Interaction between Aspterric Acid and Indole-3-acetic Acid on Reproductive Growth in *Arabidopsis thaliana*

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Application of indole-3-acetic acid (IAA) with a pollen growth inhibitor, aspterric acid (AA), results in the recovery of normal pollen development. In contrast, application of gibberellin (GA_3) with AA do not induce normal pollen growth. In addition, application of different concentrations of IAA with AA shortens the period of growth from bolting to first flowering as compared to that treated with AA alone. Furthermore, stem length and number of flower bud treated with IAA and AA were similar to those of control. These results suggest, that IAA may play an important role in reproductive growth of *A. thaliana*.

Key words: Aspterric Acid, Indole-3-acetic Acid, *Arabidopsis thaliana*

Introduction

Many aspects of plant growth and development such as germination, root growth, stem elongation, ripeness, and senescence are dependent on the flow of plant hormones. However, the role of plant hormones in the reproductive organogenesis is still poorly understood, because the development of floral organs is a complex phenomenon (Martinez-Zapater *et al.*, 1994; Clark and Meyerowitz, 1994). Appropriate molecular tools for the growth regulation of floral organs are necessary for the characterization of the role of plant hormones in the reproductive growth.

Arabidopsis thaliana is currently a model system for plant molecular biology of flower development in dicotyledonous plants (Vieira *et al.*, 1990). This plant has been developed as a convenient screening plant to detect plant growth regulators that induce changes in flower development (Clark and Meyerowitz, 1994; Dawson *et al.*, 1994, 1993; Dennis and Surridge, 2000; Brown, 1972). Application of *N*-1-naphthylphthalamic acid revealed that the plant hormone auxin acts as a morphogen directing regional patterning in the developing gynoecium of *A. thaliana* (Sessions *et al.*, 1997; Nemhauser *et al.*, 2000). However, auxin is not known to play a role in the developing androecium.

Aspterric acid (AA), an inhibitor of pollen development in *A. thaliana*, has been isolated from the fungus *Aspergillus terreus* (Tsuda *et al.*, 1978; Shimada *et al.*, 2002). This study uses a technique of applying a pollen growth inhibitor, AA, to develope *Arabidopsis* flower bud to further characterize the role of plant hormones in the reproductive oraganogenesis. We describe that the plant hormone indole-3-acetic acid (IAA) acts as a growth regulator in the developing androecium of *A. thaliana.*

Materials and Methods

Plant material

Seeds of *A. thaliana* were sown in plastic pots (8 cm inner diameter) filled with a mixture of perlite and vermiculite (1:1, v/v) and germinated in a growth chamber maintained at 25 °C under a continuous light (100 μE/m^2 s). Liquid fertilizer was applied once a week. The fertilizer contained 5 mM KNO_3, 2.5 mM KH_2PO_4, 2 mM $MgSO_4$, 2 mM $Ca(NO_3)_2$, 50 μM Fe-EDTA, 70 μM H_3BO_3, 14 μM $MnCl_2$, 0.5 μM $CuSO_4$, 1 μM $ZnSO_4$, 0.2 μM $NaMoO_4$, 10 μM NaCl, and 0.01 μM $CoCl_2$.

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Treatment with AA and plant hormones

Chemicals to be tested were each formulated as an aqueous solution containing 0.1% Tween-80 as a wetting agent and 2% EtOH to aid their solubility. Each solution was sprayed on all leaves with an atomizer at the rate of 1 ml per three pots. The chemicals were applied once in two days for a total of 6 treatments from the period rosette leaf formed (17 d after sowing). Triplicate experiments were conducted.

Data collection

Stem length and the number of flower buds were measured everyday from the start of treatment. The date of visible flower buds and blooming were also recorded everyday. The stamens were stained with I_2-KI solution and observed under a light microscope after anthesis.

Results and Discussion

Microscopic examination of anthers

Anthers from freshly opened flowers were stained with I_2-KI solution, and the condition of pollen in anthers treated with or without chemicals was examined by a light microscope (Fig. 1). While the pollen grains within the untreated anthers were densely stained, the anthers treated with 100 μM AA contained collapsed structures and no pollen. The anthers treated with 100 μM AA plus different concentrations of IAA contained the stained pollen grains, but the anthers treated with 100 μM AA plus 10^{-6} M gibberellin (GA_3) contained no pollen and 100 μM AA plus 10^{-10} M GA_3 or 10^{-8} M GA_3 inhibited anther growth. Those results suggest that application of IAA with AA results in the recovery of the normal pollen development but application of GA_3 with AA do not induce the normal pollen development.

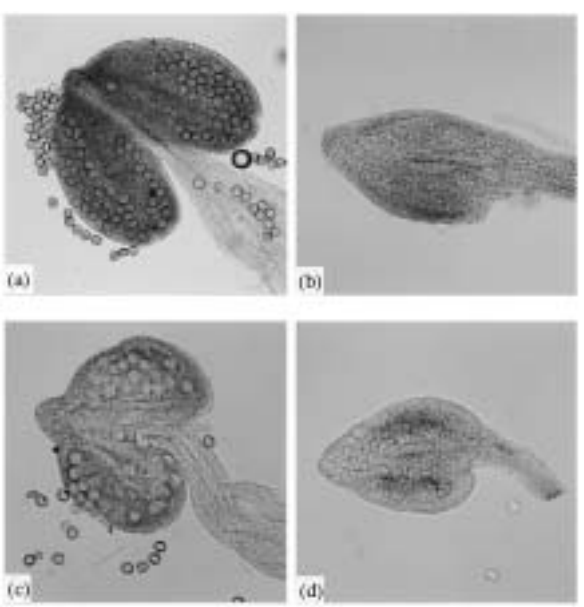

Fig. 1. Anthers treated with AA and plant hormone. (a) to (d) anthers just after anthesis: (a) untreated anthers, (b) 100 μM AA, (c) 100 μM AA plus 10^{-6} M IAA, (d) 100 μM AA plus 10^{-6} M GA_3.

Comparative effects of AA and combined treatments with IAA and GA_3

The effects of various chemical treatments on growth and flowering of *A. thaliana* were shown in Figs. 2 and 3. Fig. 2a shows the period of the growth from rosette leaf formation to bolting in both untreated and treated plants. AA significantly delayed the growth from rosette leaf formation to bolting at a concentration of 100 μM. Treatment with 100 μM AA delayed the growth twice as long as that of untreated plants. Application of 100 μM AA plus different concentrations of IAA delayed the growth similar to that of 100 μM AA alone. Similarly, 100 μM AA plus GA_3 delayed the growth similar to that of 100 μM AA alone.

Fig. 2b shows the period of the growth from bolting to first flowering in both untreated and treated plants. Treatment of 100 μM AA delayed the reproductive growth 3.5 times as long as that of untreated plants. All treatments of 100 μM AA plus IAA showed the growth similar to that of untreated plants. In contrast, the treatment of 100 μM AA plus 10^{-10} M GA_3 delayed the growth similar to that of 100 μM AA alone, and treatment of 100 μM AA plus 10^{-8} M or 10^{-6} M GA_3 showed weak inhibitory activity against the growth.

Fig. 3a shows stem length to first flowering in both untreated and treated plants. AA inhibited stem elongation to 10% of control at a concentration of 100 μM. All treatments of 100 μM AA plus IAA showed weak inhibitory activities against stem elongation. Similarly, all treatments of 100 μM AA plus GA_3 showed weak inhibitory activities against stem elongation.

Fig. 3b shows the number of flower buds to first flowering in both untreated and treated plants. Plants treated with 100 μM AA significantly inhibited flower bud formation. Applications of 100 μM

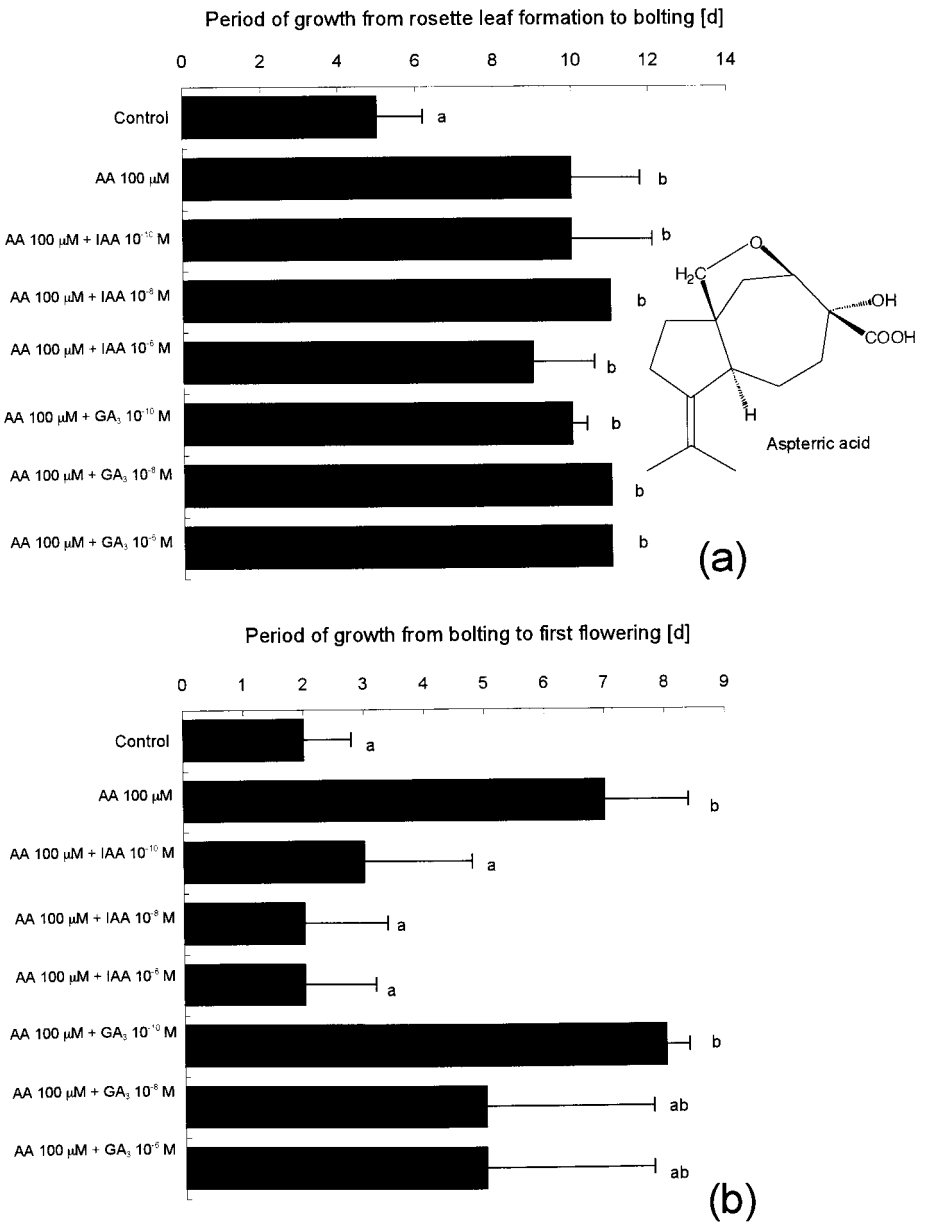

Fig. 2. Effects of AA and combined treatments with AA and IAA or GA_3 on the periods of growth (a) from rosette leaf formation to bolting and (b) from bolting to first flowering. All chemicals were applied 6 times (once in two days) from rosette leaf formation. Bars indicate standard error of the mean. The significance of the difference was evaluated by Duncan's multiple-range test, $P < 0.05$.

AA plus different concentrations of IAA showed similar flower bud formation to the untreated plants except for 100 μM AA plus 10^{-10} M IAA. In contrast, application of 100 μM AA plus 10^{-10} or 10^{-8} M GA_3 showed weak inhibitory activity against flower bud formation, and 100 μM AA plus 10^{-6} M GA_3 inhibited flower bud formation similar to that of 100 μM AA alone.

Applications of different concentrations of IAA with AA reveal to shorten the period of growth

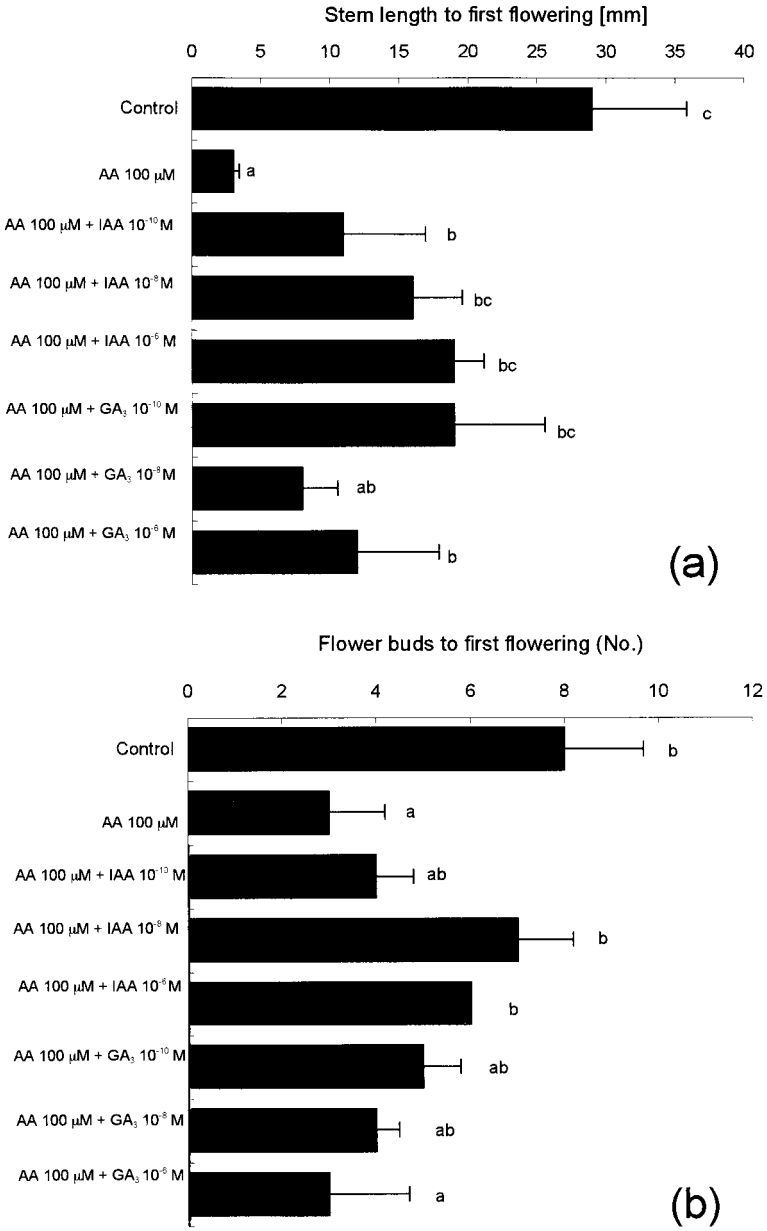

Fig. 3. Effects of AA and combined treatments with AA and IAA or GA_3 on (a) the stem length and (b) the number of flower buds to first flowering. Bars indicate standard error of the mean. The significance of the difference was evaluated by Duncan's multiple-range test, $P < 0.05$.

from bolting to first flowering as compared to that treated with AA alone. Furthermore, stem length and number of flower bud treated with IAA and AA were similar to those of control. Those results suggest that application of IAA with AA results in the recovery of the normal reproductive growth of *A. thaliana*.

Applications of plant hormones with AA reveal that IAA acts as an accelerator on the reproductive growth of *A. thaliana.* In a recent study, GA_3 regulates the development and fertility of *Arabidopsis* flowers and regulates the cellular developmental pathway of anthers leading from microspore to mature pollen grain (Cheng *et al.*,

2004). However, GA_3 could not recover pollen growth inhibited by AA in our study. In contrast, IAA regulates pollen development at meiosis because AA is known to inhibit pollen development at meiosis (Shimada *et al.*, 2002). Thus, IAA may play an important role in the pollen development.

Interaction between IAA and 6-hydroxymellein, another pollen growth inhibitor isolated from *Aspergillus terreus*, will be clarified to establish the role of IAA on plant reproductive growth in the near future.

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