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BRIEF COMMUNICATION

Inhibitory effect of brassinosteroids on the flowering of the short-day plant *Pharbitis nil*

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Abstract

The effect of exogenous brassinosteroids (BR) on the flowering induction of *Pharbitis nil* was examined. Generally plants treated with brassinolide and castasterone form less number of flowers than control plants, but degree of flowering inhibition was depended on the concentration and the method of BR application as well as the length of the inductive dark period. In plants regenerated from sub-induced apices treated with brassinolide at concentration of 1 and 10 μM the flower formation was inhibited completely.

Additional key words: brassinolide, castasterone, photoperiod.

The plant transition from the vegetative to the generative growth phase is a process regulated by many internal and external factors (Levy and Dean 1998). The basic environmental factors regulating flowering are photoperiod and the temperature (Colasanti and Sundaresan 2000, Reeves and Coupland 2000). Many years of experience have shown that morphogenesis of the shoot tip is controlled by substances produced under suitable conditions in the leaves. However, the character of these substances has never been unequivocally determined. In our search for the substances responsible for the induction of flowering, we start to interest in brassinosteroids, a new group of growth regulators.

These substances occur in plants in exceptionally low concentrations, but exert a strong influence on the plant growth and development (Bishop and Yokota 2001, Clouse and Sasse 1998). Brassinosteroids stimulate the growth of the coleoptiles and stems of grass, the hypocotyls and stems of dicotyledonous plants (Katsumi 1985, Meudt 1987, Mandava 1988, Sasse 1990) and prolong a deep dormancy of an apical meristems of potato tubers (Korableva *et al.* 2002). They stimulate formation of xylem in *Helianthus tuberoses* (Clouse and Zurek 1991) and participates in the production of partogenetic, haploid seeds, of both mono- and dicotyledonous plants (Kitani 1994). However, there is little research showing the role of brassinosteroids (BR) and other steroids compounds in the control of flowering. Suge (1986) showed that the direct application of brassinolide (BL) to the staminate inflorescence of *Luffa cylindrica* induced bisexual and pistilate flowers. There are also data showing that typical steroid hormones, which occur naturally in plants, participate in the control of morphogenesis and sex differentiation of flowers (Kopcewicz 1970, 1971, Kopcewicz and Poraziński 1974).

Thus, the aim of this work was to study the effect of brassinosteroids on the flowering of model short-day plant *Pharbitis nil*.

Seeds of short-day plant *Pharbitis nil* Chois cv. Violet (*Marutane Seed Co.*, Kyoto, Japan) were scarified and soaked for 24 h in distilled water (25 ± 1 °C). The swollen seeds were sown in a mixture of wet sand and vermiculite (2:1). The plants were grown for 4 d at 26 °C and continuous irradiance of 130 μmol $m^{-2}s^{-1}$ (cool white, fluorescent tubes, *Polam*, Warsaw, Poland) or 3 d in the darkness and 1 d at low irradiance (40 μmol m^{-2} s^{-1}). Flowering of *Pharbitis nil* was induced by a 12, 16 or 24 h dark period. Brassinolide and castasterone at

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Abbreviations: BL - brassinolide; BR - brassinosteroids; CS - castasterone.

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concentration of 0.01 and 1.0 μM, dissolved in water containing 0.05 % *Tween 20*, were applied to cotyledons (about 0.05 cm^3 per plant) using a soft paintbrush before (4 h) and during (at 0, 4, 8, 12, and 16 h) the inductive darkness. The control plants were treated with 0.05 % Tween 20 solution only. After the treatments, the plants were grown in a growth chamber under continuous irradiance of 130 μmol m^{-2} s^{-1}at 26 ± 1°C for 14 d. The number of flower buds per plant and the percentage of plants with flowers was determined using a dissection microscope. Ten to fifteen plants were used in each experiment, which was repeated at least 3 times.

For *in vitro* experiments, seeds of *Pharbitis nil* were scarified and sterilized for 30 s with 70 % ethanol and for 20 min with a 15 % solution of sodium hypochlorite, and then rinsed well in sterile water. The swollen seeds were then sterile sown and grown on Murashige and Skoog (1962; MS) medium supplemented with 3 % sucrose and 0.25 % *Gelrite* (*Merck*, Rahway, USA) in growth chamber at 25 ± 1 °C, under continuous irradiance of 80 μmol m^{-2} s^{-1} (cool white fluorescent tubes). Sub-induced apices were obtained from seedlings cultured for 7 d under continuous irradiance and then exposed to a single 12-h dark period. Six hours after the end of the dark period, shoot tips were excised. Apices (about 6 mm in length) were cultured on 1 cm^3 of liquid 80 % MS medium containing 2 % sucrose and tested BR in a 0.1 to 10 μM concentration. After 5 d of treatment, apices were transferred onto 5 cm^3 of liquid 80 % MS medium with 5 % sucrose and cultured for 3 weeks in continuous irradiance at 25 ± 2 °C. The controls were the apices excised from seedlings exposed to a single 12-h period of darkness, cultured on the medium without BR. At the end of that 3-week growth, the buds were observed using a dissecting microscope. Flowering responses of the developed plantlets were expressed as the percentage of plantlets with flowers and the number of flower buds per plantlet.

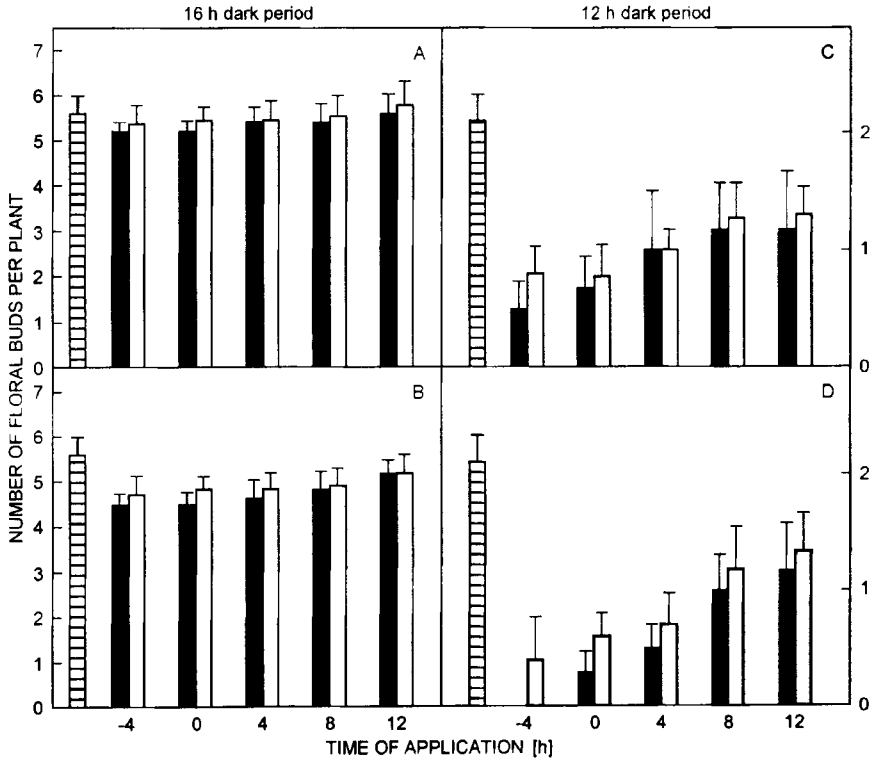

Fig. 1. Effect of brassinolide (*black columns*) and castasterone (*white columns*) in different concentration (*A*, *C* - 0.01 μM; *D*, *E* - 1.0 μM) on flowering of *Pharbitis nil*. Compounds were applied on cotyledons of 4-d-old seedlings before and during 16-h (*A*, *B*) and 12-h (*C*, *D*) inductive dark period. Control plants were exposed to 16- or 12-h-long darkness (*stripped columns*). Means of three independent experiments ± SD.

It was stated that a smaller number of flowers was obtained in all both pot and *in vitro* BR-treated plants in comparison with the control plants. The degree to which flowering was inhibited depended on the BR concentration, the length of the inductive dark period, and the method of the BR application. BR in concentrations exceeding 100 μM strongly inhibited the growth of seedlings.

Flowering was weekly inhibited when BR were applied to the cotyledons of 4-d-old seedlings cultured in continuous irradiance and later in for 16 h of darkness. At these conditions BR applied at a concentration of 1 μM

4 h before a 16 h dark period inhibited flowering by about 20 %, and CS by about 16 % (Fig. 1*B*). Flowering was inhibited to a stronger degree when the treated plants were not fully induced (12 h dark period) (Fig. 1*C,D*) or were cultured at low irradiance (72 h darkness and 24 h of low irradiance) (Fig. 2). In the first case, BL applied 4 h before a dark period at a concentration of 1 μM inhibited flowering completely, while castasterone (CS) reduced the number of flowering plants by about 80 %. In the second case, 1 μM BR inhibited flowering by from 40 to 50 %. Flowering was most strongly ihibited by BR when the shoot tips were regenerated from seedlings subjected to suboptimal induction. In these conditions 80 % of the control plants form generative buds (Fig. 3), while plantlets growing on a medium containing 1 and 10 μM BL formed only vegetative buds. Plantlets exposed to the 0.1 μM BL formed generative buds only in 30 % of cases.

The results of experiments shows that both BR and CS proved to be factors that inhibited the flowering of the short-day plant *Pharbitis nil*. This effect, although with varying intensity, occurred both during the application of compounds to the cotyledons of induced 4 d seedlings and in cultures *in vitro*. Auxins also proved to inhibit the flowering of *P. nil* (Kulikowska-Gulewska *at al.* 1995). Similar as BR, also in the case of exogenous IAA the inhibiting effect was stronger when the hormone was applied before or during the first hours of the dark period. This confirms the previous observations of the general similarity of the actions of IAA and BR in various developmental processes (Clouse and Sasse 1998). The research carried out does not allow it to be demonstrated unequivocally whether brassinosteroids play a direct role in shoot tips changing the pattern of their differentiation or whether they are responsible for the formation of some kind of flowering inhibitor in cotyledons. Studies using marked compounds show that BR can be transported in the plant (Adam and Schneider 1999), although it is not certain whether this process takes place natively. In order to solve this problem, the endogenous level of BR in individual organs of *Pharbitis nil* during the inductive night would have to be determined.

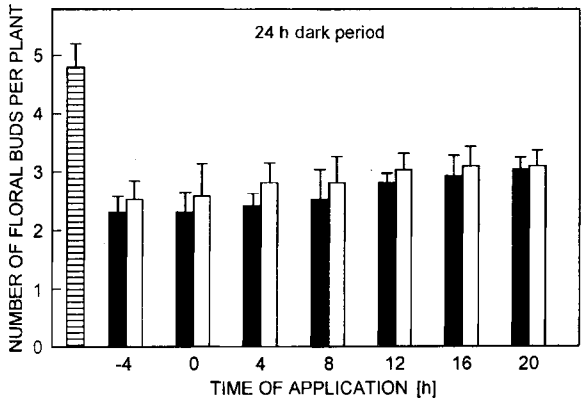

Fig. 2. Effect of brassinolide (*black columns*) and castasterone (*white columns*) on flowering of *Pharbitis nil*. Seedlings were grown for 3 d in the darkness and 1 d under low irradiance (40 μmol m^{-2} s^{-1}) and then exposed to a 24-h-long inductive dark period. Compounds were applied on cotyledons 4 h before and during 24 h inductive dark period. Control plants were exposed to 24-h darkness (*stripped columns*). Means of three independent experiments ± SD.

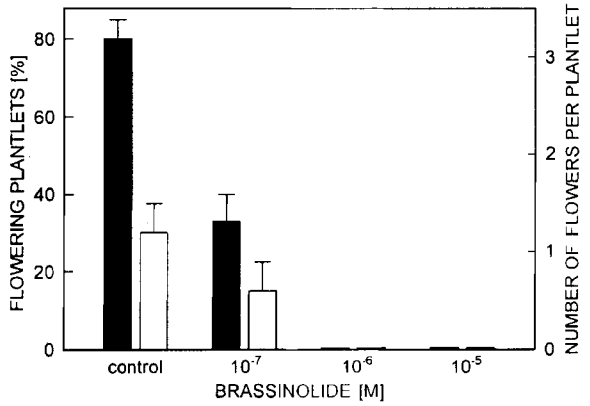

Fig. 3. Effect of brassinolide on floral bud formation (percent of flowering plantlets - *black columns*, number. of flowers per plantlet - *white columns*) in *Pharbitis nil* apex cultures. Apices were obtained from seedlings cultured for 7 d under continuous irradiance, exposed to a single 12-h sub-inductive dark period and cultured for additional 3 weeks (see Materials and methods). Means of three independent experiments ± SD.

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