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Photomorphogenesis in *Arabidopsis thaliana* (L.) Heynh

THRESHOLD INTENSITIES AND BLUE-FAR-RED SYNERGISM IN FLORAL INDUCTION[1,2]

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ABSTRACT

***Arabidopsis* seeds were germinated on sterile mineral agar supplemented with 1% glucose and cultured under continuous light regimes. With 4-hour incandescent plus 20-hour monochromatic illumination in the region from 400 to 485 nanometers there was effective floral induction at an intensity of 100 microwatts per square centimeter. Exclusion of far red wave lengths from the 4-hour incandescent period sharply reduced the effectiveness of subsequent monochromatic blue light in promoting floral induction. Delayed floral induction occurred under continuous incandescent light lacking far red and was attributable to the blue wave lengths. Continuous 485 nanometer (100 microwatts per square centimeter) exposure without any white light treatment during the postgermination growth period was ineffective in floral induction and meristem development. Light at 730 nanometers under the same conditions was partially effective, whereas energy between 500 and 700 nanometers was completely ineffective. When continuous monochromatic light at a 3-fold higher energy level was administered, all photomorphogenic responses were accomplished with 485 nanometer light, including germination and 100% floral induction without any white light treatment at any time during the experiment. Almost equal quantum effectiveness was calculated when equivalent quantum flux densities in the region from 710 to 740 nanometers or at 485 nanometers were used. It is postulated that floral induction in *Arabidopsis* may be the result of a continuous excitation of a stable form of far red-absorbing phytochrome localized in or on a membrane, and that excitation can be either by direct absorption of energy by far red-absorbing phytochrome or by transfer from an accessory pigment.**

The action spectrum of germination for *Arabidopsis* was obtained by Shropshire, Klein, and Elstad (18). However, for floral induction in this quantitative long day species, no such detailed action spectrum has been established. Meijer (13), reporting on floral induction, found a positive effect with a 16-hr photoperiod of blue and blue plus far red and a negative effect with 16 hr of red light. These results, although not in agreement with established red light-induced photomorphogenic responses, do agree with earlier work of Funke (7), who found that in many long day species of the Crucifereae, blue glass-filtered sunlight was equivalent to white light in floral induction, while red light in its effect simulated darkness. Blue light without detectable far red contamination was found to promote flowering in the long day plant *Hyoscyamus niger* by Wassink *et al.* (19), whereas red light, regarded as the predominating photoregulatory component in white light, was completely ineffective.

While a possible circadian rhythm of photophile and scotophile phases in which a given red/far red ratio alternately promotes or inhibits floral induction may reconcile some anomalies in experiments on photoinduction of flowering (4), no such photophile and scotophile phases could be detected by Hussey (11) in *Arabidopsis*. This species responds quantitatively to light in that it "accumulates" light until a threshold is reached that triggers the irreversible floral induction without regard for the periodicity with which successive fractions of the total required exposure are delivered. In *Arabidopsis thaliana* strain Estland flowering occurs most rapidly under continuous light.

With improved interference filter monochromators, continuous high spectral purity monochromatic light can be generated to meet the quantitative energy requirements originally established with continuous white light. *Arabidopsis thaliana* is particularly well suited to such studies because of its small size, genetic and phenotypic uniformity, rapid development, obligate photomorphogenetic light requirement, and ease of culture throughout its life cycle on aseptic agar media in which necessary photosynthates can be replaced by the addition of glucose.

Previous studies (14) have established the time sequence and dramatic nature of the meristematic changes at the onset of floral induction under continuous white light, thus providing parameters for relating induction stages under monochromatic light.

This report presents the spectral dependence of floral induction in a light regimen of 4 hr of white light followed by 20 hr of monochromatic light. The studies demonstrate no correlation between photocontrol of meristem activation and photosynthesis and photocontrol of floral induction. The contribution of incandescent white light to the response obtained with sequential blue light is shown to depend on the far red contribution in white light. The quantum effectiveness, the wave lengths, and approximate threshold intensities for floral induction under continuous monochromatic irradiation have been determined.

MATERIALS AND METHODS

Plant Material and Culture Media. Dry seeds of *Arabidopsis thaliana* (L.) Heynh. race Estland (12) were surface-sterilized

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by immersion in a solution of equal parts 95% ethanol and 3% hydrogen peroxide for 3 to 10 min and then sown singly with an inoculating loop on the surface of 4.5 ml of sterile agar media in 150- × 16-mm outside diameter culture tubes. The standard culture media contained 0.003 M KNO_3, 0.002 M $Ca(NO_3)_2$, 0.002 M NaH_2PO_4, and 0.0015 M $MgSO_4$.

Trace elements were prepared as 2 liters of a 400× concentrated stock solution chelated with 10.0 g of disodium EDTA and added (10 ml in 2 liters macro-element solution) to give final concentrations as follows (mg/liter): 12.06 $FeSO_4 \cdot 7H_2O$; 2.3 $MnSO_4 \cdot 4H_2O$; 0.24 $CuSO_4 \cdot 5H_2O$; 0.29 $ZnSO_4 \cdot 7H_2O$; 1.86 H_3BO_3; 0.03 ammonium molybdate. The mineral solution (pH 5.6) was solidified with 0.78% (w/v) of Oxoid Ionagar No. 2 (Consolidated Labs Inc., Chicago Heights, Ill.) and supplemented with 1% (w/v) anhydrous glucose.

Culture tubes were autoclaved after dispensing the culture solution and capped with Pyrex caps which permitted gas exchange while maintaining aseptic conditions. Tubes were supported in styrofoam blocks holding 40 tubes arranged within a radius of 3 inches. The tubes were transferred within 1 hr after sowing to dark cabinets at 4 C for a 48-hr imbibition period prior to commencement of a light regime. Germination and growth in all experiments were carried out at 24 C in a controlled environment room in which the white light, dark, and monochromatic growth cabinets were located. The monochromatic irradiation system consisted of eight interference-filter monochromator units mounted on wood cabinets as previously described (20). The following monochromatic sources were used for action spectra determinations: 365, 385, 400, 415, 420, 445, 455, 485, 500, 520, 550, 600, 660, 700, 710, 720, 730, 740, 750, 760, and 800 nm. In each unit the filament current of a 1500-w projection lamp could be monitored and the radiant flux varied by means of variable transformers. The interference filters (Baird Atomic Inc., Cambridge, Mass.) had transmission characteristics of ±5 nm half-band width about the designated wave band. The projected light passed through 10 cm of distilled water before reaching the filters. Where experiments were designed in which the far red component of the radiation treatment was to be removed, a $CuSO_4$ solution filter was used in place of the 10-cm path length of distilled water (22). Radiant energy at the level of the seedlings was measured by means of a calibrated thermopile (22) or with an ISCO spectroradiometer (Instrumental Specialities Inc., Lincoln, Neb.) equipped with a quartz fiber probe, so that total energy in the filter band pass could be calculated. The values obtained with the ISCO agreed within 10% with values obtained with the thermopile and also provided a further check on the spectral purity. From the measured irradiance expressed in μwatts/cm^2 at the peak wave length of an interference filter, the quantum flux density was calculated from the relation quantum/sec = $(1987 \times 10^{-23}/\text{Å})$ μwatts. These values were converted to pico-Einsteins (pE/cm^2/sec) using the value 6.025×10^{11} quanta/sec = pE/sec.

A standard experimental procedure was established in which all samples were given 4 hr of white light, followed by 20 hr of continuous monochromatic radiation. Continuous white light and 4 hr white light + 20 hr darkness were used as controls. All intensities were adjusted to 100 μw/cm^2. The 4 hr + 20 hr light regimen was followed each day. Observations were always made during the 4-hr white light exposure.

In experiments where the photomorphogenic effectiveness of solely monochromatic light was studied, after the 48-hr imbibition period, the seeds were exposed for 15 min to 100 μw/cm^2 of 660 nm light to induce germination, followed by 42 hr of darkness, after which time the continuous monochromatic exposure was begun. Irradiation was continued for 21 days at 100 μw/cm^2, and at the end of that time a final harvest was made.

Standard white light was provided in controlled environment cabinets with six 150-w reflector floodlamps in large cabinets or with one 300-w reflector floodlamp in smaller cabinets. The light in these cabinets passed through a water filter, and lamp heat was dissipated by a reflux coil (21). The height of plant growth level inside the cabinets was adjusted so that equal energy was available to plants in both large and small cabinets. Transfers of plants from white light to monochromatic light cabinets required only a few seconds.

Growth of plants in test tube culture under each light source was highly uniform. Floral induction was detected by daily dissection of plants sampled during each experimental run. Occurrence of induction was confirmed by the appearance of macroscopically visible buds and elongation of stems, changes observable without removing plants from light cabinets. Hypocotyl growth, number of leaves, primary leaf length, and stem length following induction were measured. Fresh and dry weights were measured at final harvests. Chlorophyll concentration was determined by the methods of Arnon (1). Photomicrographs of dissected apical meristems were made using bright and dark field illumination of freshly dissected whole mounts in water; representative vegetative and reproductive apices are shown in Figure 1.

For fresh seed stock used in all floral induction experiments, plants were grown to maturity under a white light source consisting of both fluorescent and incandescent lamps. Seeds were used within 3 months of harvest.

RESULTS

Observations were made on seedling development under continuous light, comparing normal plants with the albino mutant *im* (16) grown on 1% glucose. This mutant is essentially albino under white light. Its growth under these conditions is a useful index of the efficacy of glucose as a substitute for photosynthesis.

Normal plants in continuous white light produced primary leaves 3.6 mm in length within 8 days, whereas albino plants averaged 0.6 mm. Normal plants under 4 hr white light only had 0.1 mm leaf development. Both red and far red light activated germination, but neither activated meristem development to the extent that visible leaf primordia were produced in an 8-day growth period. Continuous 660 nm light did produce greening, cotyledon expansion, and hypocotyl elongation, while continuous far red light produced no greening but did suppress hypocotyl elongation. It appears, therefore, that the light requirement for germination is distinct from that of postgermination light-induced morphogenesis.

Germination responses to monochromatic light after 2 and 3 days of exposure is shown in Figure 2. Germination under the 4 hr + 20 hr regime after two light cycles is influenced by the different monochromatic wave lengths, but this differential is minimized after three light cycles. Figure 3 illustrates that hypocotyl elongation and leaf expansion measured after 11 cycles are likewise influenced by the quality of monochromatic light. The role of the monochromatic exposure in photosynthesis is reflected in leaf development.

Dissection of plants grown in continuous white light revealed primary bud primordia on the 8th day (Fig. 4) and the time of floral induction, based on the previous studies (14), was fixed at 7 days. Floral primordia were found at 12 days on dissection of 455 nm regimen plants; induction time was fixed at 11 days. Daily sample dissection continued until the first floral primordia were observed or until 32 days after sowing. Observations of postinduction growth, as shown in Figure 4,

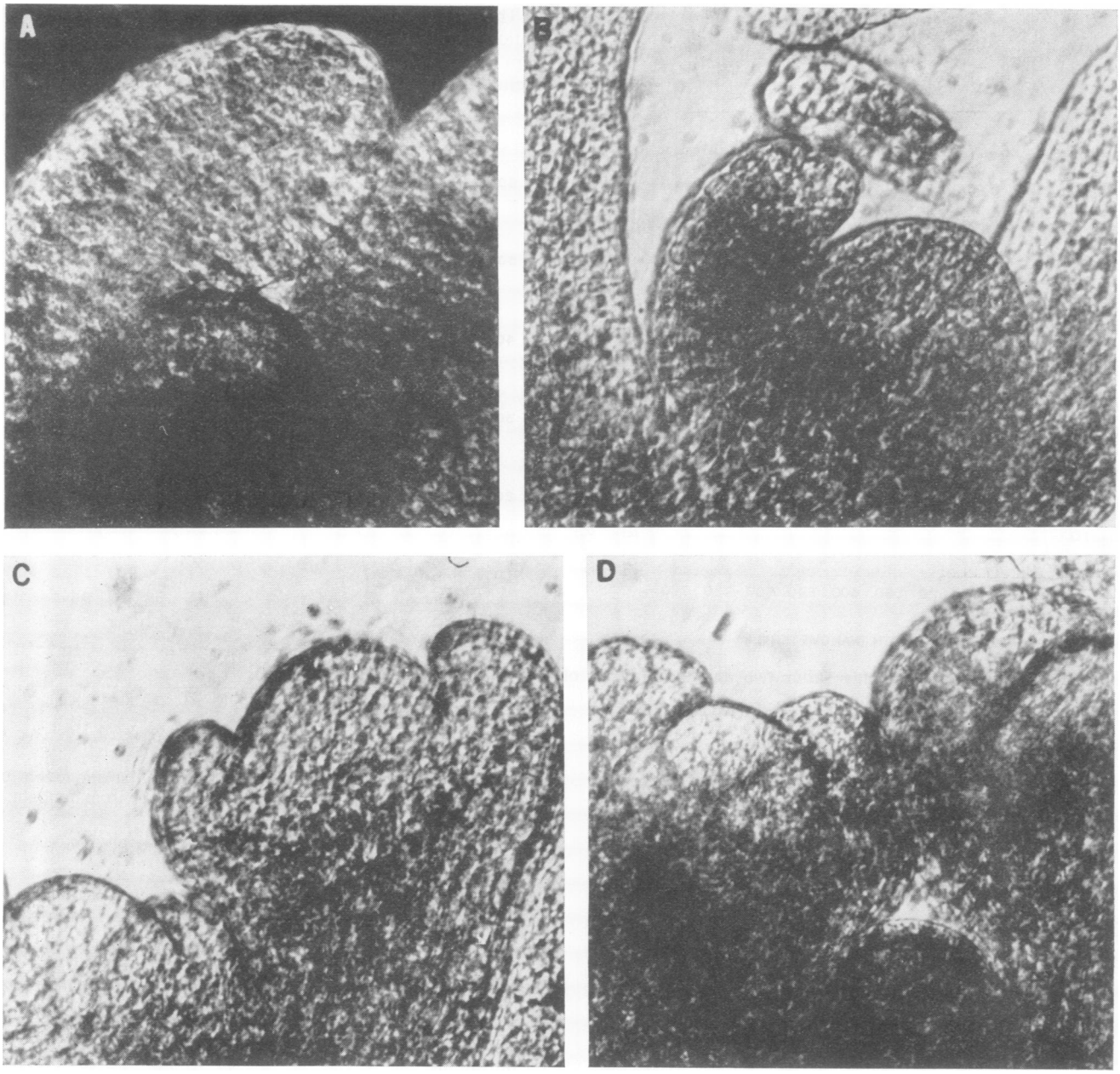

FIG. 1. Representative dissected *Arabidopsis* meristems at different developmental stages, wet mounts. × 1125. A: Phase contrast, vegetative meristem (shallow central dome); B: bright field, transitional to floral meristem (deeply convex central dome); C and D: stages in development of primary bud primordia from floral meristem.

were made on all remaining plants. After 32 days, a period encompassing the complete life cycle of plants grown in continuous white light, the experiment was terminated. The results are shown in Table I and Figure 4.

As shown in Table I, a 4-hr white light exposure followed by 20 hr of low intensity 660 nm light (100 μw/cm^2) did not produce flowers even though the plants were protosynthetically active, as indicated by the level of chlorophyll and vegetative development. However, the same light regimen employing either 455 or 730 nm energy did induce flowering. This indicates that there is no correlation between photosynthetic capacity and floral induction in *Arabadopsis*.

The responses to blue, far red, their interaction with and relationship to white light effects were further examined. Photomorphogenesis in blue wave lengths following 4 hr of white light with and without a far red component were compared (Table II). In addition to the blue wave length monochromators two additional units were used for far red wave lengths of 780 and 800 nm with distilled water filters to provide information on photomorphogenesis accomplished with photoconversion, as opposed to thermal (dark) reversion of Pfr[4] formed in the white light photoperiod. Monochromatic energies of 100 μw/cm^2 were possible, except at 365 to 385 nm, where the intensity was 18 μw/cm^2, and at 400 nm, where the intensity was 60 μw/cm^2. Lamps had to be replaced daily in the near ultraviolet wave lengths.

[4] Abbreviations: Pfr and Pr: far red- and red-absorbing forms of phytochrome, respectively.

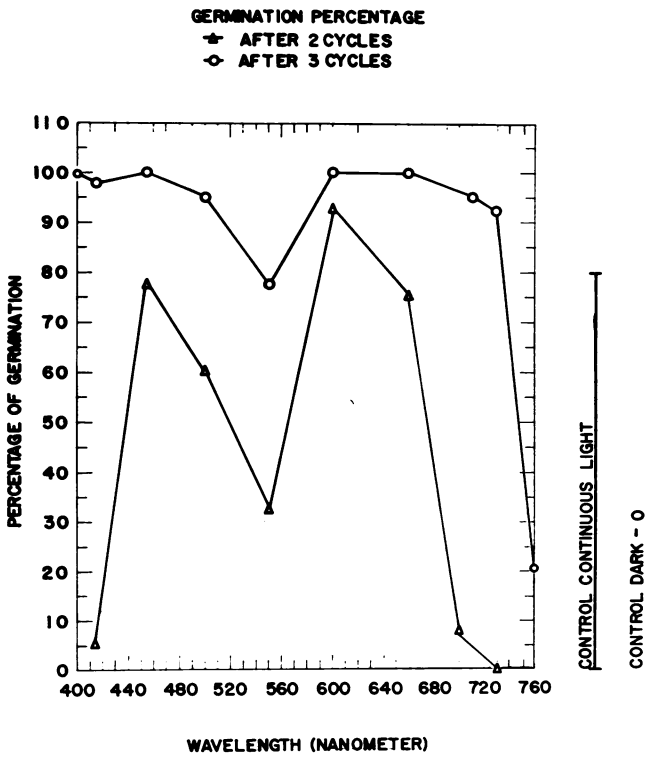

FIG. 2. Germination percentage after two and three cycles of 4 hr white and 20 hr monochromatic light.

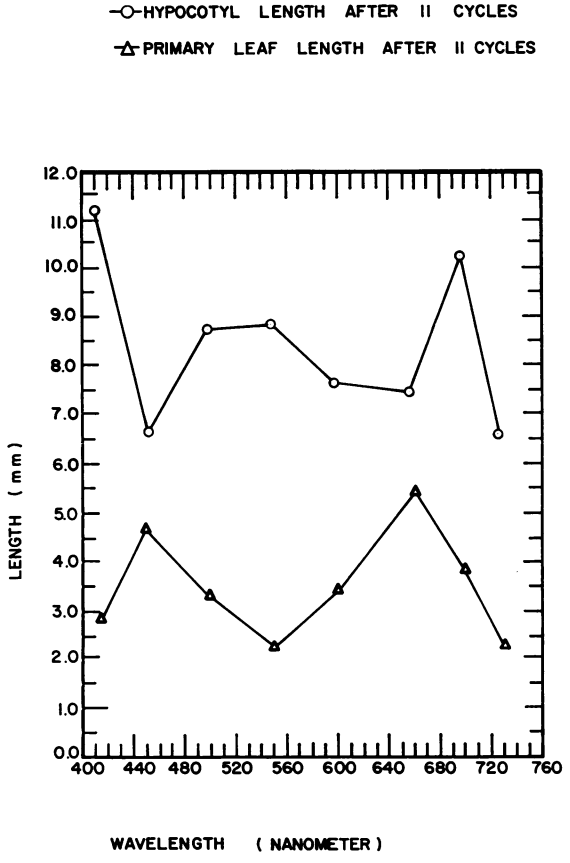

FIG. 3. Mean hypocotyl length and primary leaf length after 11 light cycles of 4 hr white and 20 hr monochromatic light.

The results of these light treatments produced 100% germination of seeds after three light cycles in all light regimens except those with 780 and 800 nm light. After seven light cycles, including the daily 4-hr white light treatment, only 27% of

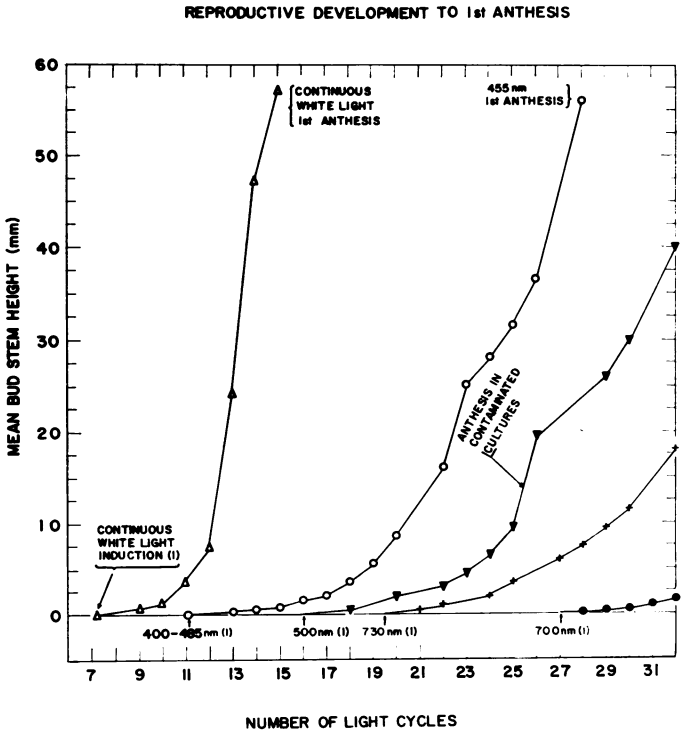

FIG. 4. Reproductive development from first induction under continuous white or 4 hr white and 20 hr monochromatic light.

Table I. *Morphogenesis under 4 hr White, 20 hr Monochromatic Light*

The incident energy of the monochromatic light was 100 μw/cm^2.

Light Treatment	Days to 1st Floral Primordia	Fresh Weight	No. of Rosette Leaves	Dry Weight	Chlorophyll Fresh Weight	
					ch *a*	ch *b*
		mg/plant[1]		*mg/plant*	*μg/100 mg*	
Continuous white	7	32.7	5.1	2.06	56	21
4 hr white 20 hr 455 nm	11	27.9	4.8	1.81	68	40
4 hr white 20 hr 500 nm	15	21.0	5.0	1.26	39	6
4 hr white 20 hr 550 nm	Nil at 32	20.4	9.2	1.57	54	14
4 hr white 20 hr 600 nm	Nil at 32	26.8	10.5	1.42	70	20
4 hr white 20 hr 660 nm	Nil at 32	32.5	11.3	1.72	47	33
4 hr white 20 hr 700 nm	27	25.7	8.2	1.67	49	14
4 hr white 20 hr 730 nm	20	12.4	4.5	0.59	19.6	15
4 hr white 20 hr dark	Nil at 32	9.1	4.2	0.45	51	16

[1] Fresh weight at 1st anthesis in continuous light, weights at harvest after 32 days in others.

Table II. *Effect of Blue and Near Ultraviolet Wave Lengths after 4 hr White Light with and without Far Red Components*

Observations were made after 22 days of continuous irradiation.

20 hr Monochromatic Light, Peak Wave Length	Incident Energy	4 hr White Light with 0.5% $CuSO_4$ Filter			4 hr White Light with H_2O Filter		
		Days to floral induction	Rosette leaves	1st leaf length	Days to floral induction	Rosette leaves	1st leaf length
	$\mu w/cm^2$			mm			mm
Continuous white light	3000	16	5.4	c.12	7	3.9	c.12
365 nm	18	...[1]	0	0.1	...	2.0	4.9
385 nm	18	...	2	0.1	...	2.6	5.2
400 nm	60	...	2.7	3.8	11	3.7	c.10
420 nm	100	...	2.1	2.4	12	3.5	c.10
445 nm	100	18	2.8	3.5	11	4.0	c.12
485 nm	100	18	3.6	5.5	11	4.4	c.12
V52 albino mutant continuous white light	3000	18	5.0	3.4	16	5.0	2.8
20 hr darkness		...	2	0.1	...	2.2	3.4

[1] Vegetative at 22 days.

these far red-irradiated seeds had germinated; dark germination was zero. These same ungerminated seeds gave 100% germination following transfer to continuous white light. The 27% of seeds which did germinate under the 780 and 800 nm regimes showed no significant postgermination development and appeared similar to seedlings kept in darkness, despite the fact that they received 4 hr of white light in each 24-hr cycle. When the far red irradiation schedule was replaced with white light after 20 days, seedlings developed expanded cotyledons and normal meristematic activity.

The details of development under light treatments with and without far red light, as shown in Table II, indicate that floral induction occurred within 7 days in continuous incandescent white light, but where incandescent light was filtered through the far red-absorbing 0.5% $CuSO_4$ filter, plants sampled were still vegetative. Monochromatic 20-hr blue light regimes in conjunction with 4 hr of incandescent light passing through distilled water only, showed bud primordia at 12 days, corresponding to induction at 11 days. The 400 nm wave band with only 60 $\mu w/cm^2$ energy was as effective as the other blue wave bands where the monochromatic energy was 100 $\mu w/cm^2$. The 365 and 385 nm wave bands (18 $\mu w/cm^2$), though showing an increment of growth above that of 20 hr darkness, did not induce any primordia even after 22 days. When the monochromatic blue wave bands were alternated with 4 hr white light from which the far red component had been removed by the 0.5% $CuSO_4$ filter, floral induction required 18 days and occurred only at the 445 nm and 485 nm stations. The albino plants, although completely devoid of chlorophyll, flowered in 16 to 18 days in the absence or presence of the far red component of the white light treatment.

Table III presents results of seedling development with solely monochromatic light except for a brief exposure (15 min) to 660 nm for inducing germination. Seedlings grown in continuous 485 and 500 nm light, though possessing chlorophyll, normal cotyledons, and reduced hypocotyls, had a very restricted development compared to the previous cultures grown in 4-hr white, 20-hr monochromatic light cycles (Tables I and II), and no seedlings became reproductive under continuous 485 to 660 nm irradiation at 100 $\mu w/cm^2$. Vigorous vegetative development but little hypocotyl suppression occurred at 660 nm.

A test of higher photon flux densities revealed that monochromatic light at higher energy levels would induce germination without white or red light pretreatments (Table IV). One hundred per cent germination occurred under the 485, 660, 730, and 720 nm illumination, but extreme differences in the photoblastic and photomorphogenic effectiveness of far red illumination occurred within 20 nm on either side of 730 nm wave length.

This 3-fold increase in the intensity at 485 nm also raised floral induction from 0 to 100%, and it occurred in the same time as when 4 hr of incandescent white light was alternated with 20 hr of monochromatic light. Similarly, the 3-fold increase at 730 nm increased the percentage of activated meri-

Table III. *Photomorphogenesis under Continuous Monochromatic Light at 100 $\mu w/cm^2$ for 21 Days*

All samples were exposed to red light pretreatment to give 100% germination. Parentheses indicate vegetative plants.

Wave Length	Flowering	Days to Floral Induction	Fresh Weight	Rosette Leaf Number	Length of 1st Leaf	Hypocotyl Length	Bud Stem Height
nm	%		mg		mm		
485	0	...	(0.99	2	1.8	8.0	...)
500	0	...	(1.10	2.1	1.5	13.0	...)
520	0	...	(0.90	Nil	0.1	14.6	...)
550	0	...	(0.90	Nil	0.1	13.0	...)
660	0	...	(7.75	6.6	10.0	14.2	...)
700	35	19	5.30	5.2	12.0	13.8	2.0
			(5.0	5.8	12.0	12.0	...)
710	48	11	6.0	4.0	8.0	9.2	7.2
			(1.4	2.0	1.0	7.0	...)
730	65	11	5.9	3.8	7.0	6.5	8.0
			(2.2	2.4	1.4	5.0	...)
Dark	0	...	(0.7	Nil	Nil	16.7	...)
White light	100	6	18.0[1]	4.0	10.0	4.5	57.0[1]

[1] Measured at anthesis.

Table IV. *Photomorphogenesis under Continuous High Intensity Monochromatic Light for 22 Days*

No light pretreatment for germination induction. Parentheses indicate vegetative plants.

Wave Length	Incident Energy	Germination (No Light Induction Treatment)	Flowering	Days to Floral Induction	Fresh Weight	Rosette Leaf Number	1st Leaf Length	Hypocotyl Length	Bud Stem Height
nm	$\mu w/cm^2$	%	%		mg		mm		
485	299	100	100	12	11.6	3.9	7.6	5.8	25.3
660	198	100	0	23+	(11.7	7.2	12.5	13.7	...)
700	182	36	0	23+	(9.3	5.3	12.3	11.6	...)
710	190	63	64	13	9.2	4.0	7.6	8.0	7.3
					(1.3	2.0	0.5	8.5	...)
720	183	100	89	13	6.3	4.2	6.3	5.0	3.5
					(5.0	4.0	5.0	4.0	...)
730	190	100	100	13	5.8	4.0	6.1	5.5	5.2
740	200	95	80	13	6.5	4.5	10.6	7.0	3.0
					(2.9	4.0	3.5	6.0	...)
750	122	66	25	21	4.5	4.0	17.0	9.0	2.0
					(0.6	...	0.1	7.1	...)

Table V. *Total Quantum Requirement to First Floral Induction Observed under Continuous Monochromatic Irradiation, Commencing with Irradiation of Imbibed Seeds*

Wave Length	Quantum Flux	Days to Induction	Total Quanta
nm	$\mu E \cdot cm^{-2} \cdot sec^{-1}$		$E \times 10^{-3} \cdot cm^{-2}$
485	1210	12	1.254
660	1100	>23	>2.18
700	1063	>23	>2.11
710	1126	13	1.264
720	1100	13	1.235
730	1157	13	1.299
740	1234	13	1.386
750	764	21	1.386

stems and floral induction from 65% to 100%. However, no increased effectiveness, but rather a complete suppression, of floral induction occurred when the flux of continuous 700 nm light was doubled. The complete ineffectiveness of 660 nm light in floral induction was also maintained at a doubled flux density, even though vegetative growth was enhanced.

The total quanta required for occurrence of first observed floral induction were calculated and are shown in Table V. As indicated, there is a remarkable similarity in quantum effectiveness of the blue and far red wave lengths.

DISCUSSION

An explanation of the results in terms of phytochrome may be attempted if it is postulated that continuous red irradiation results in depletion and eventual elimination of the phytochrome pool because of the photolabile nature of Pfr (3), so that photoinductive control of morphogenesis is lost owing to destruction of photoregulatory phytochrome during seedling development. In white light, because of the greater quantum efficiency of red light photoconversion, phytochrome is predominantly in the labile Pfr form. However, the influence of the far red component of the incandescent light should conserve phytochrome by providing for continuous recycling back to the stable Pr form.

The results of experiments using white light treatments with and without a far red component (Table II) support the concept of phytochrome control derived from spectrophotometric data (2), namely that far red irradiation at 760 nm and beyond is inactive in photomorphogenesis. At these wave lengths, total photoconversion of phytochrome to inactive Pr occurs. Despite the Pr:Pfr photoequilibrium established in the sequential 4-hr white light periods, evidently insufficient product of Pfr action accumulates to activate germination. This contrasts with the photomorphogenic effectiveness of 730 nm light and suggests that interpretation of far red irradiation effects can be made only when the far red exposure is of a known and narrow wave band.

The close agreement in time of floral induction at 445 and 485 nm following 4 hr of white light without far red light to induction in continuous white light without far red light indicates that the blue region of the spectrum controlled this photoresponse (Table II). The striking decrease in the irradiation time required when far red light was present during the white light exposure demonstrates the synergistic effect of blue light with far red light. It should also be noted that the photosynthetic pigments are not essential for photomorphogenesis, as evidenced by the fact that the albino mutant, totally devoid of chlorophyll, could be cultured to flower bud formation in 16 days.

It is possible that blue light, which has been shown to stimulate RNA and protein metabolism (15), may influence the synthesis *de novo* of phytochrome. Blue monochromatic light following a 4-hr photoperiod would be relatively ineffective as compared to white light. Nevertheless, it would be sufficient to maintain a low Pfr concentration in an excited state and thus maintain an effective level of Pfr, as proposed by Hartmann (10).

The very low but positive effect of 700 nm light, following a 4-hr white light treatment, on floral induction is more difficult to explain (Table I). Absorption of phytochrome is much higher at 700 nm than in the blue region, and a Pfr:Pr balance should be maintained by cycling. The low efficiency in floral induction under 4-hr of white light plus 20 hr at 700 nm may result from an inhibitory or "supercritical" concentration of Pfr which is slowly reduced to the critical, as would occur more rapidly under continuous white light. The concept of a supercritical level of Pfr gains support from the experiments of Hartmann (10). The effectiveness of continuous white light, as compared to 700 nm, required postulating a greater photodestruction in white light to bring Pfr levels down below the critical concentration, despite the preponderance of Pfr in white light.

The sharp drop in effectiveness in the blue-green region, where the steady state ratio of Pfr:Pr and the absorption efficiency should not differ appreciably, casts some doubt on phytochrome as the primary photoreceptor in this region. Absorption spectra and the calculated action spectra for photoconversion, determined by Butler *et al.* (2) on isolated purified phytochrome, are near minimal at these wave lengths and do not show sufficient differences between wave lengths from 455 to 520 nm to account for extreme differences in the effectiveness in this region.

At a sufficiently high radiant flux, all photomorphogenic responses from germination to flowering can be accomplished by 485 nm light, as well as by 730 nm light, with equal quantum effectiveness (Tables IV, V). Neither of these wave lengths should appreciably change the seed phytochrome content in the direction of increasing the concentration of Pfr, and neither wave length would be efficiently absorbed by inactive "dark phytochrome" Pr (absorbance maximum, 660 nm). Neither blue nor far red light alone is as effective as white light, yet it is evident that the red, yellow, and green regions of the spectrum are ineffective.

It is to be noted that at higher intensities, induction does occur at both 740 and 750 nm with equal effectiveness (Table IV). The floral meristems at 750 nm are probably a result of the photomorphogenic effect of 740 nm and lower wave length flux passing through the filter rather than to the nominal 750 nm peak wave length. If the Hartmann hypothesis (9, 10) that Pfr is the photoreceptor for the far red effect is accepted, then one would expect the 750 nm wave length to be completely ineffective. However, at high flux densities, the overlap between half-band widths of the filters prevents precise resolution of wave lengths. Nevertheless, effectiveness of 710 nm and ineffectiveness of 700 nm light is evident and in agreement with the Hartmann hypothesis.

It remains possible that the controlling molecule is phytochrome, and that blue light excites a flavin or carotenoid photoreceptor which can transfer excitation energy to phytochrome, thus preventing thermal reversion of Pfr to Pr and maintaining a critical concentration of excited Pfr.

The evident synergism between the blue and far red regions in white light and the nature of the action spectrum in the blue region strongly suggest that, analogously to the two-pigment systems of photosynthesis, there are two photoreceptors for photomorphogenesis which are coupled to interact synergistically. In this view, it is postulated that the blue region photoreceptor is a flavin or carotenoid pigment able to transfer excitation energy to phytochrome. Phytochrome is the "effec-

tor" chromoprotein, but only a small amount of the total constitutes the "photomorphogenic reaction center," this being "stable Pfr" which has been hypothesized to be from 1 to 3% of the total phytochrome. This stable Pfr may not revert to Pr even in the dark, but its effectiveness in the "high energy responses" of photomorphogenesis depends on its receiving excitation energy, either directly by absorption of 710 to 740 nm quanta, or indirectly by resonance transfer from the "blue region photoreceptor." In accord with recent evidence and views (5, 8), stable Pfr and its flavin or carotenoid accessory pigment system are probably integrated in membrane structures. The permeability of membranes could then be changed when stable Pfr phytochrome is energized by light. Normally sequestered reactants could then interact and their products accumulate, but only while the photoperiod continued. The time, intensity, and wave length dependence, and the quantitative long day characteristic of floral induction in *Arabidopsis* and other species (6) which respond to far red light can be interpreted in terms of this model. Other photomorphogenic responses (17) have also been explained in terms of two photoreceptors.

The conclusion of Clarkson and Hillman (3), following their observation in *Pisum* of "apparent synthesis" of phytochrome following depletion in continuous red light, that phytochrome levels under continuous red light should be adequate for all photomorphogenesis, clearly cannot be applied to these results obtained with *Arabidopsis*. Nevertheless, the distinction made by Funke (7) between groups of plants where red light was as effective as white light in floral induction and the group of plants, predominantly members of the Crucifereae (as is *Arabidopsis*), in which red light was ineffective is significant. The distinction might lie in the protection of phytochrome from red light-sensitized "photodestruction" in some species but not in others.

Our results do not indicate that leaf area or photosynthetic capacity influences floral induction. The possibility that the meristem cells themselves are adequate receptors for the photomorphogenic light should be considered.

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