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

IV. Further Studies on the Light Preceding the Inductive Dark Period

by Atsushi TAKIMOTO* and Katsuhiko IKEDA*

Received July 3, 1959

Low-intensity light preceding the inductive dark period of 16 hours inhibits flower initiation in *Pharbitis* seedlings, however, the low intensity light including little far-red light inhibits only a little and that including far-red light does so heavily¹). Flower inhibitory effect of this far-red light is reversed to some extent by a brief red light given just before the dark period^{1,2}). On the other hand, low-intensity light with or without far-red light preceding a dark period of 12 hours or less, promotes flowering response remarkably^{2,3}).

In *Pharbitis* seedlings, a 16-hour dark period can induce maximum flowering response and further lengthening of the dark period hardly increases the responses, but a 12-hour dark period is not so inductive as to bring about a maximum flowering response, and further lengthening of the dark period increases flowering response greatly. It was supposed that the first process of the inductive dark period proceeds under low-intensity light or far-red light as well as in darkness, and that the far-red light prevents the subsequent dark process^{2,3}).

Far-red light preceding a dark period of 12 hours or less promotes flowering responses. If brief red light is given between the far-red light and the dark period, there is a question whether the flowering response increases or not. Considering that the red light can reverse the flower-inhibitory effect of the far-red light, a greater increase in flowering response might be expected. On the contrary, it is also conceivable that the red light following the far-red light may have a "light-break" effect, i.e. a flower inhibitory effect. The light break is known to be most effective in the first hours of a long dark period^{4,5,6,7}), and the first process of the inductive dark period presumably is undisturbed by far-red light^{2,3}).

Low-intensity light preceding a 16-hour dark period inhibits flower initiation to some extent even if it contains little far-red light^{1,2}). If the brief red light is given between the low-intensity light and the dark period, the inhibitory effect of low-intensity light may or may not be reversed. The first process of the inductive dark period is considered to proceed under such a low-intensity light. It is also possible that the brief red light following the low-intensity light has a light-break effect, and inhibits the flowering response.

The effect of a brief red light inserted between the inductive dark period and the preceding low-intensity light, far-red light or darkness was investigated here.

Material and Methods

Material used was the seedling of *Pharbitis Nil*, strain "Violet". Methods of experiments were similar to those described in a previous paper¹). Light filters and light sources used were also described there.

* Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University, Kyoto, Japan.

Experiments and Results

Experiment 1. Plants were placed in darkness for 2, 4 and 8 hours, at the end of which time they were exposed to red light (600–700 $m\mu$) of 3000 erg/cm.²/sec. for 5 minutes, which inhibits flower initiation when given at the middle of a 16-hour dark period. Subsequently one group of the plants (Group 1) was subjected to 16 hours of darkness, which is enough to induce a maximum flowering response, and the other group (Group 2) to 12 hours of darkness, which is not so inductive as to bring about a maximum flowering response. Control plants were subjected to 16- and 12-hour darkness preceded by sun light. Results are shown in Table 1.

Table 1. Flowering response of plants which were exposed to 2 to 8 hours of darkness, 5 minutes of red light of 3000 erg/cm.²/sec., and 12 or 16 hours of darkness, successively.
(Treated on May 1 and dissected on May 15, 1958)

Group	Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
1	16 ^h d	38	100	5.0	100
	2 ^h d→5'R→16 ^h d	38	100	4.8	100
	4 ^h d→"→"	39	100	4.9	100
	8 ^h d→"→"	32	87.5	1.8	0
	24 ^h d	36	100	5.2	97.2
2	12 ^h d	38	100	1.1	0
	2 ^h d→5'R→12 ^h d	38	100	4.5	94.7
	4 ^h d→"→"	39	100	4.8	100
	8 ^h d→"→"	36	25.0	0.4	0
	20 ^h d	40	100	4.9	97.5

2^hd→5'R→16^hd: 2-hour darkness, 5 minutes of red light and 16-hour darkness were given successively.

These notations will be used hereafter.

In Group 1, 2- or 4-hour darkness preceding 5 minutes of the red light had little effect on flowering response, but with 8 hours of darkness preceding the red light, flower initiation was heavily inhibited. This is very similar to the results obtained with *Xanthium*^{5,6}), in which the light interruption does not inhibit flowering at the 2nd to 4th hour of the long dark period but inhibits it strongly at the 6th to 8th hour.

In Group 2, when a 12-hour dark period was preceded by another dark period of 2 or 4 hours duration, the two dark periods being separated by 5 minutes of red light, flowering response was promoted remarkably, but when preceded by 8 hours of darkness and 5 minutes of red light, it was inhibited. A 12-hour dark period can not induce maximum flowering response, and further lengthening of dark period increases flowering response strikingly; therefore, if 2 or 4 hours of darkness precede the 12-hour darkness, even if the red light was inserted between them, flowering response is increased, for the flower-inducing dark process is stable to the brief light during the first 4 hours of the dark period. After 8 hours of darkness, the flower-inducing dark process becomes light-sensitive; therefore, flowering response is reduced strikingly if an 8-hour dark period precedes the 12-hour dark period with intervening red light.

Experiment 2. Two groups of the plants were exposed to daylight fluorescent light of 50 erg/cm.²/sec. (FL) containing little far-red light. The exposure lasted 8 hours preceding a 6- to 16-hour dark period, and one group was subjected to red light for 5 minutes just before the dark period, while the other one was not. Control plants were given 6 to 16 hours of darkness preceded by sun light. Results are shown in Table 2.

Table 2. Flowering response of plants which were exposed to 8 hours of daylight fluorescent light of 50 erg/cm.²/sec., 5 minutes of red light of 3000 erg/cm.²/sec. and a dark period of various hours, successively.

FL: Daylight fluorescent light of 50 erg/cm.²/sec.
(Treated on May 26 and dissected on June 8, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
8 ^h FL→5'R→16 ^h d	40	82.5	1.1	0
" → " → 14 ^h d	40	2.5	0.0	0
" → " → 12 ^h d	40	0	0	0
" → " → 10 ^h d	40	0	0	0
" → " → 8 ^h d	38	0	0	0
" → " → 6 ^h d	40	0	0	0
8 ^h FL → 16 ^h d	39	100	4.5	94.9
" → 14 ^h d	39	100	4.9	100
" → 12 ^h d	40	100	3.9	67.5
" → 10 ^h d	39	100	4.4	74.4
" → 8 ^h d	40	100	2.6	17.5
" → 6 ^h d	39	61.6	0.7	0
16 ^h d	39	100	4.3	100
14 ^h d	39	100	1.9	2.6
12 ^h d	39	5.1	0.1	0
10 ^h d	40	0	0	0
8 ^h d	40	0	0	0
6 ^h d	40	0	0	0

Eight-hour FL did not inhibit the flowering when followed by a 16-hour dark period, and promoted remarkably when followed by a dark period of 14 hours or less. In the previous papers^{1,2)}, it was reported that the 8-hour FL preceding 16 hours of darkness inhibited flowering to some extent. In the present experiment, plants were very sensitive to photoperiodic induction and in such a case the flower inhibitory effect of the FL may become obscure. In another experiment which is not presented here, the plants were not so sensitive and the flower inhibitory effect of FL was more obvious*.

If 5 minutes of red light was given following the 8-hour FL (preceding dark period), flowering response was reduced remarkably, irrespective of the duration of darkness. This suggests that, after the 8-hour FL, under which the first process of the inductive dark period proceeds, the reactions inducing flower initiation become light-sensitive, probably producing some light-sensitive substance which is destroyed by 5 minutes of red light. The same may also be the case in complete darkness (cf. Experiment 1).

Experiment 3. Plants were treated in the same way as in Experiment 2, but

* Photoperiodic sensitivity of *Pharbitis* seedlings varies with the seasons, being influenced by unknown internal and external factors. From spring to early summer, the sensitivity for photoperiodic induction is the highest.

far-red light (700–100 $m\mu$) of 120 erg/cm.²/sec. was mixed simultaneously with the FL of 8 hours. As has been reported previously, under this light the first process of the inductive dark period is believed to proceed, but this light inhibits flower initiation by preventing the subsequent dark processes^{2,3}). Table 3 shows the results.

Table 3. Flowering response of plants which were exposed to 8 hours of FL+FR, 5 minutes of red light of 3000 erg/cm.²/sec. and a dark period of various hours, successively.

FL+FR: Daylight fluorescent light of 50 erg/cm.²/sec. mixed with far-red light of 120 erg/cm.²/sec.

(Treated on May 27 and dissected on June 11, 1958)

Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
8 ^h (FL+FR)→5'R→16 ^h d	39	94.9	2.3	7.7
" → " → 14 ^h d	40	2.5	0.0	0
" → " → 12 ^h d	39	0	0	0
" → " → 10 ^h d	38	0	0	0
" → " → 8 ^h d	40	0	0	0
" → " → 6 ^h d	40	0	0	0
8 ^h (FL+FR) → 16 ^h d	30	61.5	0.6	0
" → 14 ^h d	40	70.0	0.8	0
" → 12 ^h d	38	36.9	0.4	0
" → 10 ^h d	40	37.5	0.4	0
" → 8 ^h d	40	42.5	0.5	0
" → 6 ^h d	36	0	0	0
16 ^h d	43	100	4.3	100
14 ^h d	40	100	1.9	2.5
12 ^h d	40	32.5	0.3	0
10 ^h d	39	0	0	0
8 ^h d	39	0	0	0
6 ^h d	39	0	0	0

When a 16-hour dark period was preceded by 8-hour FL mixed with far-red light (FL+FR), flower initiation was inhibited, but this inhibition was reversed to some extent by the red light of 5 minutes given just before the dark period. This reversing effect of red light was reported previously^{1,2}). The first process of the inductive dark period proceeds under the FL+FR, and red light has a light-break effect which tends to counteract this process and thereby inhibits flowering. In the present case, however, the reversing effect of red light for the flower-inhibitory effect of far-red light evidently exceeds the light-break effect, and the net result is some stimulation of flowering. When the dark period is 12 hours or less, 8-hour FL+FR preceding the dark period promoted flower initiation to some extent, and if the red light of 5 minutes was given just before the dark period, flowering response was inhibited. Flower inhibitory effect of the red light, i.e. the light-break effect, is believed to exceed the reversing effect, in this case.

Experiment 4. Plants of the first group were subjected to darkness and those of the second group to FL for 2, 4, 6 and 8 hours, respectively. Subsequently both groups were exposed to red light of 3000 erg/cm.²/sec. for 5 minutes, and thereafter to 12 hours of darkness. Plants of the third group (control plants) were subjected to 8-, 12-, 14-, 16- and 20-hour dark periods preceded by sun light. Results are shown in Table 4. Flowering responses of the first group are very similar to those of the plants in Experiment 1. A light break at the 2nd to 6th hour of the dark period is

Table 4. Flowering response of plants which were exposed to darkness or FL of 2-8 hours, 5 minutes of red light of 3000 erg/cm.²/sec. (R) and a 12-hour dark period, successively.

FL: Daylight fluorescent light of 50 erg/cm.²/sec.
(Treated on June 12 and dissected on June 26, 1958)

Group	Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
1	2 ^b d→5'R→12 ^b d	37	100	3.6	13.5
	4 ^b d→"→"	38	100	4.1	29.0
	6 ^b d→"→"	37	94.6	3.6	21.6
	8 ^b d→"→"	38	0	0	0
2	2 ^b FL→5'R→12 ^b d	38	100	3.5	31.6
	4 ^b FL→"→"	39	100	4.3	84.7
	6 ^b FL→"→"	40	95.0	2.5	7.5
	8 ^b FL→"→"	39	0	0	0
3	8 ^b d	40	0	0	0
	12 ^b d	38	84.3	1.6	0
	14 ^b d	38	100	4.0	13.2
	16 ^b d	39	100	4.7	84.7
	20 ^b d	40	97.5	3.5	27.5

less effective, but that at the 8th hour inhibited flowering response completely.

The FL and darkness have exactly the same effect when followed by 5 minutes of red light and subsequent 12-hour dark period. This supports again the hypothesis that the first process of the inductive dark period proceeds under FL as easily as under complete darkness^{2,3}).

Experiment 5. The same experiment as Experiment 4 was performed, but the far-red light of 120 erg/cm.²/sec. was mixed with the FL (FL+FR). Results are shown in Table 5.

In this experiment, too, the flowering response of the first and the second group

Table 5. Flowering response of plants which were exposed to darkness or FL+FR of 2-8 hours, 5 minutes of red light of 3000 erg/cm.²/sec., and a 12-hour dark period, successively.

FL+FR: Daylight fluorescent light of 50 erg/cm.²/sec. mixed with far-red light of 120 erg/cm.²/sec.

(Treated on May 26 and dissected on June 8, 1958)

Group	Treatment	No. of plants dissected	% of plants with flower buds	No. of flower buds per plant	% of plants with terminal flower bud
1	2 ^b d→5'R→12 ^b d	40	100	2.0	2.5
	4 ^b d→"→"	40	100	4.0	47.5
	6 ^b d→"→"	39	100	3.7	43.6
	8 ^b d→"→"	40	52.5	0.7	0.
2	2 ^b (FL+FR)→5'R→12 ^b d	40	97.5	2.7	2.5
	4 ^b (FL+FR)→"→"	40	105	4.6	95.0
	6 ^b (FL+FR)→"→"	40	97.5	2.9	20.0
	8 ^b (FL+FR)→"→"	39	2.6	0.0	0
3	12 ^b d	40	72.5	0.9	0.
	14 ^b d	40	100	2.8	2.5
	16 ^b d	38	100	4.2	57.9
	20 ^b d	39	97.4	4.5	74.4

showed no remarkable difference. This implies that the first process of the inductive dark period can proceed under FL+FR as easily as under FL or darkness, and that the flower inhibitory effect of far-red light which exerts an influence upon the following dark process is reversed by 5 minutes of red light; thus, the results become similar to those obtained in Experiment 4.

Only the plants exposed to the red light following an 8-hour FL+FR initiated considerably fewer flower primordia than those darkened for 8 hours preceding the red light, probably owing to insufficient reversing effect of the red light for the flower inhibitory effect of 8-hour FL+FR. It was reported in a previous paper, that far-red light of 2~4 hours preceding the inductive dark period is not so effective for flower inhibition but that of 8 hours is very effective²⁾.

Discussion and Conclusions

From the results mentioned above, it is concluded as follows:

The first process of the inductive dark period proceeds under FL, FL+FR and darkness, and this process is stable to red light of 5 minutes (3000 erg/cm.²/sec.). But the process taking place 6-8 hours after the beginning of the first process is light-sensitive and destroyed or nullified by the brief red light. Probably, a light-sensitive substance, which is destroyed by the red light, is produced after 6-8 hours of FL, FL+FR or darkness.

As has been reported previously, far-red light preceding the inductive dark period has a dual effect, i.e. flower-promoting and flower-inhibitory effect^{2,3)}. The former is attributable to the fact that the first process of the inductive dark period can proceed under far-red light, and the latter to the effect on the "red-far-red absorbing pigment system", for the flower-inhibitory effect of far-red light is reversed by red light to some extent.

Red light following the far-red light is also considered to have a dual effect, i.e. a light-break effect and a reversing effect on the "red-far-red absorbing pigment system". The former is flower inhibitory and the latter is flower promoting. The observable effect of the red light following far-red light depends on which of these two effects is the stronger.

Summary

1) Five minutes of red light of 3000 erg/cm.²/sec. given at the 2nd to 4th hour of a long dark period does not inhibit flower initiation, but that given at the 8th hour does so.

2) Eight-hour daylight fluorescent light of 10 lux (FL) preceding the dark period inhibits flower initiation a little when the dark period is 16 hours or more, but promotes when the dark period is 12 hours or less. Red light of 5 minutes following the 8-hour FL inhibits flowering response strongly irrespective of the duration of darkness. Probably, the red light has a light-break effect, because the first process of the inductive dark period is considered to proceed under the FL.

3) Eight-hour FL mixed with far-red light of 120 erg/cm.²/sec. (FL+FR) preceding the dark period inhibits flower initiation if the dark period is 14-16 hours, but promotes if the dark period is 10 hours or less. The red light of 5 minutes inserted between them reduced the flowering responses when followed by a dark period of 14 hours or less, but increased flowering to some extent when followed by a 16-hour dark period.

4) Red light following the FL+FR is considered to have a dual effect:

i) It reverses the flower inhibitory effect of FL+FR preceding the inductive dark period.

ii) It has a light-break effect, i.e. a flower inhibitory effect, through counteracting the first process of the inductive dark period, a process which presumably proceeds under FL+FR.

The observable effect of red light following FL+FR depends on which effect is the stronger.

5) FL, FL+FR and darkness have almost the same effect on flower initiation when given for 2-6 hours preceding a 12-hour dark period, with 5 minutes of red light intervening.

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

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摘 要

アサガオの花芽形成を支配する光条件について.

IV. 暗期前の光について. 続報

滝本 敦・池田勝彦

1) 長い暗期 (16 時間以上) の最初の 2~4 時間目に 5 分間 3000 erg/cm.²/sec. の赤色光を与えても花芽形成は抑制されない. しかし 8 時間目に与えるといちじるしく抑制される.

2) 暗期前 8 時間 10 ルックスの昼光色蛍光燈光 (FL) を与えると, 暗期が 16 時間以上の場合には, わずかに花芽形成が抑制され, 暗期が 12 時間以下の場合には花芽形成が促進される. 8 時間 FL 照射後, 5 分間赤色光を与えると暗期の長さに関係なく花芽形成は抑制される.

暗期反応の初期段階は FL 下で進行し得るものと考えられるので, この赤色光の効果は恐らく光中断効果によるものであろう.

3) FL に 120 erg/cm.²/sec. の近赤外光を混ぜた光 (FL+FR) を暗期前 8 時間与えると, 暗期が 14 時間以上の場合には花芽形成が抑制され, 暗期が 10 時間以下の場合には促進される.

FL+FR と暗期の間に与えた 5 分間の赤色光は暗期が 16 時間以上の時には花芽形成を促進し, 暗期が 14 時間以下の場合には花芽形成を抑制する.

4) FL+FR 照射後に与えた赤色光は二重の効果を有するものと考えられる.

i) 暗期前に与えた FL+FR の花芽形成抑制効果を消却する.

ii) 暗期反応の初期段階は FL+FR 下で進行し得るものと考えられるので, この赤色光は光中断効果 (花芽形成抑制効果) を有する.

FL+FR 後に与えた赤色光の効果としては, 上記二重効果の差が現われてくる.

5) 12 時間の暗期前に 2~6 時間, FL, FL+FR または暗期を与え, 両者の間に 5 分間の赤色光を与えると, 開花反応はいずれの場合もほとんど同じである. すなわち, 暗期反応の最初の 2~6 時間は, FL, FL+FR 下で暗黒中におけると同様に進行しているものと考えられる. (京都大学農学部応用植物学研究室)