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Rhythmic Changes in Photoperiodic Flowering of *Pharbitis nil* Choisy

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Abstract

Flowering response to an inductive dark period in *Pharbitis* seedlings cultivar Kidachi grown under fluorescent light at night and sunlight in the day in a glasshouse depends on the time of an inductive dark period given.

The rhythmic alternation in the floral response is entrained to about a 24 h period and is reset daily by the transition from the fluorescent light to sunlight at dawn. The characteristics of the circadian rhythm are related to the duration of light after the transition between light of low and high intensity. Thus, under natural-light conditions Bünning's scotophilic or inducible phase would occur in the evening and *Pharbitis* plants could be induced to flower in response to dusk.

Key words: Circadian rhythm · Evening · Flowering · Light-on signal at dawn · *Pharbitis*

I. Introduction

The floral response of *Pharbitis nil* has been studied extensively. This plant can be induced to flower with a single dark period. The sensitivity to photoperiodic induction varies with light and temperature just before the dark period as well as with age of the plant[9,12]. The floral response of *Pharbitis* plants grown outdoors, being supplemented with fluorescent light at night, depends on whether or not the inductive dark period is given in the evening. However, plants generally subjected to daily alternation between sunlight and darkness often behave rhythmically with a period of about 24 h. If such a rhythm persists even when ambient lighting is constant, it is called an endogenous circadian rhythm. Such rhythmic phenomena have been reported on leaf, petal and stomatal movements and many other responses[3].

We studied circadian diurnal rhythmicity of flowering in response to an inductive dark period in *Pharbitis* plants, dwarf cultivar Kidachi which were grown in a glasshouse with supplemental light overnight. The flowering response of this Kidachi grown under alternation with sunlight and fluorescent light was greater than that under continuous fluorescent light[12]. This Kidachi plant forms a limited number of flower buds and no terminal flower bud at a normal temperature[12]. These features make suitable for examination of fluctuations in flowering by the exposure to inductive dark periods at different times at a normal temperature.

II. Materials and Methods

The experiments were carried out using the methods reported previously[11]. Seeds of *Pharbitis nil*,

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cultivar Kidachi, were treated with concentrated H_2SO_4 for 40 min, then washed thoroughly in running water for 20 h. Then they were allowed to sprout for 20 h on moist sand in a petri dish at 25°C. The seeds just germinated were planted in a clay pot filled with garden soil. The seedlings were grown for 5 or 6 days in a glasshouse under which light conditions were 30,000–100,000 lux of sunlight during the day supplemented with day-light fluorescent light of 1,500 lux at night and the temperature was kept at 28±2°C. At 18:00 (Japan Standard Time), all plants were transferred to the growth room with illuminance of 1,500 lux from fluorescent lamps (28°C) and placed for various durations ranging from 0 to 66 h until a 16-h inductive dark period was given. After the flower-inductive treatments plants were returned to the glasshouse, and raised for three weeks until dissection for determination of the flowering response. In some cases, the plants were grown under continuous illumination of the light from the fluorescent lamps giving an intensity of 2,500 lux. Light intensity was measured with a photocell illuminometer (SPI-5, Toshiba). Each experimental lot was consisted of 2 or 3 pots with 14 to 21 plants. Values of number of flower buds per plant in the figures are means ± the standard error of the mean.

III. Results

1. *Flowering in response to inductive dark period starting at various times*

Flowering in response to an inductive dark period given at different times (3 h intervals) preceded by 0 to 66 h of fluorescent light is shown in Fig. 1. The peaks of flowering response were found at 0 to 3 h, 24 and 45 h, and these are referred to as inducible phases. Flowering reached the bottom at 12 h, 33–39 h and 57–66 h, the non-inducible phases. This kind of fluctuations is typical pattern of an endogenous circadian rhythm. Flowering and shoot elongation of the cultivar of *Pharbitis* used in the present experiment were known to be stimulated dramatically by the treatment with gibberellin A_3 (GA_3) before the dark period[11]. The effects of exogenous GA_3 on the occurrence of rhythmic flowering were examined. An aqueous solution of GA_3 was

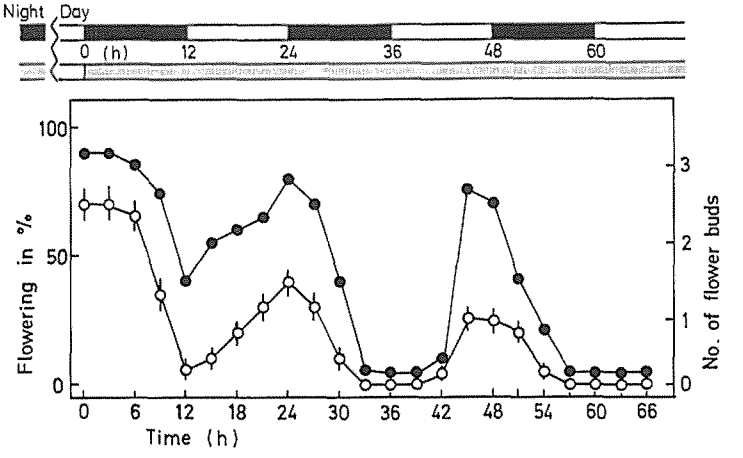

Fig. 1. Flowering of *Pharbitis* grown in the glasshouse in response to 16-h inductive dark period given at different times (0 to 66 h) preceded by fluorescent light. Percentage of plants flowering (●) and number of flower buds per plant (○). Night and day are indicated by closed square (■) and open square (□), respectively, and illumination with the fluorescent light is indicated by netted square (▨).

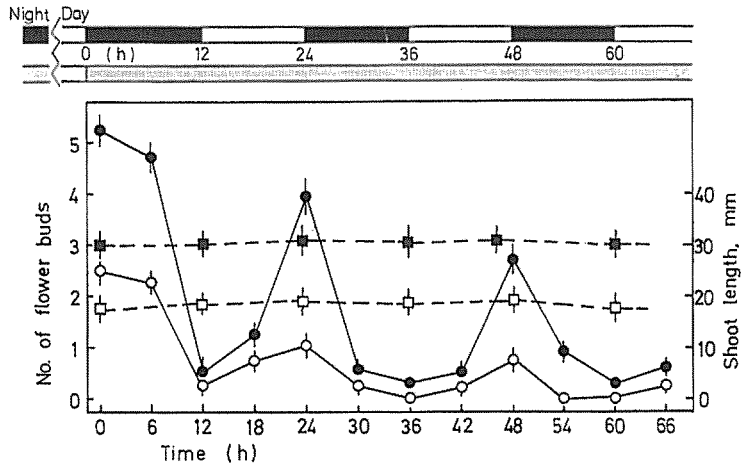

Fig. 2. Flowering (○ ●) and shoot length (□ ■) of *Pharbitis* grown in the glasshouse in response to 16-h inductive dark period given at different times preceded by fluorescent light (0 to 66 h). Immediately after the transfer to the fluorescent light some plants were given GA_3 (● ■) and the control plants were not (○ □). Night and day are indicated by closed square (▬) and open square (▭), respectively, and illumination with the fluorescent light is indicated by netted square (▨).

dripped on to the shoot apex (0.12 μg/apex) with glasscapillary tube immediately after the transfer of the plants from the glasshouse to the continuous fluorescent light. Control plants were given distilled water. As shown in Fig. 2, the rhythmic pattern of flowering was not affected by GA_3, but flowering was stimulated in the inducible phases. GA_3 also stimulated shoot elongation equally in both the inducible and the non-inducible phases. Circadian rhythm in flowering was also found in other cultivars of the same species (data not shown).

2. *Various durations of the inductive dark period*

Plants grown in the glasshouse were transferred at 18:00 to the room that provided an illuminance of 1,500 lux from the fluorescent lamps. They were exposed to an inductive dark period of various durations of 12 to 48 h, starting from different times of a day, 18:00 (0 h) to the same time of the following day (24th h). As shown in Fig. 3, inductive dark periods of 18 to 48 h starting at 18:00 on the first evening caused highest flowering

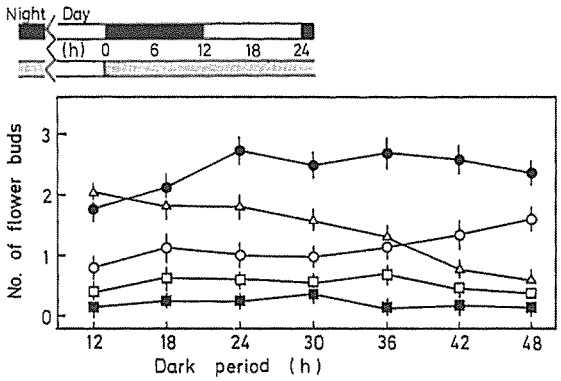

Fig. 3. Flowering of *Pharbitis* grown in the glasshouse in response to inductive dark period of various durations (12 to 48 h) given at different times preceded by fluorescent light; 0 h (●), 6 h (○), 12 h (■), 18 h (□) and 24 h (△). Night and day are indicated by closed square (▬) and open square (▭), respectively, and illumination with the fluorescent light is indicated by netted square (▨).

response. Twelve to 24 h inductive dark periods starting from the next evening still induced to flower at higher reseponse level, but lengthening dark periods resulted in less flowering. Plants exposed to dark periods starting from the 12th and 18th h flowered much less even after exposure to longer durations of darkness, although a slight increase of flowering occurred after a longer dark period starting from the 6th h.

3. *Comparison between plants grown in the glasshouse and those of continuous fluorescent light*

One group of plants grown for 6 days in the glasshouse was transferred at 18 : 00 to a continuous fluorescent light of 2,500 lux. Another group of plants was grown from planting to the same age under 2,500 lux fluorescent lights. Both were exposed to the inductive dark period at different times. As shown in Fig. 4, the plants grown in the glasshouse flowered, showing a clear rhythmic pattern, but those grown in continuous fluorescent light did not. This shows that the circadian rhythm of flowering of plants grown in the glasshouse for a few days was caused by the alternation between fluorescent light at night and sunlight during the day.

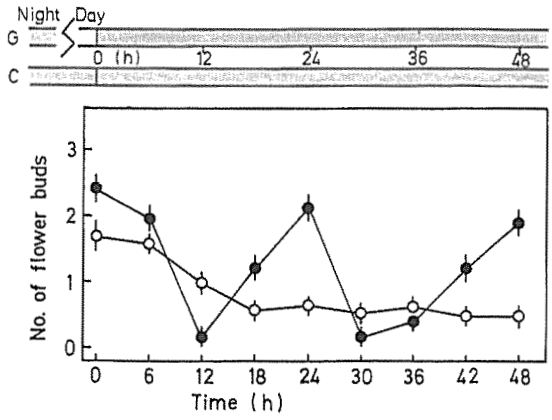

Fig. 4. Flowering of *Pharbitis* grown in the glasshouse (G, ●) or under fluorescent lights (C, ○) in response to the inductive dark period beginning at different times in continuous fluorescent light (0 to 48 h). Illumination with the fluorescent light and with sunlight are indicated by netted square (▨) and open square (□), respectively.

4. *Transition between low and high intensity light*

Plants grown in continuous fluorescent light of ca. 4,000 lux were divided into two groups. The one was exposed once to 12 h light of 30 lux (Fig. 5, No 1), and the other group was exposed twice to 12 h light of 30 lux, intervening 12 h light of 2,500 lux (Fig. 5, No 2). Both were then exposed to light of 2,500 lux for various durations before giving a 16-h inductive dark period. Control plants were exposed to 2,500 lux light for 12 and 36 h and without exposing to low intensity of 30 lux they were given the inductive dark period. The results are shown in Fig. 6. There was a circadian rhythm of flowering with peaks at 12 and at 36 h, the latter was usually greater than the former. This rhythm was the same pattern found in plants grown in the glasshouse (Figs. 1, 2 and 6). Thus, rhythmic flowering was caused by a single exposure to low intensity light.

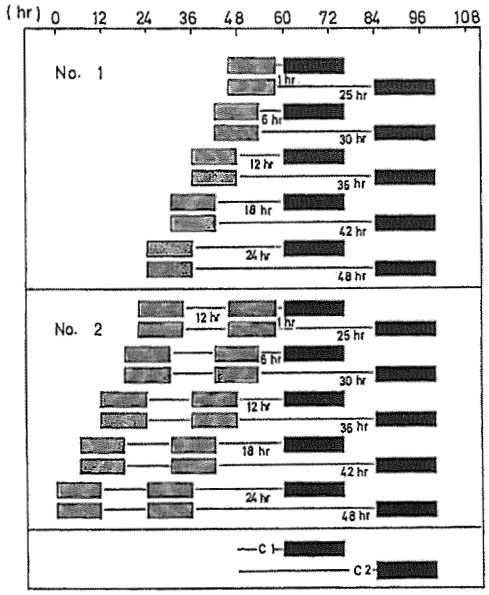

Fig. 5. Experimental schedule of exposure once (No 1) or twice (No 2) to 12 h light of 30 lux (▒) followed by fluorescent light of 2,500 lux for various durations from 1 to 48 h, and then a 16-h inductive dark period (■) was given. Control plants without exposing to 30 lux light received 2,500 lux light for either 12 h (C 1) or 36 h (C 2) preceding the inductive dark period. Lateral lines indicate 2,500 lux light period.

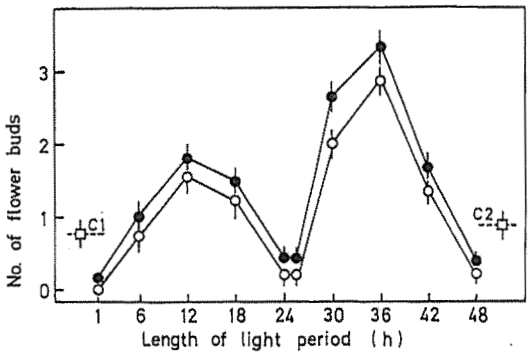

Fig. 6. Flowering of *Pharbitis* as a function of the duration of the fluorescent light (1 to 48 h) after once (○) or twice (●) exposure to 30 lux light. The control (C 1, C 2) and treatment scheme are outlined in Fig. 5.

IV. Discussion

To explain how diurnal rhythms control many phenomena including photoperiodic flowering, Bünning (1936, 1960)[1,2] proposed that there are two alternating phases, which are called photophilic (light loving) and scotophilic (dark loving). The former corresponds to the day phase and the latter to the night phase in a circadian rhythm. His theory was based, in part, on the observation that exposure to dark period during the scotophilic phase promoted flowering but inhibited it during the photophilic phase in short-day plants[3,4,5,6,7]. In the present study with *Pharbitis* plants grown in a glasshouse, flowering response to an inductive dark period fluctuated greatly with the time of exposure to the inductive dark period. The period of this oscillation was about 24 h, circadian, and in accordance with Bünning's hypothesis, higher levels of response was brought about greatest during the scotophilic phase (in the evening) and lower one was during the photophilic phase, (in the morning) (Fig. 1). This circadian pattern of rhythm was not affected by GA_3 applied to the shoot apex. GA_3 promoted flowering over the narrow inducible scotophilic phase, but shoot elongation was promoted equally in both phases (Fig. 2). Because GA_3 is only active in the flower induction taking place at the shoot apex of *Pharbitis*[11], these data taken imply that processes occurring at the shoot apex are not responsible for generation of the floral rhythm. However, a objectionable conclusion was suggested by King[10], who studied the effects of GA_3 on flowering in

another short-day plant, *Chenopodium rubrum*. In that instance daily temperature changes had established a circadian oscillation in cell division at the shoot apex. Also, the rhythmic responses found in *Chenopodium* after GA_3 was applied at different times might well occur in *Pharbitis* if a similar treatment was imposed.

If the dark period occurred during an inducible phase (0 or 24 h, Fig. 3), a relatively short dark period was enough to induce flowering (12 to 24 h dark period, Fig. 3), but even the dark period did not induce flowering if it began during a non-inducible phase (12 h, Fig. 3). These data show that there is a rhythmic change in the physiological state necessary for commencement of early floral processes in cotyledons, and that this state exists soon after the beginning of the dark period.

Paraska and Spector[14] and Heide et al.[8] reported that flowering of *Pharbitis* fluctuated rhythmically and depended on the length of light period after the seedling emerged from the soil. Various rhythmic physiological phenomena of early seedlings could be synchronized with light exposure time of seedling at emergence from the soil. As shown in the present experiments, the rhythm also is generated by exposure to alternation between night and day (Fig. 4), and between low and high intensities of light (Fig. 6).

The evidence presented here shows that flowering of *Pharbitis* plants in response to an inductive dark period is controlled by a clock with a circadian oscillation, which is reset by the alternations in light intensity as described by Bünning[2,3]. Papenfuss and Salisbury[13] showed that the clock in *Xanthium* could be rephased, suspended, restarted or delayed depending on the experimental conditions. Salisbury[15] also suggested that in *Xanthium* light only affects the operation of the clock, and that synthesis of flowering hormone occurs only when the clock is in an inducible phase. The diurnal flowering rhythm of *Pharbitis* must have been entrained to a circadian period with a different phase when the plants grown in the open air are exposed to sunlight at dawn. Bünning's scotophilic or inducible phase of the circadian rhythm would occur in the evening and flowering processes in the cotyledons of *Pharbitis* plants could start at dusk.

References

1) BÜNNING, E. Die endogenous Tagesrhythmik als Gründlage der photoperiodischen Reaktion. Ber. dt. Bot. Ges. 54: 590–607 (1936).
2) BÜNNING, E. Circadian rhythms and the time measurement in photoperiodism. *In* Biological Clocks, Cold Spring Harb. Symp. quant. Biol. 25: 249–256 (1960).
3) BÜNNING, E. The Physiological Clock. pp. 4–29, 54–78, 111–123. Springer-Verlag. Berlin (1964).
4) CUMMING, B. C. Circadian rhythms of flower induction and their significance in photoperiodic response. Can. J. Bot. 47: 309–325 (1969).
5) COULTER, M. W. and K. C. HAMNER. Photoperiodic flowering response of Biloxi soybean in 72 hour cycles. Plant Physiol. 39: 848–856 (1964).
6) ENGELMANN, W. Endogenous rhythmik und photoperiodische Blüteninduktion bei *Kalanchoe*. Planta, 55: 496–511 (1960).
7) HALABAN, R. The flowering response of coleus in relation to photoperiod and the circadian rhythm of leaf movement. Plant Physiol 43: 1894–1898 (1968).
8) HEIDE, O. M., R. W. KING and L. T. EVANS. The semidian rhythm in lowering response of *Pharbitis nil* in relation to dark period time measurement and to a circadian rhythm. Physiol. Plant. 73: 286–294 (1988).
9) IMAMURA, S. Photoperiodic induction and the floral stimulus. *In* Physiology of flowering in *Pharbitis Nil*. S. Imamura (ed.) pp. 15–28 Jap. Soc. Plant Physiol. Tokyo (1967).
10) KING, R. W. Multiple circadian rhythms regulate photoperiodic flowering responses in *Chenopodium rubrum*. Can. J. Bot. 53: 2631–2638 (1975).

11) OGAWA, Y. Stimulation of the flowering of *Pharbitis nil* Chois. by gibberellin A_3: Time dependent action at the apex. Plant Cell Physiol. 22: 675–681 (1981).
12) OGAWA, Y. Influences of radiation intensity, plant age and benzyladenine on photoperiodic flowering of *Pharbitis nil* seedlings. Bull. Fac. Bioresources, Mie Univ. 7: 83–90 (1992).
13) PAPENFUSS, H. D. and F. B. SALISBURY. Properties of clock resetting in *Xanthium*. Plant Physiol. 42: 1562–1568 (1967).
14) 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).
15) SALISBURRY, F. B. Xanthium strumarium L. *In* Induction of Flowering, Some Case History. L. T. Evans (ed.). pp. 14–61. MacMillan Comp. (Australia) (1969).

アサガオの光周性花成のリズム的な変化

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アサガオ，品種キダチを昼間は太陽光で（最高，3万–10万 lux），夜間は蛍光灯の照射下（約 1,500 lux）のガラス室で育てた幼植物を約 1,500 lux の連続照射下に置いて，各種の時間後，花成誘導暗期を与えた。

花成の強さは，暗期の開始時刻によって変化し，概日性リズムの変動を示した。この花成リズムは，ガラス室内で受けた朝明け時の蛍光灯から太陽光の照射光の変化（点灯信号），あるいは蛍光灯連続照明下で生育中に受けた低い照度（30 lux）から高い照度（2,500 lux）の変化によって生じた。

この実験結果から，戸外で生育しているアサガオの葉は，朝明けの太陽光の点灯信号によって花成反応の概日性リズムを生じ，夕方，Bünning 説の概日性リズムの好暗期に入り，薄暮になって暗期花成反応が開始すると思われる。