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Involvement of Abscisic Acid and Indole-3-acetic Acid in the Flowering of *Pharbitis nil*

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Abstract. The involvement of abscisic acid (ABA) and indole-3-acetic acid (IAA) in the regulation of flowering of *Pharbitis nil* was investigated through exogenous applications and analyses of endogenous levels. Both hormones inhibited the flowering of *P. nil* when they were applied before or after a single 15-h dark treatment. The inhibitory effect of ABA and IAA was significant when they were applied before the dark treatment, and the application to plumules was more effective than that to cotyledons. In all applications, the inhibitory effect of IAA was stronger than that of ABA. Endogenous levels of ABA and IAA in the plumules were compared between flower-inductive (15-h dark treatment) and noninductive (continuous light) light conditions. There was no significant difference in the ABA level between light and dark conditions, whereas the level of IAA was decreased by the dark treatment. These results suggest that biosynthesis and/or catabolism of IAA is affected by the light treatment and therefore may be involved in the regulation of early flowering processes in the apex. The inhibitory effects of ABA and IAA were reversed by an application of gibberellin A_3, indicating that gibberellin A_3 counteracts the flowering processes affected by ABA and IAA. Application of aminoethoxyvinylglycine restored the flowering response inhibited by IAA, which suggests the possibility that the inhibitory effect of IAA is the result of enhanced ethylene biosynthesis.

Key Words. *Pharbitis nil* Chois—Flowering—Indole-3-acetic acid—Abscisic acid—Gibberellin—Ethylene biosynthesis

Flowering is a fundamental turning point in the life cycle of most plants. Flowering can be induced by external factors such as light, temperature, and nutrition. A single dark treatment induces flowering in *Pharbitis nil* up to 7 days after germination. It has been speculated that a flowering stimulus is synthesized in the cotyledon during the dark treatment and transported to the apex via phloem to induce flower buds (Imamura 1967). Plant hormones must be also involved in this process, since they are known to affect differentiation and development of cells, tissues, and organs. In our previous study it was demonstrated that endogenous gibberellins (GAs) play a promotive role in the early process of flowering in *P. nil* (Wijayanti et al. 1996).

The effect of abscisic acid (ABA) and indole-3-acetic acid (IAA) in the flowering of *P. nil* has been studied by exogenous applications. IAA showed an inhibitory effect (Harada 1967, Ogawa and Zeevaart 1967) whereas ABA was promotive (El-Antably and Wareing 1966, Harada et al. 1971, Nakayama and Hashimoto 1973). On the other hand, Marumo et al. (1990) reported an inhibitory effect of ABA. We analyzed endogenous levels of ABA and IAA in the phloem exudates of *P. nil* to find whether their levels were changed by a dark treatment (Wijayanti et al. 1995). These results suggest the possibility that ABA and IAA are involved in the flowering of *P. nil.* To study this hypothesis, investigations on the biological effects, endogenous levels, and biosynthesis of these plant hormones are necessary.

In the present study, the effects of the exogenous application of ABA and IAA are examined, and their endogenous levels in the plumules under different light conditions are analyzed. The interactions of ABA and

Abbreviations: $GA_{(n)}$, gibberellin (A_n); ABA, abscisic acid; IAA, indole-3-acetic acid; AVG, aminoethoxyvinylglycine; IPA, indole-3-propionic acid; HPLC, high performance liquid chromatography; R_t, retention time; GC-SIM, gas chromatography-selected ion monitoring.

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IAA with other plant hormones, namely GA and ethylene, are also reported.

Materials and Methods

Plants

Seeds of *P. nil* Chois. strain Violet were purchased from Marutane Co. (Kyoto, Japan). They were treated with concentrated sulfuric acid for 60 min, washed under running tap water for 6–7 h, then sown in wet vermiculite and incubated in a growth chamber under light (5,000 lux) at 25°C. Four-day-old seedlings were transferred into pots (four seedlings/pot), and 4 days later they were subjected to a single 15-h dark treatment. For analyses of endogenous ABA and IAA levels, plumules were collected before or after the dark treatment. Otherwise, seedlings (treated or not treated with ABA or IAA) were allowed to grow for 2 weeks, and then the flowering response was examined by a dissecting microscope (Wijayanti et al. 1996). Plants were maintained by daily watering with 0.1% nutrient solution (Hyponex 5:10:5) until the end of the experiments.

Application of Plant Hormones and Aminoethoxyvinylglycine (AVG)

The application of ABA and IAA was done by placing their methanol solution (1 μL) onto plumules or cotyledons by a microsyringe. When the interaction of ABA and IAA with GA was investigated, the methanol solution of GA_3 (1 μL) was placed onto the plumules of the seedlings pretreated with ABA or IAA. An involvement of ethylene biosynthesis in the inhibition of flowering by IAA was investigated by applying AVG (dissolved in 50% acetone, 1 μL/plant) to the plumules prior to the IAA treatment. The times of applications of hormones and AVG are described in legends of figures and table.

Analytical Procedures for ABA and IAA

Plumules (from 100 seedlings, 1.0–1.5 g, fresh weight) were homogenized in a chilled mortar and pestle and then extracted twice with 80% methanol (10 mL each). The extracts were combined, then IPA (5 ng) and [6-$^2H_3$]ABA (10 ng) were added to the extracts as internal standards. The extracts were concentrated and purified by solvent fractionation and HPLC on a Nucleosil 5 C_{18} column as described previously for IAA analysis (Kobayashi et al. 1989). In the HPLC, ABA was eluted between IAA (R_t 6.3 min) and IPA (R_t 8.9 min), and eluates were collected from R_t 5.0 to 10.4 min. This fraction was subjected to HPLC analysis using a Nucleosil 5 $N(CH_3)_2$ column (100 × 6 mm) with a fluorescence detector (excitation, 280 nm; emission, 350 nm). The column was eluted with 0.2 or 0.3% acetic acid in methanol (flow rate, 1.5 mL/min). IAA was quantified by the method described previously (Kobayashi et al. 1989). The ABA fraction was collected from R_t 4.0 to 6.0 min (elution with 0.2% acetic acid in methanol), dried up in vacuo, methylated with an excess amount of diazomethane, and analyzed by GC-SIM (Wijayanti et al. 1995).

Results and Discussion

Effect of Exogenous Application of ABA and IAA

The effect of ABA and IAA on the flowering of *P. nil* was examined by an exogenous application of these hormones to young seedlings under flower-inductive conditions. As shown in Fig. 1, both ABA and IAA inhibited flowering, and the inhibitory effect of IAA was stronger than that of ABA. The inhibitory effect was reduced when ABA or IAA was applied after the dark treatment. When ABA or IAA was applied to the cotyledons just before the dark treatment, the inhibitory effect was weaker than that in the application to the plumules (Fig. 2). The number of flower buds was decreased in accordance with the amount of ABA and IAA applied.

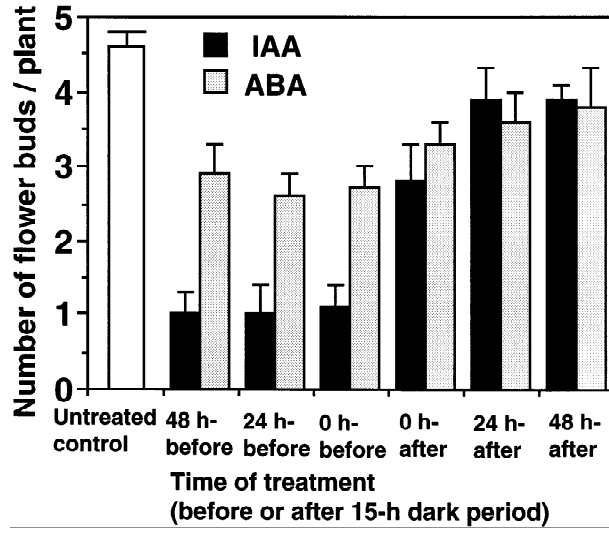

Fig. 1. Effect of ABA and IAA on the flowering response. ABA and IAA (each 1 μg) were applied to the plumules before or after the 15-h dark treatment.

The inhibitory effect of ABA in the flowering of *P. nil* is consistent with the result reported by Marumo et al. (1990). However, the works by El-Antably and Wareing (1966), Harada et al. (1971), and Nakayama and Hashimoto (1973) presented a promotive effect of ABA. This inconsistency may be caused by the differences in environmental conditions, growth stage, application method, and amount of ABA applied. Because we applied ABA directly to the plumules before the dark treatment and found the inhibition, it can be concluded that ABA has an inhibitory effect on the early process of the flowering in the apex of *P. nil.*

As has been reported by Ogawa and Zeevaart (1967) and Harada (1967), IAA showed an inhibitory effect on the flowering of *P. nil.* Our present study showed that the inhibitory effect of IAA was significant when it was applied to the plumules. In the report by Ogawa and Zeevaart (1967), however, IAA showed an inhibitory effect only when applied to the cotyledons, suggesting that IAA inhibits the synthesis of flowering stimulus. Setting aside this argument, the present study clearly presents evidence for the inhibitory effect of IAA on the flowering of *P. nil.*

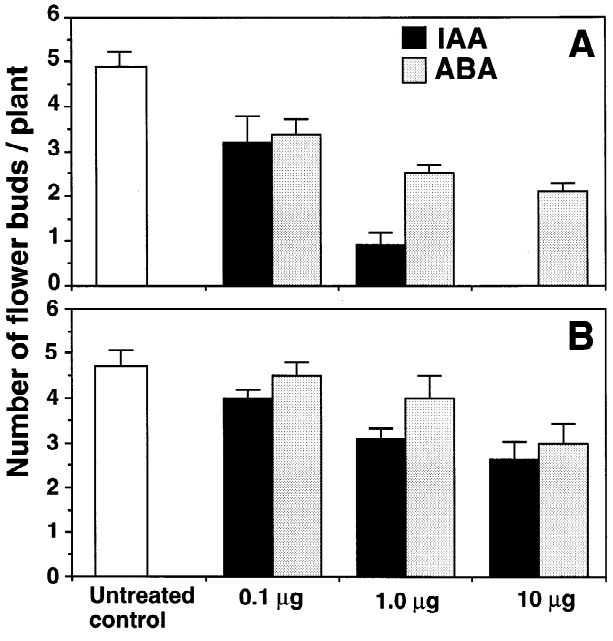

Fig. 2. Effect of ABA and IAA applied to the plumules (*A*) or cotyledons (*B*) on the flowering response. ABA and IAA were applied just before the 15-h dark treatment.

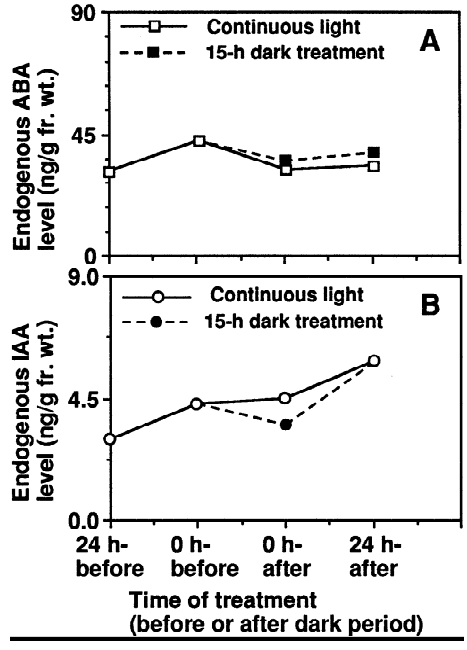

Fig. 3. Effect of 15-h dark treatment on the level of ABA (*A*) and IAA (*B*) in the plumule.

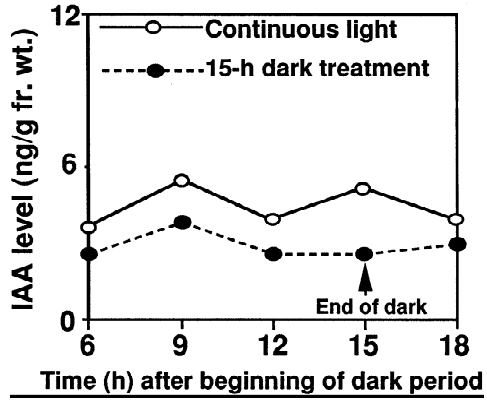

Fig. 4. Effect of 15-h dark treatment on the level of IAA in the plumule.

Endogenous Levels of ABA and IAA in Plumules under Different Light Conditions

Endogenous levels of ABA and IAA were analyzed in the plumules of the seedlings grown under flower-inductive (treated with a 15-h dark period) and non-inductive (continuous light) light conditions. When compared just after the dark period, the IAA level of the plants treated with dark period was always lower than that of untreated plants, whereas ABA levels were almost similar (Fig. 3). The experiment was repeated three more times to show that the IAA level of the treated plants was 22–40% less than that of untreated plants at the end of the dark period. Thus, the fluctuation pattern of the IAA level during the dark period was analyzed (Fig. 4). The IAA level in the plumules was always lower than that of control plants (grown under continuous light) throughout the dark period. The maximum difference was observed at the end of the dark period. These results suggest that the biosynthesis and/or catabolism of IAA is affected by the light condition. Since IAA applied to the plumules showed an inhibitory effect on the flowering, the decrease in the endogenous IAA level by the dark treatment provides a less suppressive condition for the flowering. Endogenous IAA may be involved in the early processes of flowering as a negative regulator.

Interaction of Plant Hormones

Our previous study revealed that GA plays a promotive role in the early process of flowering (Wijayanti et al. 1996). Thus, the early flowering processes in the apex may be regulated by interactions between hormones (i.e. compete with each other and/or affect biosynthesis or catabolism of other hormones). From this point of view, the effect of GA on the inhibition of flowering caused by ABA and IAA was studied. As shown in Fig. 5, application of 1 μg of GA_3 just before the dark treatment overcame the inhibitory effect of ABA and IAA. As expected, the effect of GA_3 was much weaker when it was applied after the dark treatment. The inhibition by ABA was overcome completely by GA_3, but that by IAA (10 μg) was not (Table 1). The opposite interaction between GA and ABA can be predicted because their opposite interactions at some physiologic processes such as

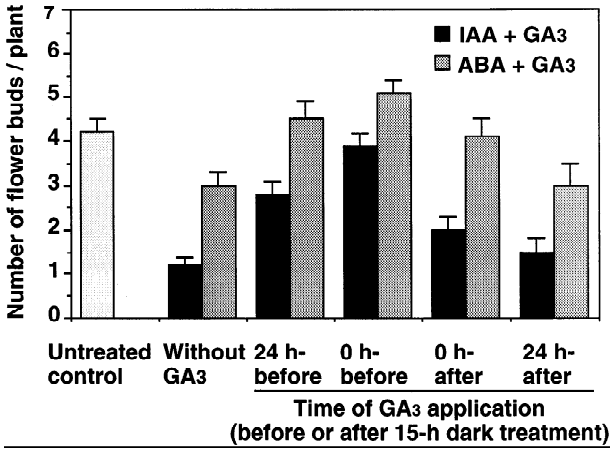

Fig. 5. Effect of coapplication of IAA or ABA with GA_3 on the flowering response. IAA and ABA (each 1 μg) were applied to the plumules 24 h before the 15-h dark treatment. GA_3 (1 μg) was applied to the plumules at the time indicated.

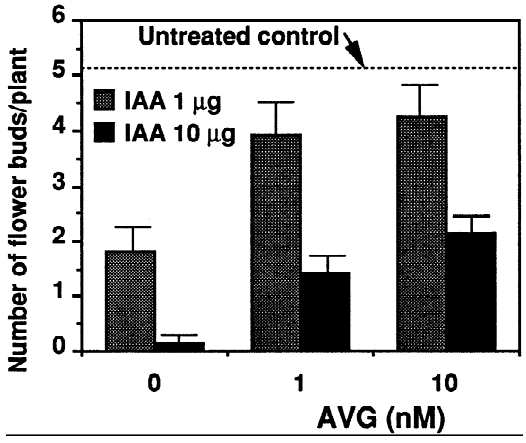

Fig. 6. Effect of coapplication of IAA and AVG on the flowering response. IAA and AVG were applied to the plumules just before the 15-h dark treatment.

Table 1. Effect of coapplication of GA_3[a] and ABA or IAA[b] on the flowering response (number of flower buds/plant ± S.E.).

	IAA		ABA	
GA_3 (μg)	1 μg[c]	10 μg[d]	1 μg[c]	10 μg[d]
0	1.0 ± 0.2	0.2 ± 0.1	2.3 ± 0.2	2.2 ± 0.3
0.1	3.1 ± 0.5	1.9 ± 0.6	4.4 ± 0.2	4.2 ± 0.4
1.0	4.6 ± 0.3	2.2 ± 0.5	5.1 ± 0.2	4.8 ± 0.4

[a] GA_3 was applied to the plumules just before the 15-h dark treatment.
[b] ABA and IAA were applied to the plumules just before the 15-h dark treatment.
[c] Value for untreated control, 4.1 ± 0.4.
[d] Value for untreated control, 4.2 ± 0.3.

seed germination have been reported (Jacobsen et al. 1995). GA and ABA may act at the same site in the flowering process in an opposite manner. The interaction of IAA with GA in the flowering process is a novel finding; however, the inhibition by excess application of IAA (10 μg) was not restored completely by the GA application. The epinasty of leaves was observed in the seedlings treated with IAA, indicating evocation of ethylene by excess IAA. Since ethylene has been known to inhibit the flowering of *P. nil* (Suge 1980), IAA may inhibit flowering via enhanced ethylene biosynthesis. This possibility was investigated using AVG, an inhibitor of ethylene biosynthesis.

The application of AVG just before the dark treatment almost restored the flowering response inhibited by 1 μg of IAA (Fig. 6). From this result, it was suggested that one of the modes of action of IAA in inhibiting the flowering is the induction of ethylene biosynthesis. The site of action of ethylene is still under question, but it has been reported that ethylene inhibited the formation of flowering stimulus in cotyledons (Suge 1980). The involvement of ethylene does not exclude a possibility that IAA also inhibits the flowering by a mechanism independent from ethylene, since the inhibitory effect of 10 μg of IAA was only partially overcome by the AVG treatment (Fig. 6).

The present results indicate that the early processes of flowering in *P. nil* are regulated through interactions among the different hormones, i.e. ABA, IAA, GA, and ethylene. Although the endogenous levels of these hormones are not always influenced by the dark treatment, the balance of hormone levels may be important for the flowering process.

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