Bull. Fac. Bioresources, Mie Univ.
No. 11: 185–190
December 24. 1993

Generation of Circadian Flowering Rhythm of *Pharbitis nil* Choisy in Response to Dark-Light Period

Yukiyoshi OGAWA

Faculty of Bioresources, Mie University

Abstract

The effects of a phasing dark period and an intervening light period on circadian flowering response to an inductive dark period were studied with *Pharbitis*, cultivar Kidachi, grown in continuous fluorescent light.

A single light-on signal by the transition from dark to light period established a circadian rhythm of flowering, which lasted for at least 48 h. The generation of the rhythm and the phase pattern were not affected by exogenous gibberellin A_3 (GA_3), benzyladenine (BA) or indoleacetic acid (IAA), though the two formers stimulated flowering during the inducible phases of the rhythm. The same circadian rhythm was detected when the phasing dark period was longer than 4 h, but the response level of flowering became higher with lenghening duration of the dark period. The circadian rhythm became evident if the light intensity of the intervening light period was less than 5,000 lux, though the flower-response level became generally lower with lowering intensity of the light. The flowering was influenced by the phase of the rhythm during which the plants were exposed to the inductive dark period.

A significance of the phasing dark period and the intervening light period is discussed in relation to the first dark-light periods in the photoperiodic flowering of short-day plants that require several cycles of dark-light period to flower.

Key words: Circadian flowering rhythm · Intervening light period · *Pharbitis* · Phasing dark period

I. Introduction

It has been reported that rhythmic changes in flowering of *Pharbitis*, cultivar. Violet could be reset by a light-on signal. Takimoto and Hamner[13] reported a light-on rhythm with 8- and 12-h photoperiods after an 8 h dark period. Paraska and Spector[12] and Heide et al.[3] found the circadian rhythm that initiated at the time of seedling emergence. King et al.[4] showed that a circadian rhythm in dark-grown seedlings was related to the timing of a red light pulse after a saturating exposure to red light. Lumsden et al.[5] reported that a rhythmic response to a red light interrupting an inductive dark period was influenced by the previous exposure to light. Ogawa[8] showed that *Pharbitis*, cultivar Kidachi grown in a greenhouse with alternation of light intensity between day and night exhibited a circadian rhythm of flowering for several days even when they were placed in continuous light.

In the present experiments we manipulated the duration of the dark period that synchronizes endogenous diurnal rhythms, the intensity of the intervening light period and the duration of the inductive dark period in order to determine their effects on the circadian floral rhythm in *Pharbitis*, cultivar Kidachi grown in continuous fluorescent light.

Accepted June 16, 1993

II. Materials and Methods

Germinating seeds of *Pharbitis nil* Choisy, cultivar Kidachi, were planted in clay pots filled with garden soil as reported previously[8]. They were grown for 5 or 6 days at 28±1°C and in the light of ca. 4,000 lux from daylight fluorescent lamps. They were then exposed to a subcritical dark period of 8 h unless stated otherwise as a phasing dark period. Then they were lighted with fluorescent light of 2,500 lux for various durations (1 h to 48 h) of intervening light period, and finally exposed to a 16-h inductive dark period. As a control, plants without subjecting to the phasing darkness were exposed to the light of 2,500 lux for 12 h or 36 h preceding the inductive dark period. Each phasing dark period was started at different times so that the inductive dark period could be given at the same time with 24 h intervals (Fig. 1). After the inductive dark period, they were grown in a glasshause kept at 28±2°C subjecting to the sunlight during the day and fluorescent light of 1,500 lux at night. Two or three weeks later the plants were dissected to measure the floral response. In some cases, an aqueous solution of GA_3 was dropped onto the plumule (0.12 μg/plant) with a glasscapillary tube, and a solution of benzyladenine (BA) or indoleacetic acid (IAA) was applied to the cotyledons (1.5 μg/plant) with a paint brush. Control plants were given distilled water. Two or three pots with 14 to 21 plants were used for each experiment. The flowering response is expressed as the means of the numbers of flower buds per plant±the standard error of the mean.

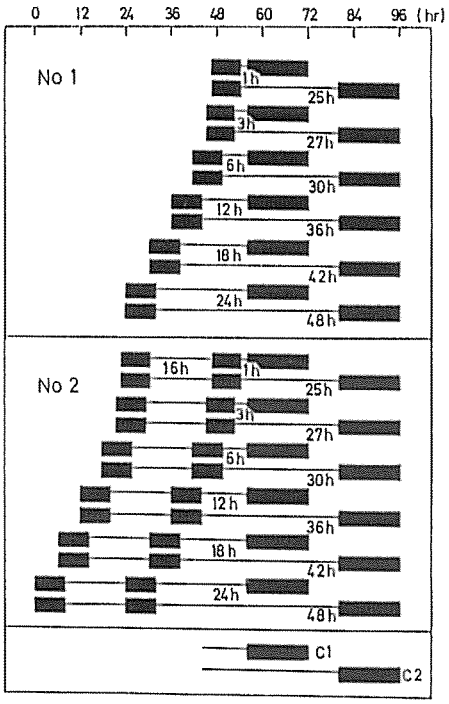

Fig. 1. Schedule of treatments involving once (No 1) and twice (No 2) exposure to an 8-h phasing dark period (■), followed by various durations of 2,500 lux fluorescent light (—, 1 h to 48 h), and then by a 16 h-inductive dark period (■). Control plants without an exposure to the phasing dark period were exposed to 2,500 lux light either 12 h (C 1) or 36 h (C 2) before giving the inductive dark period.

III. Results

1. *Effect of phasing dark period*

As detailed in Fig. 1, plants were exposed to an 8-h phasing darkness either once (No. 1) or twice (No. 2)

with 16-h intervals. Then they were exposed to various durations of the intervening light period before a 16-h inductive dark period. The results are shown in Fig. 2-a. The higher level of flowering response did occur 12 and 36 h after the end of the phasing dark period and the phases are referred to as inducible phase, and after reaching each peak they declined to the phase of low level of flowering at 24–25 h and 48 h, the non-inducible phase, showing a distinct circadian rhythmic fluctuation. Only once exposure to the phasing dark period was adequate to trigger the floral rhythm. This rhythm is the same as that found in plants exposed to sunlight during the day and fluorescent light at night, and to the fluorescent light transferring from 30 to 2,500 lux, as previously reported[8].

GA_3 and BA promoted flowering when they were applied before the inductive dark period as reported by Ogawa[7], and Ogawa and King[9], but IAA inhibited it as seen in the previous report[6]. Then, we studied the effects of these exogenous growth substances on the generation of the circadian rhythm. Aqueous solution of GA_3, BA or IAA was applied just before the exposure to an 8-h phasing darkness followed by an intervening light

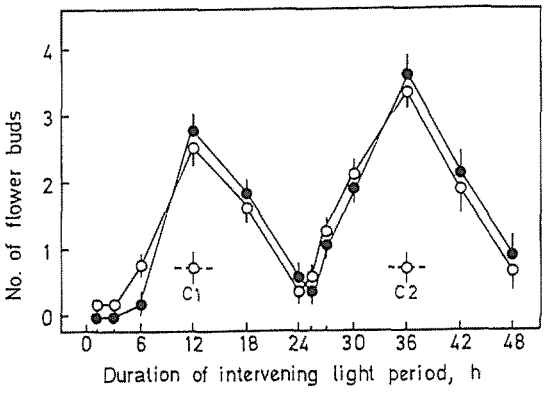

Fig. 2-a. Floral response of *Pharbitis* as a function of the duration of the intervening light period preceded by one (●) or two (○) 8 h-phasing dark periods. The control (C 1, C 2) and treatment schedules are shown in Fig. 1.

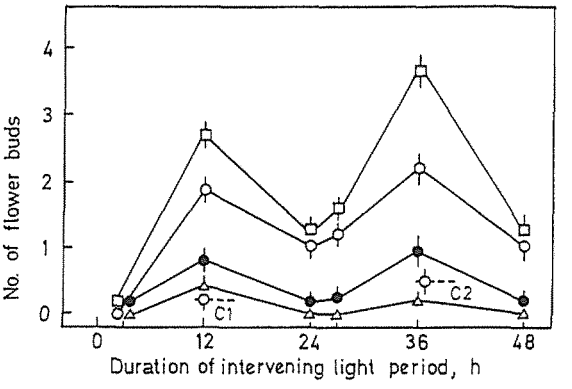

Fig. 2-b. Floral response of *Pharbitis* applied with GA_3 (□), BA (○), IAA (△) and distilled water (●) as a function of the duration of the intervening light period preceded by 8 h-phasing dark period. The control (C 1, C 2) and treatment schedules are shown in Fig. 1.

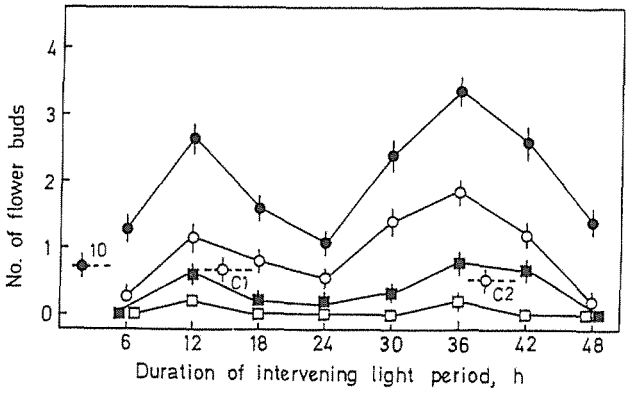

Fig. 2-c. Floral response of *Pharbitis* as a function of the duration of the intervening light period preceded by the phasing dark periods of various duration; 4 h (□), 6 h (■), 8 h (○), and 10 h (●). Response to 10-h phasing dark period with no inductive dark period; (--●--). The control (C 1, C 2) and treatment schedules are shown in Fig. 1.

period of various durations, and then a 16-h inductive dark period was given. The results are shown in Fig. 2-b. These substances had no effect on the generation of the rhythm and the phase pattern. GA_3 and BA promoted flowering to a greater extent at 12 and 36 h during the inducible phase, and to a lesser extent at 24 and 48 h during the non-inducible phase. IAA inhibited flowering of both phases.

The effects of the duration of phasing dark period on generation of the circadian rhythm were examined. The plants were exposed to a 4-, 6-, 8- and 10-h phasing darkness before giving various durations of 2,500 lux light followed by an inductive dark period. The results are shown in Fig. 2-c. Again, the same pattern of floral rhythm was observed independent on the length of phasing darkness. However, the number of flowers became greater as the dark period was lengthened, especially greatest at 12 and 36 h intervening light.

2. *Effect of light intensity during the intervening light period*

After subjecting the plants to an 8 h-phasing darkness, the light intensity during an intervening light period was varied from 1,000 to 7,000 lux and then followed by the inductive dark period. The results are shown in Fig. 3. The circadian floral rhythm with peaks at 12 and 36 h was found only when the intervening light intensity was 1,000 to 3,000 lux. The flowering remained at higher level with the irradiance of light intensity more than 5,000 lux and did not decrease even at 24 and 48 h.

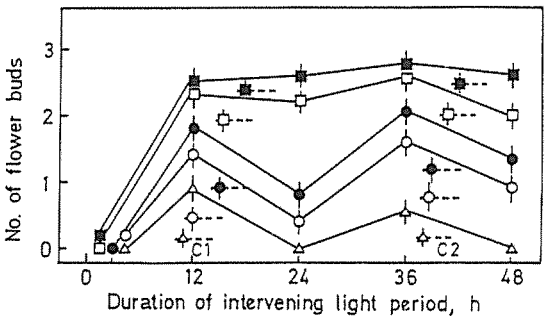

Fig. 3. Floral response of *Pharbitis* as a function of the duration of the intervening light period of different intensities; 1,000 lux (△), 2,000 lux (○), 3,000 lux (●), 5,000 lux (□), and 7,000 lux (■), preceded by 8 h-phasing dark period. Plants not subjected to the phasing dark period were exposed for either 12 h (C 1) or 36 h (C 2) to the light at 1,000 lux (--△--), 2,000 lux (--○--), 3,000 lux (--●--), 5,000 lux (--□--) or 7,000 lux (--■--).

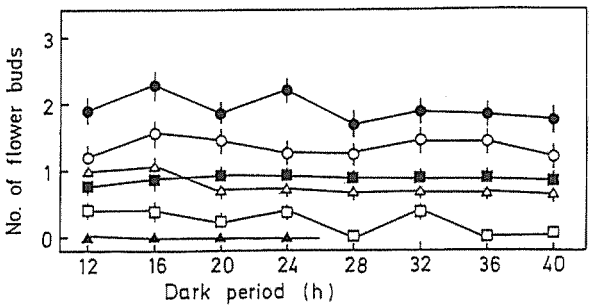

Fig. 4. Floral response of *Pharbitis* subjected to intervening light periods of various durations as a function of the duration of the inductive dark period (12 to 40 h). The intervening light period; 6 h (▲), 12 h (○), 36 h (●) and 48 h (■) preceded by 8-h phasing dark period. Control plants (△) were not exposed to the phasing dark period.

3. *Effect of duration of the inductive dark period*

The plants grown in 2,500 lux fluorescent light were exposed to an 8-h phasing darkness which was followed by an intervening light period lasting 6, 12, 24, 36 and 48 h. The inductive dark period finally given were varied in length from 12 to 40 h. The control plants were subjected to the inductive dark period of various length without exposing to the phasing darkness as well as the intervening light period. As shown in Fig. 4, 12- and 36-h intervening light periods could bring about high level of flower response after every application of inductive

dark period, showing higher level of response with intervening 36 h light. In contrast, 24 and 48 h intervening light period caused only low level of flower response regardless of the duration of the dark period. Six hours of the light period gave no flowering at all.

IV. Discussion

Effect of the duration of the light period before the inductive dark period had been studied by Hamner with Biloxi soybean[1] and by Harder and Gümmer with *Kalanchoe*[2]. These plants require several photoperiodic cycles to bring about to flower. Flowering of those plants increased with an intervening light of 12 h and decreased with an intervening light of 24 h. Papenfuss and Salisbury[11] reported that in *Xanthium* exposure to a 12-h light period preceded by a 7.5-h dark period caused the maximum flowering in response to an inductive dark period. In the present study, flowering in *Pharbitis* was also promoted with intervening light periods of 12 and 36 h, the latter duration being usually more effective that the former (Fig. 2-a). For the other short-day plants mentioned above, their optimal 12 h light-12 h dark period cycles for flowering may coincide with 12 h intervening light during the inducible phase in flowering rhythm of *Pharbitis*.

The circadian rhythm of flowering in *Pharbitis* is probably generated with the light-on signal which caused by the transfer from the phasing dark period to the light period, and with only a single pulse of the signal the rhythm can sustain for 48 h at least (Fig. 2-a).

The phase pattern of the flowering rhythm was not affected by GA_3 applied to the shoot apex in agreement with our previous suggestion[8]. BA and IAA did not have also direct effects on the pattern of the flowering rhythm, though these growth substances have been indicated to act on floral processes in the cotyledon[6,9].

The phase pattern of the flowering rhythm was not affected also by the durations of the phasing dark period (Fig. 2-c), and no difference in the phase pattern was found among 8, 12 and 16 h phasing dark periods (data not shown). However, the level of flower response in the inducible phase was higher with longer period of phasing dark periods (Fig. 2-c). And, 20 and 24 h phasing dark periods did not cause the circadian rhythm (data not shown). Thus, the light-on signal at the transition from the phasing dark period to the light period may act qualitatively as well as quantitatively depending on the duration of phasing dark period.

The circadian flowering rhythm became evident only when the intervening light intensity was 1,000 to 3,000 lux, though the genuine flower response was relatively weak. In contrast, the rhythm was not evident at 5,000 lux or above (Fig. 3). There exists a possibility that the flowering rhythm might have been masked by the higher level of flower response with higher intensity of light. In general the circadian flowering rhythm of *Pharbitis* has been evident when a relatively low-intensity light period precedes the inductive dark period[8].

A relatively short inductive dark period was enough to induce a strong flowering when it was started at the inducible phase of the rhythm. However, even a sufficient length of inductive dark period could not induce flowering when started at the non-inducible phase (Fig. 4), suggesting that qualitative nature of photoperiodic flower induction could be influenced by the phase of rhythm to which the plants were subjected.

These evidences found in the present experiments lead to an idea that in plants which require several short-day cycles for floral induction, the first dark periods as phasing darkness would reset to generate the inducible phase of rhythm during which the subsequent dark periods could induce flowering. Further study should be done on this idea using the other short-day plants mentioned above.

References

1) HAMNER, K. C. Interrelation of light and darkness in photoperiodic induction. Bot. Gaz. 101: 658–681 (1940).
2) HARDER, R. and G. GÜMMER. Über die Blütenbildung von *Kalanchoe blossfeldiana* in verchiedene Licht-Dunkel-Rhythmen. Planta, 37: 12–47 (1949).
3) HEIDE, O. M., R. W. KING and L. T. EVANS. The semidian rhythm in flowering response of *Pharbitis nil* in relation to dark period time measurement and to a circadian rhythm. Physiol. Plant, 73: 286–294 (1988).
4) KING, R. W., E. SCHÄFER, B. Thomas and D. VINCE-PRUE. Photoperiodism and rhythmic response to light. Plant Cell Enviro. 5: 395–404 (1982).
5) LUMSDEN, P., B. THOMAS, D. VINCE-PRUE. Photoperiodic control of flowering in dark-grown seedlings of *Pharbitis nil* Choisy. Plant Physiol. 70: 277–282 (1982).
6) OGAWA, Y. Über die photoperiodische Empfindlichkeit der Keimpflanzen von *Pharbitis nil* Chois. mit besonderer Berücksichtigung auf dem Wuchsstoffgehalt der Kotyledonen. Bot. Mag. Tokyo, 75: 92–101 (1962).
7) OGAWA, Y. Stimulation of the flowering in *Pharbitis nil* Chois. by gibberellin A_3: Time dependent action at the apex. Plant Cell Physiol. 22: 675–681 (1981).
8) OGAWA, Y. Rhythmic changes of photoperiodic flowering in *Pharbitis nil* Choisy. Bull. Fac. Bioresources, Mie Univ. No. 11: 177–183 (1993).
9) OGAWA, Y. and R. W. KING. Indirect action of benzyladenine and other chemicals on flowering of *Pharbitis nil* Chois. Plant. Cell Physiol. 63: 643–649 (1979).
10) PARASKA, J. R. and C. SPECTOR. The initiation of an endogenous rhythm affecting flower bud formation in *Pharbitis nil.* Physiol. Plant. 32: 62–65 (1974).
11) PAPENFUSS, H. D. and F. B. SALISBURY. Light promotion of flowering and time measurement in *Xanthium.* Z. Pflanzphysiol. 54: 195–202 (1966).
12) PAPENFUSS, H. D. and F. B. SALISBURY. Properties of clock resetting in flowering of *Xanthium.* Plant Physiol. 42: 1562–1568 (1967).
13) TAKIMOTO, A. and HAMNER, K. C. Effect of temperature and preconditions on photoperiodic response of *Pharbitis nil* Plant. Physiol. 39: 1024–1030 (1964).

暗明移行によるアサガオの花成反応の概日性リズムの誘発

小 川 幸 持
三重大学生物資源学部

アサガオ，品種キダチの幼植物を用いて，光周性花成の概日性リズムの誘発における位相暗期の長さ，中間明期の光の強度，外生のジベレリン A_3 (GA_3)，ベンジルアデニン (BA) およびインドール酢酸 (IAA) の影響を調べた。

位相暗期から明期へ1回の変化，すなわち1回の点灯信号によって，少なくとも48時間にわたって花成の概日性リズムが生じた。このリズムの誘発とリズムのパターンは GA_3, BA および IAA によって影響されなかった。しかし，このリズムの好適期の花成の程度は，GA_3, BA によって増大し，IAA によって減少した。4時間以上の位相暗期によって明らかなリズムが生じた。位相暗期 (16時間以下) が長いほど，リズムの好適期での花成の程度は大きくなった。5000 lux より低い光強度の中間明期で明らかなリズムが生じた。このリズムの好適期では，いずれの長さの誘導暗期によって花成が起ったが，不適期では，花成は弱いか殆ど起らなかった。

花成誘導に数回の暗期が必要な他の短日植物を用いて，初期の暗期と明期の交替を，花成の概日性リズムの好適期の誘発における位相暗期と中間明期の観点から調べる必要がある。