ABA IN PHOTOPERIODIC INDUCTION OF *PHARBITIS NIL*

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ABSTRACT

The endogenous content of ABA in the cotyledons of *Pharbitis nil* is high during the light phase before an inductive 16-h-long dark period. During the night, however, at the beginning, the level of ABA is relatively low with the tendency to increase during the second half of an inductive dark period. The dual effect of exogenous ABA on the *Pharbitis nil* flowering has been observed. ABA applied to the cotyledons on subthreshold photoperiod (12-h-long night) stimulates flower bud formation. On the other hand, however, ABA applied during an inductive (16-h-long) dark period, as well as applied to the medium of cultured plantlets, inhibits flowering. Thus, the flowering effect of ABA is clearly dependent on the state of flower induction which is different in plants growing on various photoperiods.

KEY WORDS: flowering, abscisic acid, photoperiodic induction, *Pharbitis nil*.

INTRODUCTION

The evidence of abscisic acid (ABA) involvement in the regulation of flowering was first established in the experiments with short-day plants (SDP) growing on marginally inductive photoperiods (Krekule, Horavka 1972, Vince-Prue, Gressel 1985). Under such conditions ABA often stimulates flowering. ABA could not however induce flowering in SDP under quite non-inductive conditions (Nakayama, Hashimoto 1973). In literature there are also data concerning the inhibitory effects of ABA on flowering (Kinet et al. 1975). There are also discrepancies in data concerning the endogenous ABA level. ABA content is usually higher in mature plants than in juvenile ones (Nakayama, Hashimoto 1973, Podolnyi et al. 1989). No differences in ABA level between induced and noninduced *Perilla* leaves have been also found (Purse 1984).

Thus, there is a lack of clear view on the role of ABA in the photoperiodic induction of flowering. Therefore we decided to investigate the endogenous ABA content and the effects of exogenous abscisic acid on *Pharbitis nil* flowering in intact seedlings and in vitro cultured plantlets.

MATERIAL AND METHODS

Plant material

Seeds of *Pharbitis nil* Chois. cv. Violet (Marutane Seed Co., Kyoto, Japan) were stirred with concentrated H_2SO_4 for 1 hour, rinsed well and imbibed for 24 h in distilled water (25°C). The swollen seeds were sown into pots filled with perli-

te: sterile sand (2 : 1), and the pots were transferred to a growth chamber at 25°C with continuous irradiance (18.3 W m⁻², cool white fluorescent tubes, Polam, Warsaw, Poland) for 4 days. The plants were then treated with ABA (10⁻³ and 10⁻⁵ mol dm⁻³) and exposed to 12 h or 16 h long nights. ABA was applied to the cotyledons at 2 h or 4 h intervals before and during 12 h subinductive dark period or during 16 h inductive one. The water solutions of ABA were painted with a small soft brush (100 mm³ per plant). The control plants were treated with distilled water. After the completion of treatments the plants were grown in a growth chamber under continuous light at 25°C for 14 days. The number of floral buds per plant and the percentage of plants exhibiting terminal flowering were then determined using dissection microscope. The length of plants was also measured. Fifteen plants were used in each experiment, repeated at least 3 times. Standard errors were calculated.

Culture of shoot tips in vitro

Seeds of *Pharbitis nil* Chois. cv. Violet subjected to acid treatment were additionally sterilized with 15% solution of NaOCl for 20 min and washed well in sterile water. Then the seeds were left to stand for 24 h in distilled water and then sown on Murashige and Skoog's (MS) medium (1962) supplemented with 3% sucrose and 0.25% Gelrite (Merck). Seeds were germinated and then seedlings were cultivated in a growth chamber at 25°C ± 1°C under continuous light (18.3 W m⁻² cool white fluorescent tubes) for 7-8 days.

The apices (about 7 mm in length) were excised from the seedlings and each apex was cultured on 10 cm³ of MS me-

dium with 5% sucrose, 0.25% Gelrite without (control) or with ABA (10^{-7} - 10^{-5} mol dm $^{-3}$).

Apex cultures were maintained in growth chambers under continuous light at constant temperature of $25 \pm 1^\circ\text{C}$. The cultured apices with the first developed leaves (3-4 days after excision) were exposed to several different photoperiods. Photoperiods with 12, 14 and 16 h of darkness, sometimes repeated several times (2, 3 or 4), were used. The non-induced (0 or 8 h darkness), partly induced (12 h x 3, 14 h x 3, 16 h x 1, 16 h x 2) and strongly induced (16 h x 3, 16 h x 4) explants were selected. After the photoperiodic treatment plantlets were cultured during 3 additional weeks in a growth chamber under continuous light at 25°C . Flowering responses of the developed plantlets were expressed as the percentage of flowering plantlets (A), the percentage of plantlets with a terminal flower (B), and the number of flower buds per plantlet (C). All the experiments were repeated 3 times with 8-12 replicates for each treatment and standard errors were calculated.

Determination of endogenous ABA

Free ABA was extracted from frozen plant material (1.5 g of cotyledons) by 80% methanol, two parts of 15 cm 3 each. After the filtration the crude extract was passed through Baker-Bond Spe octadecyl C-18 column (J. T. Baker Inc. Phillipsburg, NJ 08856 USA) and evaporated to dryness in a stream of nitrogen. After the methylation with N-methyl-N-nitroso-4-toluenesulfonamide ABA in the residue was determined by gas chromatography on Shimadzu GC-14A equipped with a capilar column (0.32 mm in diameter, 25 m long) packed with SE-54-DF0.50. In all the cases the nitrogen carrier gas flow rate was 2 cm 3 min $^{-1}$, the injector heaters were set at $+260^\circ\text{C}$ and the oven temperature was $+200^\circ\text{C}$. The retention time of ABA was 8 min 8 s. The amount of ABA was established on the basis of recorder readings, calculating the area of the ABA peak. A quantitative estimations were made by comparing the peak areas of endogenous and standard ABA. The presented results were obtained from three independent experiments. The results were statistically evaluated and LSD was calculated at a significance level of 0.01 and 0.05.

RESULTS

The content of endogenous ABA in *Pharbitis nil* cotyledons is high under the light before an inductive long night. It is decreasing during the period from the 8th hour before 16-h-long night and reaches the minimum level at the 4th hour of darkness. Then the level of ABA is going up till the 12th hour of an inductive night (Fig. 1).

The application of exogenous ABA to the cotyledons of six day old intact seedlings at different times of 12-h-long subinductive dark period resulted in the slight stimulation of flower buds formation. Control plants had no flower buds at all and ABA-treated ones had about 1 flower bud per plant (Fig. 2). On the other hand the application of ABA during 16-h-long inductive night caused the inhibition of flowering. Control plants had 5.2 flower buds per plant (Fig. 3) and ABA-treated, especially at the 4th hour of darkness, had about 2 flower buds. Also, terminal flowering was reduced from 80% for control plants to 25% for ABA treated ones (Fig. 4). ABA application during the second half of inductive darkness was almost without any effect on flowering.

The *Pharbitis nil* plantlets cultured on continuous light (0) or on 8-h-long period of darkness (8 h x 1) remained comple-

Fig. 1. The content of endogenous ABA in *Pharbitis nil* cotyledons before and during 16-h-long inductive night.

tely vegetative (Fig. 5). Three 12-h or 14-h as well as one or two 16-h-long periods of darkness caused partial flower induction. A photoperiod with 16-h-long night repeated three or four times caused strong induction of flowering. The results (Fig. 5A, B, C) show that ABA treatment is without any effect on the flowering of plants cultivated under non-inductive conditions (0 or 8 h x 1). On the other hand, however, in partly induced plants (12 h x 3, 14 h x 3, 16 h x 1, 16 h x 2) and sometimes even in strongly induced ones (16 h x 3) ABA inhibited the number of flowering plants (Fig. 5A), the formation of terminal flowers (Fig. 5B) and the number of flo-

Fig. 2. Flowering response of *Pharbitis nil* seedlings to ABA application during 12-h-long subinductive dark period. The data are average for 3 replicates (15 plants each) $\pm$ SE. Control plants had (0) flower buds per plant.

Fig. 3. Flowering response of *Pharbitis nil* seedlings to ABA application during 16-h-long inductive dark period. Data are average for 3 replicates (15 plants each) $\pm$ SE. Control plants had 5.2 flower buds.

Fig. 4. Terminal flowering of *Pharbitis nil* seedlings after ABA application during 16-h-long inductive dark period. Data are average of 3 replicates (15 plants each). Control plants had 80% of terminal flowering.

wer buds per plantlet (Fig. 5C). ABA at the concentration of 10^{-5} mol dm^{-3} inhibited completely the flower bud formation (Fig. 5A, B, C) and reduced, also at the concentration of 10^{-6} mol dm^{-3} , the elongation growth of *Pharbitis nil* plantlets shoots (Table 1).

DISCUSSION

Since cotyledons or leaves are the main site of daylength perception while flowers develop in the shoot apices, the production at the leaf of a signal that acts in a remote place at the apex appears to be critical for the floral transition to occur. A long distance signaling system would also account for the

Fig. 5. Effects of ABA on the floral bud formation in *Pharbitis nil* apex cultures exposed to varying photoperiodic conditions.

- A. The percentage of flowering plantlets.
- B. The percentage of plantlets with a terminal flower.
- C. The number of flower buds per plantlet.

Apex explants were cultured on MS medium without (O) or with ABA under continuous light. When the apices developed the first leaves, they were exposed to various photoperiods. The plantlets were further cultivated under continuous light for 3 weeks, then the flowering response was estimated.

correlative influences that are implicated for the control of flower transition in all types of the plant. Chailakhyan in 1936 postulated that the flowering process is under the control of florigen, a unique and universal flower hormonal factor. This theory was later completed by the suggestion that an

TABLE 1. The influence of ABA on the stem elongation of *Pharbitis nil* plantlets exposed to various photoperiods.

Dark period [h] ABA [mol dm ⁻³]	Stem length [cm]							
	0	8 x 1	12 x 3	14 x 3	16 x 1	16 x 2	16 x 3	16 x 4
0	15.5± 2.8	15.0± 6.0	14.1± 3.6	15.6± 3.1	16.0± 3.7	16.6± 2.0	18.0± 1.2	24.5± 5.0
10 ⁻⁷	16.1± 3.0	14.0± 2.0	13.8± 3.0	13.5± 2.3	15.0± 2.4	16.0± 3.0	16.9± 1.0	19.5± 4.0
10 ⁻⁶	9.7± 3.2	5.8± 1.2	5.0± 1.2	4.5± 0.9	6.0± 0.3	7.7± 2.8	5.5± 0.7	8.5± 1.1
10 ⁻⁵	3.5± 0.2	4.4± 0.2	1.6± 0.4	1.0± 0.2	3.6± 0.2	2.2± 0.1	4.0± 0.2	5.5± 0.8

Apex explants were cultured on MS medium without (O) or with ABA under continuous light. When the apices developed the first leaves, they were exposed to various photoperiods. The plantlets were further cultivated under continuous light for 3 weeks, then their elongation growth was measured.

additional hormonal factor, the antiflorigen, should be also implicated (Lang et al. 1977). However, all the attempts to isolate and identify these flower hormones failed, leading to the emergence of alternate hypotheses. Among others Bernier, (1988) postulated that flower transition involves a multifactorial system including different widely known hormones and other substances acting in a harmonized fashion either simultaneously or sequentially to trigger different steps of flowering process. Thus, the participation of ABA, compound which is taking part in the regulation of many physiological processes, could not be excluded, either. The early information on the ABA involvement in the regulation of flowering appeared from the experiments with plants grown on marginally inductive photoperiods (El-Antably 1966). Under such conditions ABA stimulates flowering of *Pharbitis nil* (Vince-Prue, Gressel 1995), *Chenopodium rubrum* (Krekule, Horavka 1972, Krekule, Ullman 1971), *Plumbago* (Nitsch 1967, Nakayama, Hashimoto 1973), *Cannabis sativa* (Galoch 1980) and *Torenia* (Tanimoto, Harada 1981). These results have been confirmed in our experiments (Fig. 2). ABA application to *Pharbitis* cotyledons on subinductive photoperiod slightly stimulates the flower bud formation. On the other hand, however, ABA applied during 16-h-long inductive darkness inhibits flowering (Figs 3 and 4). Inhibition is especially strong during the first half of an inductive night. ABA application during later hours of darkness has been much less effective. Strong ABA inhibition of flowering at the 4th hour of darkness corresponds to low endogenous ABA content (Fig. 1). This suggests that the application of ABA during the second half of 16-h-long dark period is low effective because the endogenous content of native ABA at that time is already comparatively higher (Fig. 1). The inhibitory effect of ABA treatment on *Pharbitis nil* flowering was reported previously also by Tretyn et al. (1994). They found that ABA decreased the flowering response if applied when cotyledonary stomata were open. ABA can probably induce an increase in calcium level which precedes stomatal closure. Because ABA can modulate flowering and at the same time it is a factor regulating stomatal closure, the role of stomata movement in the mechanism of flower induction cannot be excluded.

All the obtained results seem to support the idea, that abscisic acid is involved in the control of flowering. However, the question remains whether it is involved directly in the control of flower induction or indirectly through the effect on metabolic or others (e.g. stomatal movements) phenomena. Flowe-

ring is a multistage process composed of sequences of events temporally and spatially ordered. Absciscic acid could be simply involved in the regulation of a particular event which is necessary if flowering could take place. Further investigations on the mechanism and site of ABA action in flowering are under the way.

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LITERATURE CITED

- BERNIER G. 1988. The control of floral evocation and morphogenesis. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 39: 197-219.
- CHAILAKHYAN M. Kh. 1936. On the hormonal theory of plant development C.R. /Dokl. Akad. Sci. URSS 3: 443-447.
- EL-ANTABLY H. M. M., WAREING P. F. 1966. Stimulation of flowering in certain short - day plants by abscisin. *Nature* 210: 328-329.
- GALOCH E. 1980. The hormonal control of sex differentiation in dioecious plants of hemp (*Cannabis sativa*) II. The effect of plant growth regulators upon development of excised stem tips cultured in vitro. *Acta Physiol. Plant.* Vol. II, No. 1: 3-7.
- KINET J. M., BODSON M., JACQMARD A., BERNIER G. 1975. The inhibition of flowering by abscisic acid in *Sinapis alba* L. *Zeitschrift für Pflanzenphysiologie*, Band 77, Heft 11: 70-74.
- KREKULE J., HORAVKA B. 1972. The response of short day plant *Chenopodium rubrum* L. to abscisic acid and gibberellic acid treatment applied at two levels of photoperiodic induction. *Biol. Plant.* 14: 254-259.
- KREKULE J., ULLMAN J. 1971. The effect of abscisic acid on flowering in *Chenopodium rubrum* L. *Biol. Plant.* 13: 60-63.
- LANG A. M., CHAILAKHYAN M. Kh., FROLOVA A. 1977. Promotion and inhibition of flower formation in day - neutral plant. *Proc. Nat. Acad. Sci. (USA)* 74: 2412-2416.
- MURASHIGE T., SKOOG F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 15: 473-497.
- NAKAYAMA S., HASHIMOTO T. 1973. Effect of abscisic acid on flowering in *Pharbitis nil*. *Plant and Cell Physiol.* 14: 419-422.
- NITSCH C., NITSCH J.P. 1967. The induction of flowering in vitro in stem segments of *Plumbago indica* L. II. The production of reproductive buds. *Planta* 72: 371-384.

- PODOLNYI V. Z., JOSEFUSOVA Z., KHELMISKAYA J., WEREDNIKOW S., KREKULE J., CHAILAKHYAN M. Kh. 1989. Absciseic acid as a potent regulator of the transition from juvenile to mature stage in *Xanthium strumarium*. *Biologia Plantarum* (Praha) 31: 139-144.
- PURSE J.G., 1984. Phloem exudate of *Perilla crista* and its effect on flowering of *Perilla crista* shoots explants. *J. Exp. Bot.* 35: 227-238.
- TANIMOTO S., HARADA H. 1981. Chemical factors controlling floral bud formation of *Torenia* stem segments cultured in vitro II. Effect of growth regulators. *Plant and Cell Physiol.* 22/3: 543-550.
- TRETYN A., CZAPLEWSKA J., CYMERSKI M., KOPCEWICZ J., KENDRICK R. E. 1994. The mechanism of calcium action on flower induction in *Pharbitis nil*. *J. Plant Physiol.* Vol. 14: 419-422.
- VINCE-PRUE D., GRESSEL J. 1985. *Pharbitis nil* Chois. in: Halevy A. H. Handbook of Flowering. Boca Raton CRC Vol. IV: 47-81.

ABA W INDUKCJI FOTOPERIODYCZNEJ U *PHARBITIS NIL*

STRESZCZENIE

Zawartość endogennego ABA w liściach *Pharbitis nil* jest wysoka podczas fazy świetlnej przed indukcyjnym 16-godzinnym okresem ciemnym. Jednakże na początku nocy poziom ABA jest niski, z tendencją do wzrostu w drugiej połowie indukcyjnego okresu ciemnego. Obserwowano dwójaki wpływ egzogenego ABA na kwitnienie *Pharbitis nil*. ABA zaaplikowany do liści podczas podprogowego (12 h noc) fotoperiodu stymuluje tworzenie pąków kwiatowych. Z drugiej strony, ABA podany podczas indukcyjnego okresu ciemnego (16 h noc), jak również włączony do pożywki kultur wierzchołków wzrostu, hamuje kwitnienie. Tak więc wpływ ABA na kwitnienie jest wyraźnie uzależniony od stopnia indukcji generatywnej, który jest różny u roślin rosnących na różnych fotoperiodach.

SŁOWA KLUCZOWE: kwitnienie, kwas abscyzynowy, indukcja fotoperiodyczna, *Pharbitis nil*.