IAA IN THE CONTROL OF PHOTOPERIODIC FLOWER INDUCTION OF *PHARBITIS NIL* CHOIS

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(Received: October 28, 1994. Accepted: February 4, 1995)

ABSTRACT

The endogenous content of IAA in the cotyledons of *Pharbitis nil* is low before and during the first half of the inductive 16-h-long dark period. From the 8th to the 12th hour the level of IAA increased and then again was going down at the end of a dark period. Exogenous IAA applied to the cotyledones before and during the first half of the inductive dark period inhibits flower bud formation. The application of IAA to shoot apex also resulted in the inhibition of flowering. Experiments with TIBA, an auxin polar transport inhibitor, and PCIB, an auxin action inhibitor, have shown that auxin polar transport in cotyledones and long-distance auxin transport from cotyledones to shoot apex play an important role in IAA inhibition of flower bud formation. It suggests that auxins play their role not only at the level of floral induction in cotyledones, but also in the later events of floral evocation and differentiation in shoot apex.

KEY WORDS: photoperiodic induction, flowering, *Pharbitis nil*, IAA.

INTRODUCTION

Pharbitis nil will not only initiate flowers in response to a single inductive short-day /SD/ cycle, but it is also responsive to photoperiodic induction at a very early stage of seedling growth (Vince – Prue, Gressel 1984). In routine experiments 5-6 days old seedlings are used. The evidence of auxin involvement in the regulation of flowering was first established in the experiments with IAA application to photoperiodically responsive plants. In most cases, especially with short-day plants (SDP), an inhibitory effect was observed (Lang 1961, Jacobs 1985, Simmonds 1987). However, numerous other studies have given disparate results (Bernier et al. 1981). This may be so, because of the complex nature of the flowering phenomenon which includes photoperiodic induction of leaf tissue, transport of flowering signals, evocation of apical meristems and differentiation of flowers, any or all of which may be modulated by auxin. There are discrepancies in data concerning the primary site of auxin action in flowering. It can be a leaf or a cotyledone (Krekule et al. 1985, Podolnyi et al. 1991), or a shoot apex (Krekule and Privratsky 1974). This report is an attempt to determine whether IAA influenced flowering by the effect on the photoperiodic induction in cotyledones or on post-inductive events in apical meristems. The IAA endogenous content as well as the influence of exogenously applied auxin on flower formation were investigated.

MATERIAL AND METHODS

Plant material

Seeds of *Pharbitis nil* Chois cv. Violet (Marutane Seed Co, Kyoto, Japan) were stirred with concentrated H₂SO₄ for 1 hour, rinsed well and imbibed for 24 h in distilled water (25°C). The swollen seeds were sown in pots filled with perlite: sterile sand (2:1), and the pots 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 plant growth regulators and exposed to 16 h inductive dark period. IAA (indole-3-acetic acid, 10⁻³ mol dm⁻³), TIBA (2, 3, 5-triiodobenzoic acid, 10⁻³ - 10⁻⁵ mol dm⁻³), PCIB (p-chloro-phenoxyisobutyric acid, 10⁻³ mol dm⁻³) were applied to cotyledones or shoot apex at 2 h intervals before, during and after a 16 h inductive dark period. The water solutions of the compounds were painted with a small brush (100 mm³ per plant). The application of TIBA to cotyledone petioles was done in cotton swabs. 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 flower buds per plant and the percentage of plants exhibiting terminal flowering were then determined using a dissection microscope. Fifteen plants were used in each experiment, repeated at least 3 times. Standard errors were calculated.

Determination of endogenous IAA

Free IAA was extracted from frozen plant material (1.5 g of cotyledones) by 80% methanol (two parts of 15 ml each). After the filtration the crude extract was passed through Baker-bond spe octadecyl C-18 column (J.T. Baker Inc. Phillipsburg, NJ 08865 USA) and evaporated to dryness under vacuum (35°C). The residue was dissolved using 1 ml of 100% methanol and evaporated to dryness by a stream of nitrogen. After the methylation with N-methyl-N-nitroso-4-toluenesulfonamide IAA in the residue was determined by gas chromatography on Shimadzu GC-14A equipped with a capillary

column (0.32 mm diameter, 25 m long) packed with SE-54-DF0.50. In all the cases the nitrogen carrier gas flow rate was 2 cm³/min, the injector heaters were set at +260°C and the oven temperature was +180°C. The retention time of IAA was 6 min 35 s. The amount of IAA was established on the basis of recorder readings calculating the area of the IAA peak. A quantitative estimations were made by comparing the peak areas of endogenous and standard IAA. 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.

Fig. 1. Content of endogenous IAA in *Pharbitis nil* cotyledones before and during 16 h inductive night.
Abbreviations:
L – light period before inductive night;
D – inductive night.

Fig. 2. Flowering response of *Pharbitis nil* to IAA 10⁻³ mol dm⁻³ treatment. IAA was applied to cotyledones before, during and after 16 h inductive night. The values are mean for 15 replicates ± SE.
Abbreviations:
L – light period before inductive night;
D – inductive night;
+L – light period after inductive night.

Fig. 3. Flowering response of *Pharbitis nil* to IAA 10^{-3} mol dm^{-3} treatment. IAA was applied to shoot apex during 16 h inductive night. The values are mean for 15 replicated $\pm$ SE. Abbreviations D – inductive night.

Fig. 4. Flowering response of *Pharbitis nil* to IAA 10^{-3} mol dm^{-3} and TIBA 10^{-5} mol dm^{-3} treatment. The compounds were applied to cotyledones during 16 h inductive night. The values are mean for 15 replicates $\pm$ SE. DC dark control.

Fig. 5. Flowering response of *Pharbitis nil* to IAA 10^{-3} mol dm^{-3} and TIBA 10^{-3} mol dm^{-3} treatment. IAA was applied to cotyledones and TIBA to cotyledone petioles during 16 h inductive night. The values are mean for 15 replicates $\pm$ SE. DC dark control.

Fig. 6. Flowering response of *Pharbitis nil* IAA 10^{-3} mol dm^{-3} and PCIB 10^{-3} mol dm^{-3} treatment. IAA was applied to cotyledones and PCIB to shoot apex during 16 h inductive night. The values are mean for 15 replicates $\pm$ SE. DC dark control.

RESULTS

The content of endogenous free IAA in *Pharbitis nil* cotyledons is rather low before and during the first half of the inductive 16-h-long dark period. From the 8th to the 12th h the level of IAA increased significantly and then again was going down at the end of a dark period (Fig. 1). The application of exogenous IAA to cotyledones at different times of inductive photoperiod resulted in the inhibition of flowering, especially 8 h before the beginning of an inductive dark period, during the first half of an inductive night and at the 14th h of darkness (Fig. 2A, B). Control plants had about 6 flower buds and IAA-treated ones – about 1.5-4. IAA application after an inductive dark period was almost without any effect on flowering. Terminal flowering (Fig. 2B) was reduced by IAA from 100% in control plants to even less than 10% during the first hours of darkness. When auxin was applied to shoot apex of *Pharbitis nil* seedlings it also resulted in the inhibition of flowering (Fig. 3A, B). The inhibitory effect was seen 2 h later than in the case of IAA application to the cotyledones, but the effect was stable and existed also during the second half of an inductive dark period. TIBA, applied alone to cotyledones, had no effect on flowering of *Pharbitis* seedlings. However, when TIBA was applied prior to IAA treatment, auxin inhibition of flowering was completely reversed (Fig. 4A, B). When TIBA was applied to cotyledone petioles it had an inhibitory effect on flower bud formation, especially at the 4th h of an inductive dark period (Fig. 5A, B). Simultaneous application of TIBA (to cotyledone petioles) and IAA (to cotyledones) resulted in the partial reversion of auxin inhibitory effect (Fig. 5). PCIB, applied alone to shoot apex, had almost no effect on flower bud formation (Fig. 6A, B). Simultaneous application of IAA (to cotyledones) and PCIB (to shoot apex) resulted, however, in the reversion of auxin inhibitory effect.

DISCUSSION

The obtained results confirm the inhibitory effect of exogenous IAA on the flowering of *Pharbitis nil* seedlings, especially when IAA was applied to the cotyledones 8 h before or during the first half of 16-h-long inductive dark period (Fig. 2). Strong IAA inhibition at that time corresponds to low endogenous IAA content (Fig. 1). Also, the inhibitory effect of IAA in the 14th h of an inductive dark period (Fig. 2) corresponds to the decrease of endogenous IAA content between the 12th and 16th h of darkness (Fig. 1). This suggests that the application of IAA during the second part of 16-h-long dark period is ineffective because the endogenous content of auxin at that time is already high. The inhibitory effects of IAA treatment were reported previously by Ogawa (1967), Friedman et al. (1990) and Halevy et al. (1990) for *Pharbitis nil*, by Pavlova and Krekule (1990) for SDP *Chenopodium rubrum* and by Lozhnikova et al. (1990) for SDP *Nicotiana tabacum* cv. Meryland Mammoth. IAA is also inhibitory to flowering when applied to seedlings shoot apices during an inductive night (Fig. 3). Similar results have been reported for *Chenopodium rubrum* (Pavlova and Krekule, 1990). The experiments with TIBA, an auxin polar transport inhibitor (Hatori et al. 1991), have shown that this compound applied to cotyledones prior to IAA treatment can reverse the auxin inhibition of flowering (Fig. 4). It suggests that auxin polar transport in cotyledones plays an important role in the photoperiodic control of flowering. On the other hand, TIBA applied to the cotyledone petioles was itself in-

hibitory for flowering and, to some extent, opposite to IAA action (Fig. 5). It suggests, that long distance auxin transport from cotyledones to shoot apex also plays an important role in auxin inhibition of flower bud formation in *Pharbitis nil* seedlings (Prasad et al. 1989). PCIB, known as antiauxin, applied to shoot apex alone, did not influence flowering. However, when given to shoot apex simultaneously with IAA applied to cotyledones, reversed IAA effect (Fig. 6). This means that auxin migrated from cotyledones to shoot apex where it could be inhibitory for flower initiation. The obtained results suggest strongly that auxins play their role not only in cotyledones at the level of floral induction but also in the shoot apices where flower evocation and differentiation takes place. Thus, IAA inhibits some parts of flowering processes in cotyledones (creation and transport of flower stimulus) as well as morphogenetic reactions (flower primordia initiation) in the apex. A multiplicity of processes which influence plant morphogenesis are under photoperiodic control (O'Neil 1992) and it is the correlated interaction of these processes which determines such important morphogenetic events as flowering (Kinet 1993). In this study the evidence has been presented that auxin can be a part of the control system which regulates photoperiodic flower initiation in SDP. IAA production and tissue sensitivity to auxin are probably integral components of the short-day plants response to the photoperiodic induction of flowering.

ACKNOWLEDGEMENTS

This research was supported by grant No 663799203 from the Polish Committee for Scientific Research (KBN).

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IAA W KONTROLI FOTOPERIODYCZNEJ INDUKCJI KWITNIENIA *PHARBITIS NIL* CHOIS

STRESZCZENIE

Zawartość endogennego IAA w liściach *Pharbitis nil* jest niska przed i podczas pierwszej połowy indukcyjnego 16 godzinowego okresu ciemnego. W godzinach od 8 do 12 poziom IAA wzrasta, a później znowu obniża się przy końcu okresu ciemnego. Egzogenny IAA zaaplikowany do liści przed i podczas pierwszej połowy indukcyjnej nocy hamuje tworzenie pąków kwiatowych. Aplikacja IAA do wierzchołka pędu również powodowała zahamowanie kwitnienia. Doświadczenia z TIBA, inhibitorem polarnego transportu auksyny oraz PCIB, inhibitorem działania auksyny wykazały, że polarny transport auksyny w liściach, a także długo-dystansowy transport auksyny z liści do wierzchołka pędu, odgrywają ważną rolę w hamowaniu tworzenia pąków kwiatowych przez IAA. Sugeruje to, że auksyny odgrywają rolę nie tylko na poziomie indukcji kwitnienia w liściach, ale także w późniejszych wydarzeniach ewokacji kwitnienia i dyferencjacji kwiatu w wierzchołku pędu.

SŁOWA KLUCZOWE: Indukcja fotoperiodyczna, kwitnienie, *Pharbitis nil*, IAA.