Differences between Effects of Spectral and Fluorescent or Filtered Monochromatic Lights on Inducing Photoperiodic Sensitivity of *Pharbitis nil*

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1) Red light irradiated for a relatively short time to *Pharbitis* plants grown under continuous green, blue, or far-red lights and in darkness just before a 16 hr dark treatment always initiated flowering induction, but far-red light given in the same way to red light grown plants inhibited flowering induction.

2) The effectiveness of red light given to plants grown under green light before a 16 hr dark treatment to induce photoperiodic sensitivity was compared between fluorescent light obtained from colored fluorescent lamps and spectral light beams from a grating spectrograph.

3) The effectiveness in inhibiting flowering induction of far-red light, obtained through an IR-1 filter and radiant heat cut glass from incandescent lamps, was also compared with that of spectral light beams from the far-red region by irradiating to plants grown under red light for a short time before the dark period.

4) Total energy values required for inducing 100% flowering by red light were about 37×10^6 erg/cm² and 8.9×10^6 erg/cm² in fluorescent and spectral light, respectively. Those required for complete inhibition of flowering were 8.6×10^6 erg/cm² and 9.9×10^4 in filtered far-red light and spectral light, respectively.

The present experiment indicates that the total energy required for the same photoresponse are apt to be larger in colored fluorescent lights or filtered lights than in spectral light beams. This fact must be considered in photobiological experiments using monochromatic light other than spectral light beams.

5) The regions effective for flowering induction are roughly the three regions with wavelengths of about 550–600 nm, 620–640 nm and 650–675 nm, and those for inhibition of flowering induction range from about 710 nm to 770 nm.

6) Experimental conditions for light and temperature were examined in detail. Reasons for the difference in total energy values between fluorescent light and spectral light were discussed.

Endogeneous rhythm, the "biological clock", and phytochrome pigments in tide, based on the reversible effects of red and far-red lights on photoperiodic induction, have been reported as the mechanism for inducing photoperiodism in longday and shortday plants.¹⁻³⁾ There are few reports about the occurrence of pigments taking part in photoperiodic induction. If any pigment takes part it is surely the phytochrome, though it is not known whether it makes up all or only a part of such pigments. I

have begun studies on pigments in relation to the effects of light—light quality and quantity—on their occurrence. In preliminary experiments using monochromatic light from pure colored fluorescent lamps, light conditions inducing and inhibiting flower formation in which a relatively short time of irradiation of red and far-red lights just before the effective dark treatment have been found. This made experiments using spectral beams from a spectroirradiater (biological spectrograph) possible or much

easier, because a very long spectral beam irradiation is too expensive. A paper reporting detailed results on the occurrence of the pigments responsible for photoperiodic induction by monochromatic light is in preparation.

In the present paper differences between the effects of spectral beams and monochromatic fluorescent lights on inducing photoperiodic sensitivity in *Pharbitis nil*, i.e. a much shorter irradiation or a smaller spectral beam energy in wavelength from the red region, as compared to red fluorescent light, can induce flower formation.

The significance of the purity or wavelength width of monochromatic light from a grating spectroirradiator has been reported by MATSUI *et al.* in the process for protochlorophyllide phototransformation *in vivo*.⁴⁾ Flower formation in *Pharbitis* plants under monochromatic light conditions was reported by TAKIMOTO and IKEDA.⁵⁾ In the present study a similar experiment made under purer monochromatic light conditions with a more strict distribution of wavelength and energy.

MATERIAL AND METHODS

The plant material. A typical shortday plant *Pharbitis nil* Chois, strain "Violet" was used for its following characters: Its cotyledons receive photoperiodic induction.⁶⁾ It forms floral buds under complete dark conditions, but once light is received its flower formation requires effective short conditions⁷⁾ and its photoperiodic sensitivity increases from zero at germination to a maximum degree 3 or 4 days after germination.⁸⁾

To obtain uniform germination, seeds were germinated using the procedure previously reported.⁹⁾ Seeds which had rooted for several millimeters were planted in vermicurite about 1.5 cm deep in two files of 5 seeds each, in plastic pots 15×10×15 cm deep. Plants were grown by irrigating them with 0.1% hyponex solution under continuous blue, green, red and far-red light conditions in incubators, as well as with continuous dark and white light conditions at about 25°C. After 3 or 4 days, cotyledons of plants grown under continuous white

light had completely expanded and had reached their most sensitive phase. All plants, except those grown under red light, were then subjected to red light irradiation of various durations followed by one 16 hr dark treatment after which they were placed under continuous white light conditions for about 3 weeks. Lengths of hypocotyls and petiols were measured and the number of floral buds and nodes were examined under a binocular microscope.

In experiments on the inhibition of floral induction, plants grown under continuous red light were exposed to far-red light of various durations just before the 16 hr dark treatment.

To irradiate plants with spectral beams from a grating spectroirradiator, the plastic pots were arranged on an irradiation stage after growing the plants under continuous green light for 3 or 4 days, during which each file with 5 plants was subjected to a separate beam of a particular wavelength. Wavelengths of the beams irradiated to each file was marked, after removing the pots, as previously reported.¹⁰⁾ All operations before and after spectrum irradiation were performed under dim green light.

Temperature for the growth and phototreatments, in principle, was 25°C, except 23±1°C under white light, but the actual temperature of each light condition differed somewhat due to the colored light sources.

Fig. 1 Wavelength distribution for the lights used. Measured by Iio's spectroradiometer at distances of 100 cm in White, 20 cm in Blue, Green, Red and Far-red from lamps.

Table 1 Distribution of light intensities in lux (in kerg/cm²/sec) in the incubators illuminated by red, blue, green and far-red light.

Location of measurement*	Blue		Green		Red		Far-red
	Middle (ca. 25 cm below lamps)	Base (ca. 40 cm below lamps)	Middle	Base	Middle	Base	Base
A	280	250 (6.3)	360	280 (9.7)	410	360 (4.2)	(5.6)
B	340	260 (6.6)	360	280 (9.0)	370	340 (4.2)	(4.2)
C	430	360 (8.3)	520	360 (10.1)	680	460 (5.2)	(9.0)
D	275	240 (6.0)	300	230 (8.5)	410	350 (4.4)	(4.8)
E	345	250 (6.3)	280	240 (8.8)	340	310 (3.5)	(3.8)

* Location of measurement in an incubator:

Light conditions. Blue, green, and red monochromatic lights were illuminated through 2 plates of the ceiling of incubators to the inside from eight 20W pure colored fluorescent lamps (National). Far-red light was obtained by passing incandescent light from 100W and 60W lamps through an infrared filter IR-1 (NEC) and heat rays cut glass (Oota Glass Manuf. Co.). White light irradiation was done in an artificial light room furnished with eighteen 400W metal halid lamps (Yōkō lamp, Toshiba) and twenty four 40W white fluorescent lamps (White 40H FLR W/A, Toshiba) in the ceiling which were isolated from the room with transparent plastic plates and separately air-conditioned. Illumination at plants level was about 15,000–25,000 lx.

Distributions of the wavelengths of these light, measured with a spectroradiometer (Model SRP, Iio Electric Co.), are shown in Fig. 1. Light intensity and illumination were measured with a compensated thermopile (Kipp & Zonen, CA 1) and a lux-meter (Photocell illuminometer type SPI-5, Toshiba), respectively. Values for each incubator showed fluctuations due to location both vertically and horizontally as seen in Table 1.

To change the intensity of the red fluorescent light, box-shaped black mosquito nets in one to four piles were used to cover the plastic pots. In this case, the thermopile was not very useful for measuring light intensity under the nets due to a warp in the real value from radiant heat reflected from the black nets.

Methods of making the intensity distribution

of the spectrum uniform. Irradiation with purer monochromatic light was done with spectral beams from a grating spectroirradiater (JASCO, CRM-FB) using a short-arc xenon lamp (6.5 kW) as the light source, to determine the action spectrum. Purity of the monochromatic light flux, methods for measuring the intensity *etc.*, as well as the structure of the biological spectrograph have been reported elsewhere.^(10,11) To simultaneously irradiate each specimen arranged on the stage with monochromatic light beams of the same intensity, a technique to make the intensity distribution of the spectrum on the irradiation stage uniform was devised. Strips of black cardboard, 2 to 20 mm wide, were arranged at adequate intervals on the front glass of the spectroirradiater to

Fig. 2 Energy distribution of original (upper) and uniformed spectrum in wavelength region used.

Table 2 Effects of red light given before a 16 hr dark period on flowering induction of *Pharbitis* seedlings.

Light conditions before 16 ^h D	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
D	8	0	0	0
D—1 ^h R	9	0	0	0
D—2 ^h R	9	11.1	0.1	0
D—4 ^h R	8	12.5	0.3	0
D—8 ^h R	7	28.6	0.3	0
B	10	0	0	0
B—1 ^h R	10	20	0.3	0
B—2 ^h R	10	10	0.2	0
B—4 ^h R	10	10	0.2	0
B—8 ^h R	10	30	0.6	0
G	10	10	0.1	0
G—1 ^h R	10	40	0.6	0
G—2 ^h R	8	62.5	1.4	0
G—4 ^h R	9	77.8	1.6	0
G—8 ^h R	9	100	3.0	10
FR	5	0	0	0
FR—1 ^h R	8	25	0.4	0
FR—2 ^h R	6	50	1.3	0
FR—4 ^h R	9	55.6	1.4	11.1
FR—8 ^h R	10	90	4.3	60
R	10	100	5.6	100
W	10	100	5.4	100

B, G, R, FR, and D: blue, green, red, far-red light, and darkness, respectively.

shade the light beams on their path. The resultant distribution of light intensity is shown in Fig.2. Uniform intensity was 825 erg/cm²/sec.

RESULTS

Floral induction by red light given just before a 16 hr dark period

Among plants grown from germination under continuous monochromatic blue, green, red and far-red light, only plants grown under red light could always differentiated flower buds with one effective dark treatment, the rest were able to very seldom (see Table 2). But even plants grown under blue, green and far-red light, and in darkness induced flower formation when subjected to red light irradiation for adequate durations just before a 16 hr dark period, as seen in Table 2. With an increase in the

red light irradiation period, both the numbers of plants having flower buds and a terminal flower bud, and the number of flower buds per plant increased. As to the light conditions preceding red light, green and far-red lights induced the same 100 percent flowering as did continuous red or white light with an 8 hr irradiation period; although far-red light by far induced many more terminal buds than did green light. As the energy of red light was 3690—4350 erg/cm²/sec, the minimum total energy of red light required to induce 100 percent flowering calculated from the data was $106 \times 10^6 - 125 \times 10^6$ erg/cm². But the duration of the red light irradiation required for flowering induction tended to shift in every time period of the experiment, resulting in fluctuation of the total energy.

Relations between light intensity or illumina-

Table 3 Effects of the intensity of red light given for 8 hr before a 16 hr dark period on the flowering response of *Pharbitis* seedlings.

Red light intensity		No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
erg/cm ² ·sec	lux				
3600	340	10	100	5.3	100
2245	160	10	100	4.7	50
1720	80	10	100	3.4	40
1270	40	10	80	1.4	0
980	30	10	0	0	0

tion with red light and flower formation

In flowering induction by red light irradiation, both the total energy and the light intensity of illumination must be considered. Table 3 shows results of flowering induction in plants grown under continuous green light with 8 hrs' illumination of red light using varied intensities or illuminations given just before a 16 hr dark period. The intensity was reduced by using black mosquito nets in piles of from one to four as previously described. With decreasing intensity, both the number of plants with flower buds and the number of flower buds per plant decreased to zero below 980 erg/cm²/sec or 30 lx. Thus total energy of more than 28×10^6 erg/cm² for flower initiation and a value between 37×10^6 and 50×10^6 erg/cm² for 100 percent flowering may be required.

Flowering induction by spectral beams

Plastic pots with plants grown under continuous green light were arranged on the irradiation stage so that 5 plants in a line would be parallel with the monochromatic light beams. Two cotyledons from each of 5 plants were arranged in a line. Then plants in lines were simultaneously irradiated with monochromatic light beams having wave length range of ± 3 nm of the same intensity, from about 500 nm to 700 nm for one, two and three hours. Results are shown in Fig. 3. The spectral regions effective for flowering induction are roughly the three regions with wavelengths of about 550–600 nm, 620–640 nm and 650–675 nm, though some shift in the regions was seen with irradiation times. The reasons for the shift may be that the positions arranged on the stage were not exactly the same in each

Fig. 3 Flowering induction by spectral light beams.

..... one hour irradiation, — two hours' irradiation, --- three hours' irradiation. Short thick lines show location of plants wavelength regions.

experiment, and that the wavelengths with which the cotyledons were irradiated also differed in width, of at 6 nm, because cotyledons were about 3 cm wide. In the first and last regions the percent of plants with flower buds increased with irradiation times, from 20 to 60 and 100%, respectively; whereas in the middle region of 620–640 nm, they increased with irradiation for 2 hr but decreased for 3 hr.

From the results, the total energies for flower initiation and for 100 percent flowering were calculated as about 3×10^6 erg/cm² and 8.9×10^6 erg/cm², respectively, which are very smaller in comparison to those for the preceding experiments using fluorescent

Table 4 Effects of red light given just before a 16 hr dark period on the flowering response of *Pharbitis* seedlings.

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
R-10'FR-16 ^h D	13	30.8	0.5	0
R-30'FR-16 ^h D	10	0	0	0
R-1 ^h FR-16 ^h D	10	0	0	0
R-2 ^h FR-16 ^h D	10	0	0	0

Table 5 Inhibitory effects of spectrum light beams on flowering induction of *Pharbitis* seedlings grown under continuous red light.

Intensity & total energy	Wave length Scale	647		675		710		730		750		nm	
		135	140	150	160	170	180	190	200				
825 erg.cm ⁻² .sec x ca. 37 min (1831500 erg.cm ⁻²)	No. of plants dissected	9		10		9		10		9		10	
	No. of plants w. flower buds	9		10		9		5		7		6	
	%	100		100		100		50		77.8		60	
	No. of flower buds per plant	3.9		1.9		2.4		0.9		1.3		1.1	
	No. of plants w. terminal fl. bud	4		0		1		0		0		0	
825 erg.cm ⁻² .sec x ca. 20 min (99000 erg.cm ⁻²)	No. of plants dissected			4	5	5	5	5	5	5	5	5	
	No. of plants w. flower buds			4	5	5	5	3	1	0	0	1	1
	%			100	100	100	100	60	20	0	0	20	20
	No. of flower buds per plant			4.5	4.2	1.8	2.6	1.2	0.2	0	0	0.2	0.2
	No. of plants w. terminal fl. bud			1	2	0	0	0	0	0	0	0	0

lights. In similar experiments giving short time irradiations for less than one hour, no flower formation occurred.

Inhibition of flowering induction by far-red light

Plants grown under continuous red light always induce almost 100 percent flowering with one 16 hr dark period. When plants grown under continuous red light were irradiated with far-red light for 10 min to 2 hr, flower formation was greatly inhibited. About 60% inhibition occurred with a 10 min irradiation and complete inhibition with only 30 min of irradiation (Table 4). The total energy for complete inhibition of flow-

ering was determined to be about 8.6×10^6 erg/cm².

As seen in Table 5, results of experiments using spectral light from the red and far-red regions with the same intensities indicate that the effective region for inhibition of flowering induction ranges from about 710 nm to 770 nm. Complete inhibition of flowering induction was seen in a shorter irradiation for 20 min, but in the 37 min irradiation. This contradiction will be discussed later. The result of the 20 min irradiation experiment showed that the total energy of the effective wavelength light beams for complete inhibition of flowering induction is nearly 99,000 erg/cm². This

value is also much lower than that calculated from the above results for far-red through an IR-1 filter from incandescent lamps.

DISCUSSION

In these experiments, all environmental conditions (*i. e.* light and temperature) except humidity, were controlled or measured as strictly as possible. The temperature of each incubator, taken with a thermometer protected from direct light, was adjusted to a constant 25°C. But the temperature actually received by the plants was higher than 25°C due to radiant heat from the fluorescent lamps. This temperature differed considerably depending on the light quality or the color of the light. The highest radiant heat from the colored lights came from green probably due to high temperatures of the lamp tubes, themselves. Intensity values for fluorescent monochromatic lights, measured with a thermopile can not be said to be the real values because those values were also influenced by radiant heat. As the light intensity in an incubator changed, *e. g.* when the plant height was changed about 15 cm, illumination intensity from green and red lights changed about 220 lux at the tip, so plants received a changing intensity while they were grown from germination to the time of the dark treatment. But with red or far-red light given before the dark period, a change in intensity was out of the question because of the short irradiation time.

Dark treatment was given simultaneously to all plants in the experimental lots, when they had grown under white light to their most sensitive phase. But it was not clear whether the sensitivity of plants grown under the other light conditions was higher or not. Because the growth states at the dark treatment, *i. e.* height, presence of the hook, the expansion rate and area of cotyledons, and degree of greening, differed depending on light conditions, these details will be reported elsewhere. The growth states of plants grown under red light, appeared to be similar to growth states under white light. Some plants grown, especially under green and far-red lights or in darkness occa-

sionally withered during the experiment, after their removal to white light conditions.

Photoperiodic sensitivity was apt to shift somewhat in each experimental period. This maybe due to the difference in growth state and accordingly the sensitivities of plants at the moment of dark treatment were not always uniform. Results shown in Table 5, in which a smaller total energy completely inhibited flowering induction, while a larger one in another experiment did not, may be explained by the preceding statements.

Thus, to decide the absolute value of the total energy which induces photoperiodic sensitivity or which inhibits photoperiodic induction is very difficult. Moreover, the measured value for light intensity does not always indicate the real value of pure light as stated. With the present techniques, a method for separately measuring light intensity and radiant heat was also very hard to find. Compared with light values from fluorescent lamps, the intensity values of spectral light beams must contain less radiant heat, because the light source lies at a greater distance. Therefore, values for the total energy required for the same photoresponse must inevitably be measured as larger values in colored fluorescent lights or filtered lights than in spectral beams. Thus, in the present experiment, a comparison of the total energies for fluorescent light and spectral light might not be a true one. The present experiment, however, indicates that the total energy required for a photoresponse is apt to be larger than the real value, which must be considered in photobiological experiments using monochromatic light other than spectral light beams.

Other causes for the difference in total energy values between the two kinds of light must exist, as the value of fluorescent light is much larger than that expected from the addition of radiant heat. The wavelength distribution in red fluorescent light ranges from about 610 nm to 710 nm (Fig. 1), corresponding to the spectrum light used, in which no inhibitory wavelength region is included. MATSUI and his colleagues reported that purer monochromatic light (narrower half-wavelength width light) in the effective region was very effective in the

phototransformation of protochlorophyllide. So the difference may result from the greater effectiveness of irradiation with purer monochromatic spectral light which better induces photoperiodic sensitivity. The same is true for the inhibition of flowering induction by far-red light. Causes of differences in total energy values, however, are yet to clearly determined. Some possible causes are as follows: Since the limiting sensitivity of the spectrophotometer used is roughly 10^{-5} erg/cm²/sec, a stray flux of lower intensity may possibly be included in the fluorescent red light and the far-red light, which is not measurable by the photometer and some of which might possibly reduce the effectiveness of the main lights. The far-red light obtained from incandescent light through the filters possibly includes light from a wavelength region above 800 nm, because the penetrating power of the radiant heat cut glass is 20.8%, 2.8%, and 0.6% at 800 nm, 900 nm and 1,000 nm, respectively. The action of light from a longer wavelength region is not known to be either promotive or inhibitory. The interaction, an oppositereaction among the constituent wavelength lights of the fluorescent monochromatic lights and far-red light used also might account for the differences.

Due to the possibility of broad and long term irradiation, and the ease in their use, monochromatic lights from colored fluorescent lamps or filtered from incandescent lamps are widely used in photo-biological experiments. The fact that the total energy values found in these experiments are apt to become larger than the real values must be considered for this type of experiment.

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アサガオの花成誘導における光周感受性効果に対する波長光と蛍光灯
およびフィルターによる単色光との相違

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1) アサガオ紫の光周感受性に関与する色素系に対する単色光の作用に関する予備実験の結果から、16 時間暗期の直前に短時間赤色光を照射すると、発芽時より青・緑・近赤外光および暗黒下で生育した植物は常に花成誘導が起こる。これに対し、同様に照射した近赤外光は赤色光下で生育した植物の花成誘導を阻害する。この事実は回析格子照射分光器からの波長光による実験を容易にした。

2) 暗期直前に照射する赤色光の花成誘導に対する効果について、蛍光灯（純単色）による赤色光と照射分光器からの波長光（520~680 nm）とを比較した。

3) 花成誘導の阻害については、つねに花成誘導の起こる連続赤色光下で生育した植物に対し、白熱灯を光源としてフィルター IR-1 と熱線吸収ガラスとを通して得た近赤外光と照射分光器による波長光（650~780 nm）とを、暗期直前に短時間照射することによって比較した。

4) 100% 花成を誘導するのに必要な全エネルギー量は、赤色蛍光灯では $37 \times 10^6 \text{ erg/cm}^2$ 、波長光では $8.9 \times 10^6 \text{ erg/cm}^2$ であった。花成誘導の完全な阻害についてはフィルターによる近赤外光は $8.6 \times 10^6 \text{ erg/cm}^2$ であり、波長光は $9.9 \times 10^4 \text{ erg/cm}^2$ であった。

本実験で、同じ効果を生じるのに必要な全エネルギー量は、波長光以外の光において波長光より、あるいは真の値より大きな値を示し、この事実は波長光以外の単色光を用いる実験に際して留意すべきことを提示した。

5) 花成誘導に有効な波長域はおおよそ 500~600 nm, 620~640 nm と 650~675 nm の3波長域であり、花成誘導の阻害は 710~770 nm の波長域である。

6) 考察において光・温度などの実験条件について精細に検討し、蛍光灯と波長光による全エネルギーに差のある理由について考察した。