LABILE PHYTOCHROME AND PHOTOPERIODIC FLOWER INDUCTION IN *PHARBITIS NIL* CHOIS. THE IRREVERSIBLE PHYTOCHROME HYPOTHESIS

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ABSTRACT

Seedlings of *Pharbitis nil* cultivated under non-inductive conditions of white light were subjected to generative induction applying one 16-hour-long period of inductive night. During the eighth hour the night was interrupted with 1 min of red light pulse which completely inhibited the flowering. Treating the plants with KCN blocked the inhibiting effect of red light. Because KCN lowers considerably the rate of destruction of labile P_{fr}I in some plant systems, it seems probable that red light night-break irradiation (without KCN), which blocked the flowering, leads also to the accumulation of unknown P_{fr}I destruction products (irreversible phytochrome). It also suggests that it is not the labile P_{fr}I itself but the products of its irreversible transformation that could be active in the photoperiodic control of flowering.

KEY WORDS: flower induction, phytochrome, KCN, *Pharbitis nil*

INTRODUCTION

Studies of phytochrome and photoperiodic induction have been intimately linked since the discovery of phytochrome by Borthwick, Hendricks and co-workers almost 40 years ago. One of the earliest phytochrome action spectra was for the night-break flowering response in such short day plants (SDP) as *Pharbitis* and *Xanthium*. Red/far-red photoreversibility of the night break was also demonstrated (Vince-Prue 1975). Since then the question of how phytochrome interacts with the time measuring system has been the main focus of interest in this field. Analyses of phytochrome action in photoperiodism have resulted in concluding that two or more pools of phytochrome (P) with different stability as P_{fr} (PI – labile, PII – stable) are involved in photoperiodic timekeeping (Thomas 1991). In SDP a light treatment (night-break) applied in the middle of an extended dark period can prevent flowering. It suggests that newly formed P_{fr}I is responsible for that effect. On the other hand, it might be predicted that the level of this phytochrome population would be, shortly after light night-break or during light phase, very low because of continuous P_{fr}I destruction and inhibition of PI synthesis by light (Rom-bach et al. 1982, Thomas 1991). So serious doubts exist if P_{fr}I is an appropriate candidate for the molecule that detects the end-of-day responses and inhibits photoperiodic flower induction in *Pharbitis*. Because KCN considerably lowers the rate of destruction of P_{fr}I in some plant systems (Furuya et al. 1965, Kopcewicz et al. 1983), we have decided to use this compound in order to stabilise P_{fr}I after a night-break and to determine if such a treatment can change the flowering response of *Pharbitis*.

MATERIAL AND METHODS

Seeds of *Pharbitis nil* Choisy cv. Violet (Marutane Seed Co., Kyoto, Japan) were stirred with concentrated H₂SO₄ for 45 min, rinsed well and imbibed for 20 h in distilled water (25°C). The swollen seeds were sown in sterile sand and after being transferred into a thermostat were left in the dark at 25°C for a period of 2 days. The seedlings were then planted in pots filled with perlite: sterile sand (2:1), 5-7 in each, and the pots transferred to a growth chamber at 25°C with continuous light (18.3 W m⁻², cool white fluorescent tubes, Polam, Warszawa, Poland) for 3 days. The plants were then exposed to (1) continuous light; (2) 16 h of darkness (inductive night, IN); (3) IN + KCN; (4) IN + red light night-break in the middle of dark period (R); (5) IN + R + KCN. For the red light night-break irradiation (1 min) a lamp with 2500 W Xenon burner equipped with a monochromatic filter ($\lambda_{\max}$ = 660 nm, $\lambda_{1/2}$ = 9 nm, T = 57%) was used. The irradiance was 1.38 W m⁻².

KCN (10⁻² – 10⁻⁵M) was applied at two hours' intervals during 16 h inductive dark period. The water solution of the compound was painted on the cotyledons with a small brush (100 μ l per plant). The control plants (IN, IN + R) were treated with distilled water. All manipulations with plant material and treatments were performed under safe dim-green light. After the completion of treatments the plants were transferred to a growth chamber and grown under continuous light at 25°C for 10 days. The number of flower buds on the plants were then established. Fifteen plants were used for each experiment, which was repeated at least 5 times.

RESULTS AND DISCUSSION

In SDP a night-break irradiation applied during an extended dark period can prevent flowering. The timing of the night-break irradiation is, however, crucial. In *Pharbitis* the sensitivity to red light treatments increases among 5 and 8.5 h and then decreases until 12 h (Thomas 1991). It has been postulated that phytochrome can cause inhibition of flowering in SDP if a high P_{fr}/P_{tot} ratio is maintained. A light interruption in the middle of the inductive dark period re-establishes a high P_{fr}/P_{tot} ratio and inhibits flowering (Evans and King 1969). More recently, however, it has been stated that two or more pools of phytochrome with different stability as P_{fr} are involved in the photoperiodic control of flowering (Thomas and Lumsden 1984). Labile P_{frI} is probably taking part in the control of night-break inhibition of flowering and more stable P_{frII} (in form of hetero- and homodimer) is controlling end-of-day responses and promotive to flowering reactions during an inductive long night (Thomas 1991). When P_r absorbs light and is photoconverted to P_{fr} or vice-versa, it passes through a series of intermediates (Kendrick and Spruit 1977). Under continuous illumination, considerable part of phytochrome molecules will be in the form of photoconversion intermediate. Short or long illuminations also cause very fast destruction of P_{frI} . The phytochrome is accumulated in plants growing in darkness and is rapidly destroyed when they are transferred to light. P_{fr} destruction in a dark grown plants which receive a brief irradiation is rapid with a half-life of 1-2 h (Cherry et al. 1991). The destruction of P_{frI} , as requiring ATP process, could be, at least partly, blocked by inhibitors of energy-producing reactions such as KCN, 2,4-DNP or antimycin A (Furuya et al. 1965, Kopcewicz et al. 1983). So, we decided to use KCN in order to stabilize P_{frI} after night-break illumination. The results (Table 1) show that short (1 min) red light night-break has caused inhibition of flowering in *Pharbitis*. Interestingly, KCN has blocked the inhibiting effect of red light on both lateral and terminal flower buds formation. This effect is dependent on both concentration and time of KCN application. KCN is not effective if is applied together with red light. The most effective is KCN applied during a few hours around middle of inductive dark period at concentration of $10^{-4}M$ (Table 2). So, KCN may lower the destruction rate of phytochrome causing that P_{frI} is relatively longer stable and the destruction products of this phytochrome are not available. It could also mean that it is not P_{frI} itself but its destruction or irreversible transformation products (which we call irreversible phytochrome – P_n) that are involved in the control of night-break inhibition of flowering. Taking the

TABLE 1. The effect of KCN ($10^{-4}M$) on the red light night break (R) inhibition of flowering in *Pharbitis nil*. R was applied at 8th h, and KCN at 4, 6, 8, 10 and 12th h of IN. Values are means $\pm$ SE, (n = 75).

Treatments	Flower buds per plant	% of flowering plants*	% of plants with terminal flower bud
Continuous light	0.0	0	0
Inductive night (IN)	6.7 $\pm$ 0.2	100	85
IN + KCN	6.3 $\pm$ 0.3	100	83
IN + R	0.2 $\pm$ 0.0	0	0
IN + R + KCN	6.1 $\pm$ 0.3	100	81

*more than 3 flower buds

physiological activity of irreversible phytochrome under the consideration we suggest the following model of phytochrome control of generative induction in *Pharbitis*:

1. in etiolated seedlings there is only a labile P_rI form of phytochrome,
2. the first flash of light causing the photoconversion of P_rI into P_{frI} initiates at same time its destruction and irreversible phytochrome accumulation – the factor that blocks flower induction,
3. P_{frI} and/or P_n starts the synthesis of stable phytochrome (P_{frII}),
4. during the inductive long night the level of P_n decreases as a result of the lack of photoconversion of P_rI into P_{frI} . It is symptomatic that low temperature that can probably stabilize the level of P_n during the first part of inductive night is also inhibitory to flower induction. Second part of inductive night is clearly less sensitive to low temperature (Table 3).

TABLE 3. Effect of temperature during inductive night (IN) on flowering in *Pharbitis nil*. Values are means $\pm$ SE, (n=75). R, red light night-break.

Temperature during IN	Flower buds per plant
16h 25°C	6.7 $\pm$ 0.2
16h 25°C+ R	0.0
8h 10°C + 8h 25°C	0.0
8h 25°C + 8h 10°C	2.5 $\pm$ 0.3

TABLE 2. The time course of different concentrations of KCN application during inductive night (IN) on the red light night-break (R) inhibition of flowering in *Pharbitis nil*. R was applied at 8th h of IN. Values are means $\pm$ SE, (n=75).

Hours of KCN application	Flower buds per plant					IN + R	IN
	IN + R + KCN $10^{-2}M$	IN + R + KCN $10^{-3}M$	IN + R + KCN $10^{-4}M$	IN + R + KCN $10^{-5}M$			
8	0.0	0.0	0.0	0.0			
6	0.0	0.9 $\pm$ 0.1	1.8 $\pm$ 0.1	0.0			
6, 8	0.0	1.8 $\pm$ 0.3	2.2 $\pm$ 0.2	0.0	0.0		6.7 $\pm$ 0.2
6, 8, 10	0.2 $\pm$ 0.1	3.8 $\pm$ 0.3	3.9 $\pm$ 0.3	0.0			
4, 6, 8, 10, 12	0.0	5.2 $\pm$ 0.3	6.1 $\pm$ 0.3	0.2 $\pm$ 0.1			
0, 2, 4, 6, 8, 10, 12, 14, 16	0.1 $\pm$ 0.0	4.8 $\pm$ 0.4	4.7 $\pm$ 0.3	0.1 $\pm$ 0.0			

5. Stable $P_{fr}II$ (probably homodimer $P_{fr} : P_{fr}$), which is produced during light phase, causes the formation of the flowering stimulus during a non-interrupted long night. Thus it seems highly probable that the level of a stable form of phytochrome ($P_{fr}II$) could be similar during both light- and dark-phases of inductive photoperiod (Fig. 1). During a light-phase a newly created $P_{fr}I$ is undergoing fast destruction or transformation which leads to the accumulation of P_n . The high level of P_n at that time blocks flowering.

During a non-interrupted long dark phase the level of P_n goes down, and the promotive action of $P_{fr}II$ is not disturbed

(Fig. 1, Fig. 2A). In such a case the flowering occurs. In the case of night-break light interruption, the newly synthesised $P_{fr}I$ is photoconverted to $P_{fr}II$ that undergoes irreversible transformation to P_n . Depending on the time of red light night-break irradiation the flower induction could be blocked (Fig. 2). Night-break during the first hours of long inductive night is not effective since even a low level of $P_{fr}I$ gives after the transformation only a slight increase in the level of the existing P_n (Fig. 2B). Also non-effective is a night break applied towards the end of a long night (Fig. 2D). The state of generative induction becomes an irreversible fact then and a high level of P_n is already not able to change it. The most effective

Fig. 1. The scheme of hypothetical transformations of different forms of phytochrome during light (A) and dark (B) phases in *Pharbitis nil*. Abbreviations: $P_{fr}I$, red absorbing form of labile phytochrome; $P_{fr}II$, far-red absorbing form of labile phytochrome; $P_{fr}II$, stable form of P_{fr} ; P_n , hypothetical irreversible phytochrome, product of the $P_{fr}I$ destruction.

Fig. 2. The scheme of hypothetical time course of the red light night-break effect on phytochrome and flowering in *Pharbitis nil*. A. non-interrupted dark phase, B. first hours of dark phase, C. middle hours of dark phase, D. last hours of dark phase. Abbreviations: R, red (light); $P_{fr}I$, red absorbing form of labile phytochrome; $P_{fr}II$, far-red absorbing form of stable phytochrome, P_n , hypothetical irreversible phytochrome.

tive, however, is the night-break applied somewhere around the middle of the long inductive night (Fig. 2C). The state of generative induction has not become an irreversible fact yet and a high level of accumulating P_n effectively inhibits the flowering induction stimulated by $P_{fr}II$.

Thus the generative induction in *Pharbitis* is likely to occur when for some necessary period of time (about 10 h) during a long inductive night the stimulating for flowering action of $P_{fr}II$ is not going to be blocked by the P_n . This period, crucial for flowering, is called "active night" (Fig. 2). Breaking the inductive night in the middle radically shortens the active night and is the cause of inhibition of flowering. The proposed model of phytochrome transformations (Fig. 1 and Fig. 2) is at the moment mostly hypothetical. All these hypotheses have to be experimentally verified. On the other hand, however, this model is an agreement with physiological observations (Vince-Prue 1975, Thomas 1991) and seems to explain the participation of different phytochrome populations in the control of flower induction. Pratt et al. (1974) postulate that the process of phytochrome destruction may be the result of (1) compartmentation of the plant cell, (2) association of phytochrome with cell membranes or cell organelles, (3) decomposition of a chromophoric or protein part of pigment. Thus the destruction of $P_{fr}I$ is a complex process involving biochemical modification and subcellular relocation. The redistribution of phytochrome from cytoplasmatically dispersed to being sequestered in discrete areas of the cell following irradiation appears to be an early stage in the destruction process. The mechanism of phytochrome destruction is mostly unknown. The role of sequestering and ubiquitination are postulated. Both sequestering and ubiquitination require ATP (Cherry et al. 1991, Thomas 1991a). It seems possible, however, that one of the early stages of $P_{fr}I$ destruction is the irreversible transformation of this phytochrome to unknown intermediate (P_n) which takes part in the control of photoperiodic flower induction in *Pharbitis* and possibly also in other SDP. The idea of existence and physiological activity of irreversible phytochrome could be very useful in the further search for the mechanism of phytochrome control in the photoperiodic flower induction. This hypothesis has to be experimentally verified. Such investigations are presently under way.

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FITOCHROM LABILNY A FOTOPERIODYCZNA INDUKCJA KWITNIENIA *PHARBITIS NIL* CHOIS. HIPOTEZA FITOCHROMU NIEODWRACALNEGO

STRESZCZENIE

Siewki *Pharbitis nil* uprawiane w warunkach nieindukcyjnego ciągłego oświetlenia, poddano indukcyjnej 16-godzinnej nocy. Podczas ósmej godziny ciemności noc przerwano 1 minutowym pulsem światła czerwonego, które całkowicie hamuje kwitnienie. KCN blokuje hamujące działanie czerwieni. Ponieważ KCN obniża tempo destrukcji fitochromu labilnego $P_{fr}I$, wydaje się, iż hamowanie kwitnienia przez światło czerwone (bez KCN) jest związane z nagromadzeniem się produktów destrukcji (fitochrom nieodwracalny) $P_{fr}I$. Sugeruje to, że nie fitochrom labilny ale jego produkty destrukcji są aktywne w kontroli indukcji generatywnej u *Pharbitis*.

SŁOWA KLUCZOWE: indukcja kwitnienia, fitochrom, KCN, *Pharbitis nil*.