicotyl, directed principally toward the rapid maturation of the leaves. The accelerated terminal growth places greater demands on the cotyledonary food reserves, which results in accelerated translocation to the epicotyl and leaves. In corn the far red accelerates leaf growth, but not to as great a degree as in bean.

TABLE IV

FRESH WEIGHT AND PIGMENT CONTENT OF CORN LEAVES UNDER VARIOUS IRRADIATION CONDITIONS FOR EIGHT DAYS.

Irradiation treatment	Average fresh weight	Pigment per gram fresh weight			
		Carotene	Proto-chlorophyll (P)	Chlorophyll (C)	P/C
	mg.	*μg.*	*μg.*	*μg.*	
Dark	430	65	4.8	0.00	....
Far red, 725 mμ 450 μw./cm.^{2}	720	87	5.1	0.26	20
Far red, 700 mμ 450 μw./cm.^{2}	720	75	3.1	3.9	0.8
Red, 630–700 mμ 2 μw./cm.^{2}	570	....	....	355	....
Blue, 436 mμ 2 μw./cm.^{2}	570	....	....	200	....

Chlorophyll synthesis

Some chlorophyll was formed in all the treatments with wave length of 725 mμ (figs. 4 and 6). In both bean and corn, the blue wave lengths at 2 μw./cm.^{2} produced approximately 1000 times as much chlorophyll as the 725 mμ wave lengths at 150 μw./cm.^{2} and yet plants from the two plots were comparable in photomorphogenic response. Since some photomorphogenesis occurred at a blue irradiance of 2 μw./cm.^{2}, both the blue and red values were reduced from 2.0 to 0.05 μw./cm.^{2} The results for bean are given in table V. The leaves from the higher irradiance of blue (table III) were 30% heavier than those from the dark, but those from the lower irradiance were only 7% heavier. At the lower irradiance, the plumular hook was present at harvest and the hypocotyl length was only slightly inhibited. In contrast, plants exposed to the far red at 150 μw./cm.^{2} had leaves 23%

TABLE V

FRESH WEIGHT AND PIGMENT CONTENT OF BEAN SEEDLINGS UNDER VARIOUS IRRADIATION CONDITIONS FOR 11 DAYS.

Irradiation treatment	Average fresh weight of leaves	Length of hypocotyl	Leaf pigment per gram fresh weight			Relative concentrations of anthocyanin	
			Carotene	Protochlorophyll	Chlorophyll	Hypocotyl	Cotyledons
	mg.	*cm.*	*μg.*	*μg.*	*μg.*		
Dark	83	25	100	10.0	0.0	6	4,000
Far red, 765 mμ 300 μw./cm.^{2}	85	23	160	15.5	0.0	56	6,100
Far red, 725 mμ 150 μw./cm.^{2}	112	19	165	14.4	0.25	100	6,500
Red, 630–700 mμ .05 μw./cm.^{2}	129	18	....	....	133	95	7,000
Blue, 436 mμ .05 μw./cm.^{2}	89	23	....	....	45	60	5,900

heavier than those in the dark, showing a strong photomorphogenic response, but had only ca. 1/200 of the chlorophyll present in the leaves exposed to the low energy blue wave lengths. The treatment with wave length of 765 mμ (table V) produced a definite increase in leaf weight and a complete loss of plumular hook without the synthesis of any detectable chlorophyll.

While in these experiments strong photomorphogenic responses were not induced without any detectable synthesis of chlorophyll, the complete lack of any correlation between chlorophyll synthesis and photomorphogenesis in the various spectral regions, and the very low levels of chlorophyll formed in the far red treatments, suggest that protochlorophyll and chlorophyll probably do not participate in these photochemical growth reactions. However, until the photoreceptor of the photomorphogenic responses is isolated and characterized, it will be impossible to conclude that minute traces of chlorophyll may not promote certain of these growth responses.

On the basis of experimental considerations, the use of far red radiant energy offers many unique advantages over other regions of the visible spectrum for the excitation of the various growth responses associated with photomorphogenesis. At irradiances of from 150 to 450 μw./cm.^{2}, the amount of chlorophyll formed is for most purposes quantitatively negligible. Photosynthesis is also excluded as a complicating growth factor, either as regards its intermediates or products.

Protochlorophyll and carotene

Tables III and IV show that the two lowest irradiances of 725 mμ caused an increased synthesis of protochlorophyll and carotene per unit fresh weight of leaf tissue over that formed in the dark. Even at 765 mμ, new synthesis of these pigments occurred, although the growth responses were relatively small. In two preliminary experiments with bean and two with corn, low irradiances of 725 mμ produced similar results. Since the leaves of irradiated plants were always heavier than those of the plants grown in the dark there was a still greater pigment increase per plant. Both protochlorophyll and carotene increased with irradiance at 725 mμ to a maximum value and then fell with further increase in irradiance (figs. 4 and 6). At the highest irradiance, the protochlorophyll fell below the value for both bean and corn in the dark, but the chlorophyll concentration increased with increasing irradiance. Chlorophyll synthesis, however, could account for only a small proportion of the loss in protochlorophyll and carotene. For each additional molecule of chlorophyll formed in bean by 1500 μw./cm.^{2} above that formed by the 450 μw./cm.^{2} treatment, there was an approximate loss of 4 molecules of protochlorophyll and 40 of carotene. In corn, the relative protochlorophyll decrease was 3, while the carotene decrease was 50 for each new chlorophyll molecule formed.

Figure 5 shows how the absorption spectra of the leaf pigment extracts from 600 to 700 mμ changed when the plants were irradiated with wave lengths of 700 mμ and 725 mμ respectively at 450 μw./cm.^{2} The spectrum for the extract of the plants grown in the dark contains only the absorption maximum for protochlorophyll at 625 mμ. Irradiation with 725 mμ caused both an increase in protochlorophyll and the appearance of a small chlorophyll maximum at 665 mμ, but at 700 mμ, sufficient chlorophyll synthesis occurred to depress the protochlorophyll level. Since the two low irradiances at 725 mμ caused the synthesis of only traces of chlorophyll, it is evident that the observed increases in protochlorophyll and carotene could not have been caused by products arising from photosynthesis. It appears more than likely that the increased synthesis of these pigments was the indirect result of photomorphogenesis.

The rise and subsequent fall in protochlorophyll and carotene with increasing irradiance followed a course very similar to that observed by Frank (**5**) for carotene in Avena coleoptiles. Frank speculated that the fall in carotene concentration was due to the diversion of carotene precursors into those early steps of chlorophyll synthesis where the phytol group is

added onto a porphyrin nucleus. This interpretation is difficult to reconcile with the results reported here in which the drop in molar concentration of carotene is of the order of 50 times the molar increase in chlorophyll. Even on a per plant basis where the leaves were increasing in size, the concentration of both carotene and protochlorophyll increased and then decreased. This indicates that the rise and fall in concentration of these two pigments with irradiance was the result of interacting factors controlling synthesis and decomposition which were not directly related to the biosynthesis of chlorophyll precursors.

Anthocyanin synthesis

Dark-grown seedlings of Black Valentine bean contain very high concentrations of anthocyanin in the cotyledons. Traces of purple color are visible at the cotyledonary node and in the leaf veins, but all other portions of the plant appear either white or yellow. After an exposure of eight days to the longer wave lengths of the visible spectrum, additional anthocyanin occurs in the hypocotyl and epicotyl, the most intense coloration appearing in the region of the cotyledonary node. It was observed that the corn seedlings were devoid of any visible purple coloration when grown in the dark, but the leaf bases became pink when exposed to red or far red energy.

The cotyledons of plants grown in the dark (tables III and V) contained a high concentration of anthocyanin per unit fresh weight, but the concentration of anthocyanin increased nearly 50% following treatment with the higher irradiances of far red. The hypocotyl concentration increased about 20-fold with the 1500 μw./cm.^{2}–725 mμ treatment. Even at 765 mμ, where the photomorphogenic response was small as measured by increase in leaf weight and inhibition of the hypocotyl, the anthocyanin increased about 50% in the cotyledons and almost 10-fold in the hypocotyl. The curve of relative anthocyanin in bean hypocotyl at various irradiances at 725 mμ shows that, unlike carotene and protochlorophyll, anthocyanin increased with increasing irradiance over the range used. The anthocyanin concentration closely paralleled the photomorphogenic response as measured by leaf fresh weight increase.

While several reports (**3, 12**) have demonstrated that low temperature and sugars supplied in the substrate or endogenously via photosynthesis will promote the synthesis of anthocyanin, it is evident that these are not the factors contributing to synthesis by far red radiation, as reported here. Whether the increased synthesis of anthocyanin with radiant energy is a direct or indirect photo-response is not clear as yet.

Summary

The design of a growth chamber and a water-cooled and filtered irradiation system is described for growing plants under controlled conditions of temperature, humidity, irradiation, and nutrition.

Results are presented on the effects of the following spectral regions, applied continuously, on seedling growth and pigment synthesis in bean and

corn: the blue 436 mμ mercury line, red from 630 to 700 mμ, and three far red bands of 700 to 1150 mμ, 725 to 1150 mμ, and 765 to 1150 mμ. Maximum photomorphogenesis occurred with wave lengths of 630 to 700 mμ.

Irradiances at 725 mμ of 150 to 450 μw./cm.^{2} are capable of inducing a strong photomorphogenic response with synthesis of only traces of chlorophyll. However, no combination of far red energy was found in this series of studies which induced a strong photomorphogenic response with no detectable chlorophyll. At 765 mμ, a weak photomorphogenic response was obtained with no measurable formation of chlorophyll.

Blue irradiance of 2 μw./cm.^{2} induced weak photomorphogenesis with a chlorophyll concentration about 1000-fold greater than that in plants under wave lengths of 725 mμ with an equal degree of development. From these data, it is concluded that there is no quantitative relationship between chlorophyll synthesis and the photomorphogenic responses.

Data are presented on the effect of 725 mμ irradiances at 0, 150, 450, and 1500 μw./cm.^{2} on dry weight distribution, stem lengths, and anthocyanin content in the bean plant, and fresh leaf weight, chlorophyll, protochlorophyll, and carotene in bean and corn. In both species, fresh leaf weight increased with increase in irradiance. Protochlorophyll and carotene synthesis were increased by low irradiances over the synthesis in the dark, but decreased with further increasing irradiances. All irradiation treatments used caused large increases in anthocyanin in bean hypocotyl. At 725 mμ the anthocyanin progressively increased with increase in irradiance. The synthesis of anthocyanin in bean and corn is dependent upon a photo-process which does not involve photosynthesis, as shown by marked formation of anthocyanin in the far red where photosynthesis did not occur.

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