Studies on the Light Controlling Flower Initiation of *Pharbitis Nil*.

I. Intensity and Quality of the Light Preceding the Inductive Dark Period.

by Atsushi TAKIMOTO* and Katsuhiko IKEDA*

滝本 敦*・池田勝彦*: アサガオの花芽形成を支配する光条件について
I. 暗期前の光の強さと質

Received December 22, 1959

In many short day plants, the dark period of adequate length must be preceded by high-intensity light to initiate flower primordia, indicating that a process, the so-called high-intensity light process, plays an important role in photoperiodic induction^{1), 2), 3), 4), 5)}. Liverman and Bonner⁶⁾ reported that the requirement for this high-intensity light was replaced by feeding sugar or organic acids of the TCA cycle. Requirement of CO₂ during the high-intensity light process was also reported by Parker *et al*⁷⁾. The process is, therefore, regarded as the photosynthetic one producing energy sources required for the following dark process.

In preliminary experiments with *Pharbitis Nil*, in which the sugar was supplied to the plants under low intensity light followed by long dark period, no remarkable effect of the sugar was observed. The present experiments were designed to investigate whether the flower-inhibitory effect of the low intensity light preceding inductive dark period is merely attributable to the deficiency of photosynthete or to some other factors.

At the beginning, the effective intensity and the spectral sensitivity of the low intensity light for nullification of inductive effect of the following dark period was investigated. The experiments on the spectral sensitivity revealed a remarkable flower inhibitory effect of far-red light given to the plant prior to the inductive dark period. Later experiments were designed to investigate this phenomenon in detail.

Material

The material used was seedlings of *Pharbitis Nil*, strain "Violet", which will initiate floral primordia after the application of a single dark period of adequate length. Photoperiodic behaviour of this plant in the seedling stage was reported recently by Kujirai and Imamura⁸⁾.

To obtain uniform germination, the seeds were treated with conc. H₂SO₄ for 20–30 minutes, washed thoroughly in running water for about one day, and spread on moistened sand. Two days after the treatment with H₂SO₄, germinating seeds

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.
京都大学農学部応用植物学研究室

were selected for uniformity and sown in 18 cm. clay pots containing garden soil, so that one pot contains 20 plants in each. They were then placed in greenhouse at $30 \pm 2^\circ\text{C}$ under continuous illumination supplemented with incandescent light at night. Two days after the sowing, cotyledons expanded, and one day later the seedlings were subjected to the experimental treatment.

Methods

In the experiment in which the action spectrum of the low intensity light preceding inductive dark period for floral inhibition was studied (Exp. 3), coloured light was secured by the use of interference filter combined with coloured glasses, and 3 cm. layer of water serving as a heat filter. The light source was an incandescent lamp. Spectral energy transmittances of each filter are given in Fig. 1. In this experiment, intensity of each coloured light was adjusted to $500 \text{ erg/cm}^2/\text{sec.}$

Fig. 1. Spectral energy transmittances of interference filter combined with coloured glass.

Fig. 2. Spectral energy transmittances of red and blue cellophane and water.

In other experiments, red light relatively free from far-red irradiation was secured from a pink fluorescent lamp filtered by 2 layers of red cellophane, and far-red light from an incandescent filament lamp filtered by 2 layers of red and 2 layers of blue cellophanes as described by Downs^{9),10)}. Although the red cellophane filter was capable of transmitting far-red radiation, the emittance of the fluorescent lamp in the region longer than $720 \text{ m}\mu$ was negligible. The far-red light has been improved by the addition of a 5 cm. layer of water: this served to reduce the infra-red, without an appreciable decrease of the intensity of the far-red light. Spectral energy transmittances of the cellophanes and water are shown in Fig. 2.

All light measurements of the coloured light have been made with a calibrated thermopile, a Moll type, having a sensitivity of 269 mV/w/cm^2 . As the incandescent light comprises much heat radiation, measurement with a thermopile is inadequate, therefore, the intensity was measured with a lux meter. The intensity of daylight fluorescent light was also measured with a luxmeter, for comparison with the in-

candescent light: 1 lux of the daylight fluorescent light is equivalent to about 4.4 erg/cm.²/sec..

After the experimental treatment, plants were returned to the greenhouse, grown under continuous illumination for 2—3 weeks, and then dissected under a binocular microscope for the initiation of flower primordia. Generally, 20—40 plants were used for one lot of the treatments.

The percentage of the flowering plants, that of the plants with terminal flower and number of flower primordia per plant were used as the measures for flowering response. With some irregularities these measures run almost parallel.

Experiments were performed in an air-conditioned room of $25 \pm 1^\circ\text{C}$. Each experiment was repeated several times with almost similar results, so that only the representative results are given in the following.

Experiments and Results

Experiment 1. Plants were subjected to the incandescent light of low intensity (10 lux) for various hours preceding 16 hour dark period. Control plants were exposed to sun light preceding the 16 hour darkness. Flowering responses of the plants are shown in Table 1. The low intensity light of 8 hours preceding the inductive

Table 1. Effect of low intensity light of the incandescent lamp (10 lux) preceding 16 hour dark period upon flower initiation of *Pharbitis* seedlings.

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower primordia per plant	% of plants with terminal flower bud
16bd	30	100	5.3	96.7
8hIL → 16bd	30	6.7	0.1	0
16hIL → "	21	0	0	0
24hIL → "	27	0	0	0
32hIL → "	29	0	0	0
40hIL → "	18	0	0	0
48hIL → "	28	0	0	0

IL: incandescent light of 10 lux

8hIL → 16bd: 8 hour IL is followed by 16 hour dark period.

These abbreviations will be used hereafter.

dark period reduced flower initiation heavily and only 6.7 % of the plants initiated flower primordia. The low intensity light of 16 hours or more inhibited inductive effect of the following dark period completely.

Experiment 2. Plants were exposed to 8 hour incandescent light of various intensities preceding the dark period, and examined for flower inhibitory effect of the treatment. Results are shown in Table 2. Flower inhibitory effect of the incandescent light decreased with increasing intensity of the light, but even the light of 1000 lux was not strong enough to bring about a maximum flowering response.

Table 2. Effect of intensity of the incandescent light preceding 16 hour dark period upon flower initiation of *Pharbitis* seedlings. Plants were exposed to incandescent light of various intensities for 8 hours preceding the 16 hour dark period.

Intensity of incandescent light in lux	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
5	38	0	0	0
10	36	0	0	0
25	38	2.6	0	0
50	37	2.7	0	0
100	38	7.9	0.1	0
250	38	26.3	0.3	0
500	38	57.9	0.6	0
1000	38	57.9	0.7	0
Control (Sun light)	38	100	4.4	94.6

Experiment 3. To study the spectral sensitivity of the low intensity light preceding inductive dark period for flower inhibitory effect, plants were subjected to coloured light of 500 erg/cm²/sec. for 8 hours, and subsequently given a 16 hour dark period. The coloured light used was violet (406 m μ), blue (489 m μ), red (649 m μ) and far-red (747 m μ); the spectral energy distributions of each are represented in Fig. 1.

Table 3. Effect of monochromatic light of low intensity preceding 16 hour dark period upon flower initiation of *Pharbitis* seedlings. Monochromatic light was given for 8 hours with the intensity of 500 erg/cm²/sec.

Colour of light	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
Violet	15	87	2.1	7
Blue	13	69	1.4	0
Red	14	100	2.2	7
Far-red	13	0	0	0
White*	19	100	5.1	90

* 3500 lux from daylight fluorescent lamp and incandescent lamp.

The results represented in Table 3 indicate remarkable flower inhibitory effect of far-red light as compared with the other coloured light. Red light is most ineffective in inhibition. Experiments repeated several times gave similar results.

Experiment 4. As the remarkable flower inhibitory effect of the far-red light was ascertained in the foregoing experiment (Exp. 3), the low intensity light of daylight fluorescent lamp which comprises little far-red light was investigated for its flower inhibitory effect when followed by inductive dark period.

Plants were exposed to the daylight fluorescent light of 10 lux for 0, 8, 12, 16, 24, 30, 36, 42 and 48 hours, being arranged so as to close the treatment at the same time in all lots, and subsequently given 16 hour dark period. As the control, two lots of the plants were subjected to the daylight fluorescent light of 10 lux for 24 and 48 hours and given no dark period. Results are shown in Table 4.

Table 4. Effect of low intensity light of daylight fluorescent lamp (10 lux) followed by 16 hour dark period on flower initiation 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
16bd	39	100	4.2	97.4
8bFL → 16bd	40	92.5	2.9	47.5
12bFL → "	40	87.5	2.3	5.0
18bFL → "	39	79.5	1.3	0
24bFL → "	39	97.4	2.9	20.5
30bFL → "	40	97.5	3.4	37.5
36bFL → "	38	68.4	1.2	0
42bFL → "	40	50.0	0.6	0
48bFL → "	39	79.5	1.3	0
24bFL	40	0	0	0
48bFL	40	0	0	0

FL: daylight fluorescent light of 10 lux.

Flowering responses were reduced slightly with increasing duration of the low intensity light. Even when 48 hour low intensity light of this lamp preceded the 16 hour darkness, 79.5 % of the plants initiated flower primordia. Control plants which were subjected to the daylight fluorescent light of 10 lux for 24 and 48 hours initiated no flower primordia.

In this experiment, the plants exposed to the low intensity light for 12, 18, 36 and 42 hours initiated less flower buds than the other plants. They were subjected to the low intensity light either from 6 a.m. or midnight, having been exposed previously to incandescent light of about 500 lux at night. It may be supposed that the reduced flowering responses of these plants are due to this incandescent light of low intensity. But it is also conceivable that some endogenous diurnal rhythm influences the flowering responses.

Experiment 5. Flower inhibitory effect of the low intensity light preceding dark period was again investigated with respect to light sources, comparing the effect of the incandescent and the daylight fluorescent light. Results shown in Table 5 indicate clear difference in flower inhibitory effects of both lights.

Experiment 6. Low intensity light of the daylight fluorescent light (10 lux) was mixed with the far-red light of 120 erg/cm.²/sec.. Plants were exposed to this light for 8, 12, 18, 24, 36, 42 and 48 hours and subsequently given a 16 hour dark period.

Table 5. Comparison between flower inhibitory effect of incandescent light and of daylight fluorescent light of 10 lux given to *Pharbitis* seedlings preceding 16 hour dark period.

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower primordia per plant	% of plants with terminal flower bud
16bd	39	100	4.8	79.5
4hIL → 16bd	35	80.0	1.3	0
8hIL → "	38	0	0	0
12hIL → "	37	0	0	0
18hIL → "	37	0	0	0
24hIL → "	39	0	0	0
4hFL → 16bd	39	100	4.8	89.8
8hFL → "	40	32.5	0.5	0
12hFL → "	39	38.5	0.5	0
18hFL → "	40	25.0	0.3	0
24hFL → "	38	34.2	0.5	0

IL: incandescent light of 10 lux.

FL: daylight fluorescent light of 10 lux.

Table 6. Effect of FL+FR preceding 16 hour dark period on flower initiation of *Pharbitis* seedlings.

FL+FR: daylight fluorescent light of 10 lux mixed with far-red light of 120 erg/cm²/sec.

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
16bd	38	100	3.2	2.6
8hFL+FR → 16bd	39	5.1	0.1	0
12hFL+FR → "	38	0	0	0
18hFL+FR → "	39	0	0	0
24hFL+FR → "	39	0	0	0
30hFL+FR → "	40	0	0	0
36hFL+FR → "	31	0	0	0
42hFL+FR → "	38	0	0	0
48hFL+FR → "	38	0	0	0

The results are shown in Table 6. Eight hour application of this low intensity light reduced flowering responses strikingly and only 5.1 % of the plants initiated flower primordia. When this low intensity light period was lengthened up to 12 hours or more complete flower inhibition resulted.

The results show remarkable similarity between the incandescent light and the daylight fluorescent light mixed with the far-red light in flower inhibitory effect when given at low intensity preceding the inductive dark period.

Table 7. Reversible effect of red light and that mixed with far-red light upon flower inhibitory effect of the incandescent light of low intensity preceding the inductive dark period.

Intensity of incandescent light (IL): 10 lux.

Intensity of red light (R): 3000 erg/cm²/sec.

Intensity of far-red light (FR): 5000 erg/cm²/sec.

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
16hIL	39	100	4.6	94.9
8hIL 16hIL	42	0	0	0
" → 1' R → "	39	33.3	0.5	0
" → 5' R → "	39	79.5	2.1	17.9
" → 30' R → "	40	80.0	2.1	17.5
" → 2hR → "	39	82.1	2.6	25.6
" → 1' R + FR → "	39	7.7	0.1	0
" → 5' R + FR → "	39	10.3	0.1	0
" → 30' R + FR → "	38	2.6	0.1	0
" → 2hR + FR → "	40	25.0	0.4	0

Experiment 7. From the above experiments, it is ascertained that flower inhibitory effect of the low intensity light of the incandescent lamp preceding dark period depends considerably upon the effect of the far-red light from the lamp. Recently, in many photomorphogenetic responses including photoperiodic response, antagonistic action between red and far-red light was observed^{(9), (10), (11), (12), (13), (14)}. It is probable that the flower inhibitory effect of the incandescent light of low intensity is also reversed by the red light applied just before the dark treatment.

Plants were subjected to the 8 hour incandescent light of 10 lux, the plants of the first group were subsequently given red light of 3000 erg/cm²/sce., and those of the second group the red light of the same intensity mixed with far-red light of 5000 erg/cm²/sec. for 0, 1, 5, 30 minutes and 2 hours respectively, and thereafter given 16 hour dark period. Results are shown in Table 7. One minute of red light reversed flower inhibitory effect of the incandescent light to some extent, and 5—30 min. red light reversed it more effectively. The reversing effect of the red light is reduced significantly by the simultaneous application of the far-red light. Antagonistic effect of red and far-red light is observed clearly.

Discussion

Hitherto, flower inhibitory effect of low intensity light preceding the inductive dark period has been considered to be due to the lack of photosynthates as a precursor of flowering hormone or energy sources of flowering reaction⁽⁶⁾. From the present investigation, it is seen that the low intensity light of the incandescent lamp has more flower inhibitory effect than that of the daylight fluorescent lamp

comprising little far-red light, and that the strong inhibitory effect of the incandescent light is to some extent attributable to its abundant far-red light and is reversed by the red light. The "red-far-red absorbing pigment system" postulated in many photomorphogenetic responses is assumed to play an important role in this case too.

Borthwick *et al.* reported for *Xanthium* that the inductive dark process is prevented by red light, and promoted by far-red light. Thus, the 30 min. far-red irradiation preceding the dark period could reduce the critical dark length by some 2 hours¹²⁾.

On the other hand, Nakayama reported that far-red light given just before the dark period inhibits flower initiation of *Pharbitis* seedlings and that the inhibition is reversed by red light¹⁵⁾. The same is also the case in the present experiments. Why the far-red light preceding the dark period promotes flower initiation in *Xanthium*, and inhibits it in *Pharbitis* seedlings comes into question. To elucidate this discrepancy, more detailed studies along this line remain to be made.

Low intensity light of 8 hours or more preceding the inductive dark period has some flower inhibitory effect even if it contains little far-red light. This flower inhibitory effect of the low intensity light of long duration may be due to the deficiency of photosynthates and may correspond to that reported by Liverman *et al.*, which can be reversed by feeding sugar or organic acids.

Summary

The flower inhibitory effect of low intensity light preceding the dark period of adequate length (16 hours) was investigated in *Pharbitis* seedlings.

- 1) Eight hour incandescent light of 10 lux inhibits flower formation.
- 2) The incandescent light of 10–25 lux given for 8 hours suppresses the inductive effect of the following dark period entirely, and even that of 1000 lux is not strong enough to bring about maximum photoperiodic response.
- 3) Spectral sensitivity of the low intensity light for flower inhibition was studied. Far-red light has the highest inhibitory effect and red light the lowest.
- 4) Daylight fluorescent light of 10 lux which comprises little far-red light inhibits flowering far less than the incandescent light of the same intensity.
- 5) The daylight fluorescent light of 10 lux mixed with the far-red light of 120 erg/cm²/sec. inhibits flowering remarkably when given for 8 hours.
- 6) Flower inhibitory effect of the incandescent light of 10 lux is reversed by red light applied just before the dark period. But the red light mixed with the far-red light has little reversing effect for the flower inhibitory effect of the incandescent light.

Flower inhibitory effect of the incandescent light is attributable to the action of the far-red light comprised in it. "Red-far-red absorbing pigment system" is supposed to play an important role in the reaction preceding inductive dark period.

Grateful acknowledgment is given to Professor S. Imamura for his suggestion and criticisms.

References

- 1) Borthwick, H. A. and Parker, M. W., Bot. Gaz. 100: 374 (1938). 2) Hamner, K. C., Bot. Gaz. 101: 658 (1940). 3) Lang, A., Ann. Rev. Plant Physiol. 3: 265 (1952). 4) Liverman, J. L., Ann. Rev. Plant Physiol. 6: 177 (1955). 5) Mann, L. K., Bot. Gaz. 102: 339 (1940). 6) Liverman, J. L., and Bonner, J., Bot. Gaz. 115: 121 (1953). 7) Parker, M. W. and Borthwick, H. A., Bot. Gaz. 102: 256 (1940). 8) Kujirai, C. und Imamura, S., Bot. Mag. Tokyo, 71: 408 (1958). 9) Downs, R. J., Plant Physiol. 30: 468 (1955). 10) ———, ibid. 31: 279 (1956). 11) Borthwick, H. A., Hendricks, S. B., Parker, M. W., Toole, E. H. and Toole, V. K., Proc. Natl. Acad. Sci. 38: 662 (1952). 12) ———, ——— and ———, ibid. 38: 929 (1952). 13) Liverman, J. L., Johnson, M. P. and Starr, L., Science 121: 440 (1955). 14) Piringer, A. A. and Heinze, P. H., Plant Physiol. 29: 467 (1954). 15) Nakayama, S., Ecological Rev., Sendai 14: 325 (1958).

摘 要

アサガオ子葉に 16 時間の暗期を一度与えると花芽形成が見られるが、暗期前 10 ルックスの白熱電灯光を 8 時間与えると花芽を形成しなくなる。

1) 暗期前弱光の花芽形成抑制に対する有効波長を調べると、近赤外光がもっとも抑制効果強く、赤色光がもっとも弱い。

2) 近赤外光をほとんど含まない昼光色蛍光灯光 (10 ルックス) を暗期前に与えても抑制効果は非常に弱く、これに近赤外光を混ぜると抑制効果がいちじるしくなる。

3) 近赤外光の強い光を暗期前に与えると、大変いちじるしい花芽形成の抑制が見られる。

4) 10 ルックス白熱電灯光の花成抑制効果は暗期直前に 1~5 分間赤色光を与えることによって消却される。しかし赤色光と同時に近赤外光をも与えると消却効果はいちじるしく減ずる。

白熱電灯弱光の花芽形成抑制効果はそれに含まれている近赤外光の働きによるところが大きいものと考えられる。