The effect of photoperiodic treatments on mitotic activity in *Pharbitis nil* Chois

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Abstract

The short-day plant, *Pharbitis nil*, requires only a single inductive cycle with a 16-hour dark period for flowering. The mitotic activity in the shoot apices was studied directly after the termination of the inductive photoperiod. A pronounced rise in the mitotic index was found in the 2nd and the 8th-14th hours. Control plants grown under noninductive conditions (continuous light, a light interruption in the middle of the dark period) did not flower and did not show an increased mitotic index. The increased mitotic activity in the shoot apices of *Pharbitis* seems to be causally connected with the phytochrome-controlled entry of the plants into the state of generative induction.

Key words: flowering response, mitotic index, photoperiodic induction, *Pharbitis nil*

INTRODUCTION

Photoperiodic plants are very useful models in investigations of flowering mechanisms because their mode of development can be environmentally controlled to either remain vegetative or start flowering. Usually the light/dark ratio is the key factor in evoking this transition. The two main factors involved in the plants photoperiodic flowering response are the phytochrome (P) system and the endogenous rhythm (Hamner and Hoshizaki 1974, Vince-Prue 1975). In short-day (SD) plants the phytochrome caused inhibition of flowering if a high P_{fr}/P_r ratio is maintained (Hendricks 1963). Reversion of P_{fr} to P_r

during the dark period must occur for the floral induction to proceed (Evans and King 1969). A light interruption in the middle of the inductive dark period reestablishes a high P_{fr}/P_r ratio and inhibits the plants flowering. Perception of a specific photoperiod occurs in the leaves or cotyledons (Hamner and Bonner 1938) and a floral stimulus is transmitted to the shoot apices. After arrival of a floral stimulus at the meristems one of the first events documented to take place is a rise in mitotic activity (Thomas 1963, Bernier 1971).

The present study analyzes the effects of various photoperiodic treatments on the mitotic activity in the main shoot apex of *Pharbitis*.

MATERIAL AND METHODS

Seeds of *Pharbitis nil* Chois cv. "Violet" (Mauretane Seed Co. Kyoto, Japan) were stirred with concentrated H_2SO_4 for 45 min., washed thoroughly, soaked for 20h in distilled water and placed in moist sand for 72h in a thermostat (25°C). The seedlings were then planted in pots containing soil and transferred to a growth chamber (25°C) with continuous light (8.3 W/m², cool-white fluorescent tubes) for 5 days. Eight-day seedlings were exposed to a single cycle of photoperiodic treatments shown in Fig. 1. The plants were treated with photoperiods as follows: 1) controls-continuous light (CL), 2) one inductive cycle — 8h light + 16h darkness (24h SD), 3) one inductive cycle with light break — 8h light + 16h darkness with 30 min light break in the middle of dark period (24h LB).

Fifteen plants subjected to each photoperiodic treatment were retained for floral dissection ten days later.

Fig. 1. Photoperiodic treatments; the light periods and light breaks are shown in white and the dark periods in black. 1 — 24h CL: 24h continuous light + 24h continuous light. 2 — 24h SD: 24h short day + 24h light, 3 — 24h LB: 24h short day with light break + 24h light

At the end of the dark period buds were collected at two hour intervals for 24h in continuous ligh (Fig. 1). Ten buds were analyzed for the mitotic index of each photoperiodic treatment.

The harvested apices were fixed in a 3:1 solution of ethanol: propionic acid for 48 hours, transferred to 70% ethanol and stored in a refrigerator until analyzed. The buds were hydrolyzed for 4h in 0.4 N HCl and stained by Feulgen's method (Gerlach 1972). Before preparing the microscopic slides the apices were cleared of all leaves and shoot parts. The cells were observed in a light microscope. The samples averaged 2000 cells each. Counts were taken of cells in division from late prophase to late telophase. The number of cells in division divided by the total number of cells present ($\times 100$) equals the mitotic index, and this was used to make all comparisons.

RESULTS

The floral responses of *Pharbitis* to the photoperiodic treatments are presented in Table 1. Plants subjected to 24h CL and 24h LB treatments remained vegetative. Plants receiving the 24h SD produced floral buds (Table 1).

The mitotic index for buds of plants subjected to photoperiodic treatments are presented in Fig. 2. The mitotic indexes for the shoot apices ranged from 1.5 to 4.8. The plants grown under noninductive conditions of continuous light showed low mitotic activity (Fig. 2-A).

Table 1
Effect of different photoperiods on flowering of *Pharbitis nil*

Variant	Floral buds per plant ($\pm$ standard error)	% Of flowering plants	% Of plants with apical flower bud
1. 24h CL	0	0	0
2. 24h SD	6.7 ± 0.3	100	100
3. 24h LB	0	0	0

A rapid increase in mitotic activity appeared in the apices of plants exposed to one inductive cycle in the second hour after the termination of the inductive photoperiod (Fig. 2-B). A second wide maximum of mitotic activity appeared after 8-14 hours (Fig. 2-B). An interruption of the inductive dark period by a half-hour light impulse inhibited flowering (Table 1). The mitotic activity of these plants was also low (Fig. 2-C), like that of vegetative plants (Fig. 2-A).

Fig. 2. Mitotic activity of the shoot apex of *Pharbitis nil* when subjected to specified photoperiodic treatments. The time refers to hours after the end of dark period of each photoperiodic treatment used. The numbers show the mitotic index. Each point represents the mean of the results from 10 apices

DISCUSSION

Plants exposed to 24h SD flower because the dark phase exceeds the critical dark period. Plants growing on continuous light and receiving the 24h LB treatment remain vegetative. The light interruption can shift the phytochrome to P_{fr} , which is inhibitory to flowering (Hendricks 1963, Vince-Prue 1975), and the remaining dark period is not long enough for reversion to P_r (Evans and King 1969), or the light interruption can occur during a light sensitive phase of the endogenous rhythm, inhibiting floral induction (Takimoto and Hamner 1964, Vince-Prue 1975, Palmer 1976). The mitotic activity of apices of plants from both 24h CL and 24h LB treatments is almost identical (Fig. 2-A and C). On the other hand, plants subjected to 24h SD show a sharp rise in mitotic activity and subsequently flower. Similar results were obtained by Simmons (1978) in his experiments with *Pharbitis nil* Kidachi, a dwarf gibberellin mutant. He found increased mitotic activity in induced plants in the 8th and 22nd hour after the termination of the inductive photoperiod.

Further investigations with the use of mitosis inhibitors seem necessary in order to explicitly answer question whether increased mitotic activity is the

cause or the result of the development of flower primordia in the shoot apices. On the other hand, the use of specific mitosis stimulators may throw some light on the nature of the floral stimulus moving from the cotyledons to the shoot apices. The recognition and identification of the hypothetic florigen (Chailakhyan 1936, Kopcewicz and Centkowska 1980) is the central problem of the investigations on the flowering phenomenon.

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Wpływ różnych fotoperiodów na związaną z kwitnieniem aktywność mitotyczną Pharbitis nil Chois.

Streszczenie

Roślina krótkiego dnia *Pharbitis nil* wymaga do zakwitania tylko jednego cyklu indukcyjnego z 16-godzinnym okresem ciemności. Aktywność mitotyczną w wierzchołkach wzrostu pędu badano co dwie godziny po zakończeniu fotoperiodu indukcyjnego. Stwierdzono wyraźny wzrost indeksu mitotycznego w drugiej godzinie oraz po 8-14 godzinach. Rośliny kontrolne znajdujące się w warunkach nieindukcyjnych (ciągle światło, przerwanie światłem okresu ciemności) nie zakwitwały oraz nie wykazywały wzrostu aktywności mitotycznej. Podwyższona aktywność mitotyczna w wierzchołkach wzrostu *Pharbitis nil* wydaje się być przyczynowo związana z kontrolowanym przez fitochrom wejściem roślin w stan indukcji generatywnej.