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Reproductive Development of Higher Plants as Influenced by Brassinolide

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The effect of brassinolide (BR) on the reproductive development of higher plants was investigated using three different experimental system. Direct application of BR to the staminate inflorescence of *Luffa cylindrica* induced bisexual and pistillate flowers, and eventually caused the inflorescence to develop into a shoot that was similar to the main shoot. However, the activity of BR on this sex modification was inferior to that of N^6-benzylaminopurine.

BR slightly hastened the flowering of nonvernalized radish plants, *Raphanus sativus* cv. Miyashige-sofuto, whereas no such effect was observed in vernalized plants. GA_3 promoted flowering both in nonvernalized and vernalized plants.

BR had no activity to induce flowering in a short day plant *Perilla frutescence* under noninductive conditions.

Key words: Brassinolide — Flowering — *Luffa cyrindrica* — *Perilla frutescence* — *Raphanus sativus* — Sex modification.

BR, a plant growth-promoting steroid isolated from rapeseed pollen (Grove et al. 1979), has been tested for its biological activities in comparison with auxin, gibberellin and cytokinin (Katsumi 1985, Gregory and Mandava 1982, Mandava et al. 1981, Sasse 1985, Wada et al. 1981, Yopp et al. 1981). However, no data have so far been reported for its effect on the reproductive development in higher plants, except that the growth of pollen tube in vitro was found to be promoted by a synthetic 24-epimer (Sasse 1985).

Using three different experimental systems, the effects of BR on the reproductive development of higher plants were examined in comparison with other plant hormones. *Luffa cylindrica* was used to examine the effect of BR on sex modification, since the staminate flower of this plant changes sex expression by hormone treatment (Takahashi et al. 1980). *Raphanus* and *Perilla* plants were used for testing the effect of BR on flower induction, since they have been well studied for their flowering behavior as a typical long day and short day plants, respectively (Suge and Rappaport 1968, Suge 1984).

Materials and Methods

Sex expression of staminate inflorescence in Luffa cylindrica—Seeds of *Luffa cylindrica* Roem cv. Futo-hechima were imbibed overnight in water at room temperature and sown into 7.5 cm plastic pots filled with Kureha Engei Baido (Kureha Chemicals, Tokyo, Japan), a commercial soil mixture for growing seedlings of horticultural plants, containing 0.5 g N, 1.5 g P and 0.5 g K

Abbreviations: BR, Brassinolide; BA, N^6-benzylaminopurine.

per kg. Seedlings with fully expanded cotyledons were transplanted into 15 cm plastic pots filled with the same soil and grown in a greenhouse.

Experiments were started when plants had reached the 6-leaf stage. Watering and other handlings including nutrient supply were done according to ordinary cultivation practices.

In *Luffa cylindrica*, the differentiation of flower buds usually was not detected in the cotyledonary, first and second nodes, while higher nodes bore a staminate inflorescence, and still higher nodes bore both a staminate inflorescence and a pistillate flower on each node. Thus, in the present experiment, lateral organs in the cotyledonary, first second and third nodes, if any, were removed at their initial stage, and the staminate inflorescences which developed on the 4th, 5th and 6th nodes were used for the treatment and evaluation. The main stem was cut just above the 6th node before application of chemicals.

BR was directly applied to the staminate inflorescence using a piece of absorbent cotton (1 × 1 cm) which had been immersed in a solution of three different concentrations of BR, 0.01 μg/ml (2.08×10^{-5} M), 0.1 μg/ml (2.08×10^{-4} M) and 1 μg/ml (2.08×10^{-3} M). The piece of absorbent cotton was placed on an undeveloped staminate inflorescence. BA at a concentration of 0.05 μg/ml (1.16×10^{-4} M) was also used for comparison, because this agent was reported to have potent activity to modify sex expression in this experimental system (Takahashi et al. 1980).

Treatment was started at the 6-leaf stage and repeated three times at two-day intervals. Six plants were used for each treatment. Experiments were conducted during the period from April through September, 1984. Maximum and minimum temperatures during the experiment were 35 and 18°C, respectively.

Flowering of vernalized and nonvernalized radish, Raphanus sativus *cv. Miyashige-Sofuto*—The Miyashige-Sofuto cultivar of radish, *Raphanus sativus*, was used as test material in this experiment, because flowering behavior of this variety has been extensively studied (Suge and Rappaport 1968).

Seeds were imbibed for two days at room temperature in water contained in petri dishes, and vernalized 5 and 10 days at 5°C. For nonvernalized plants, seeds were imbibed at room temperature for two days prior to the end of the vernalization treatment, thus providing seedlings of approximately the same size and appearance as the vernalized ones. Seedlings from both lots of seeds were planted in 15 cm plastic pots, 5 plants per pot, and grown in a greenhouse under natural day length conditions. Twenty days after planting both the vernalized and nonvernalized plants were divided into two groups of 10–20 plants each and subjected to chemical treatments.

The temperature of the greenhouse was not controlled except during winter when it was heated to maintain the air temperature above 13°C. Experiments were conducted during the period from August, 1984 through February, 1985. Temperatures ranged from 13 to 30°C during the experiment.

BR (1 μg/ml, 2.08×10^{-3} M) or GA_3 (0.1 μg/ml, 2.89×10^{-4} M) was applied to the apex of plants three times a week as 50 μl solution. Application of chemicals was done a total of 8 times in plants vernalized for 10 days and 21 times in plants vernalized for 5 days as well as in nonvernalized ones.

Flowering of Perilla frutescence ver crispa *under noninductive conditions*—Seeds of *Perilla frutescence ver crispa* cv. Chirimen-Aojiso, a short day plant, were sown in a plastic box filled with a 1 : 1 mixture of sand and vermiculite. When the seedlings were about 5 cm tall, they were transplanted into 7.5 cm plastic pots filled with the same mixture, one plant per pot. They were grown under 24 hour photoperiod in a green-house in which temperature ranged from 20 to 30°C. Natural day light was supplemented with fluorescent and incandescent lamps for obtaining the noninductive conditions. When plants reached the 6-leaf pair stage, the main stem

was cut just above the 6th node. All leaves and axillary buds were removed except for one pair at the 6th node, before BR application.

BR was applied directly to the cut surface of the main stem with a piece of absorbent cotton (1 × 1 cm) which had been immersed in BR solution (1 μg/ml, 2.08×10^{-3} M). Treatment was repeated twice at two-day intervals. The ability to induce flower in axillary buds was evaluated at the 6th node. In the same experimental system, grafting of induced leaf onto the cut surface of the main stem caused production of flower inflorescence in axillary buds, whereas noninduced leaf did not (Suge 1984). Thus, if the chemical applied to the cut surface has the ability to induce flowering, the axillary buds will develop into inflorescences. Five plants were used for each treatment. Experiments were conducted during the period from May through September, 1984.

Results

Sex expression of staminate inflorescence in Luffa cylindrica—BR application to the staminate inflorescence on the 4th, 5th and 6th nodes induced the appearance of bisexual and pistillate flowers only in the inflorescence developed on the 6th node (Table 1). Three different concentrations of BR were tested in this experiment and sex modification was detected at 0.1 μg/ml (2.08×10^{-4} M) and 1 μg/ml (2.08×10^{-3} M), but almost no effect was seen at 0.01 μg/ml (2.08 ×

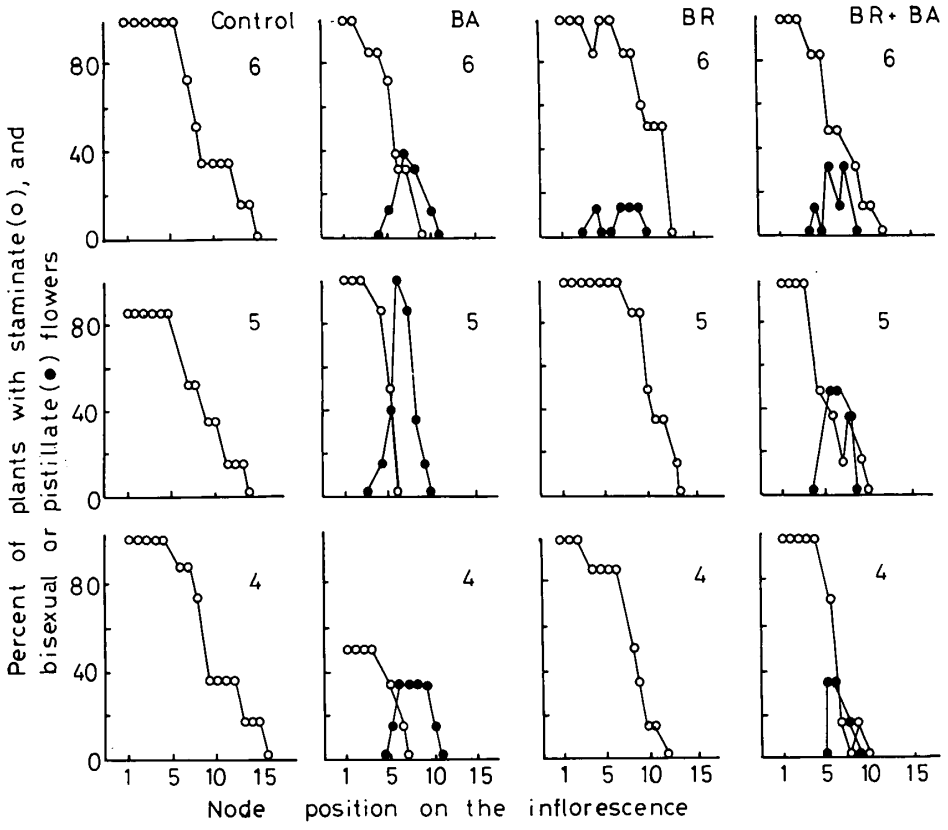

Fig. 1 Sex modification of flowers in the staminate inflorescence of *Luffa cylindrica* cv. Futo-hechima as influenced by BR and BA application. Numerals, 4, 5 and 6 in the figure indicate staminate inflorescence which developed on the 4th, 5th and 6th node, respectively. Control: lateral shoots removed, no chemical treatment. BA: BA (0.05 μl/ml, 1.16×10^{-4} M) applied to 3 inflorescences, lateral shoots removed, BR: B_R (1 μg/ml, 2.08×10^{-3} M) applied to 3 inflorescences, lateral shoots removed. BR+BA: BR (1 μg/ml, 2.08×10^{-3} M) and BA (0.05 μg/ml, 1.16×10^{-4} M) applied to 3 inflorescences, lateral shoots removed.

Table 1 Sex expression of flowers in staminate inflorescence of *Luffa cylindrica* as influenced by BR and/or BA

Chemical and concentration (μg/ml)	Staminate flowers				Bisexual and pistillate flowers			
	Number[a] in the inflorescence from							
	4th node	5th node	6th node	Total	4th node	5th node	6th node	Total
Control	55	44	53	152	0	0	0	0
BA 0.05	15	26	30	71	13	18	12	43
BR 1	44	60	60	164	0	0	4	4
BA 0.05+BR 1	36	29	36	101	5	11	10	26

[a] Total number in 6 plants.

10^{-5} M) (data not shown). The two effective concentrations of BR gave essentially the same results. Deformed flower buds characterized by abnormally developed sepals were frequently observed in the inflorescences treated with the higher concentration of BR (Table 1, Fig. 1 and 2).

BA, on the other hand, induced these sex modifications in all the inflorescences tested (Table 1, Fig. 1). Several transitional sex types induced by BR application are shown in Fig. 2D. The tops of these inflorescences developed into shoots that were similar to the main shoots. Sex modification in the staminate inflorescences was hardly affected by removal of main

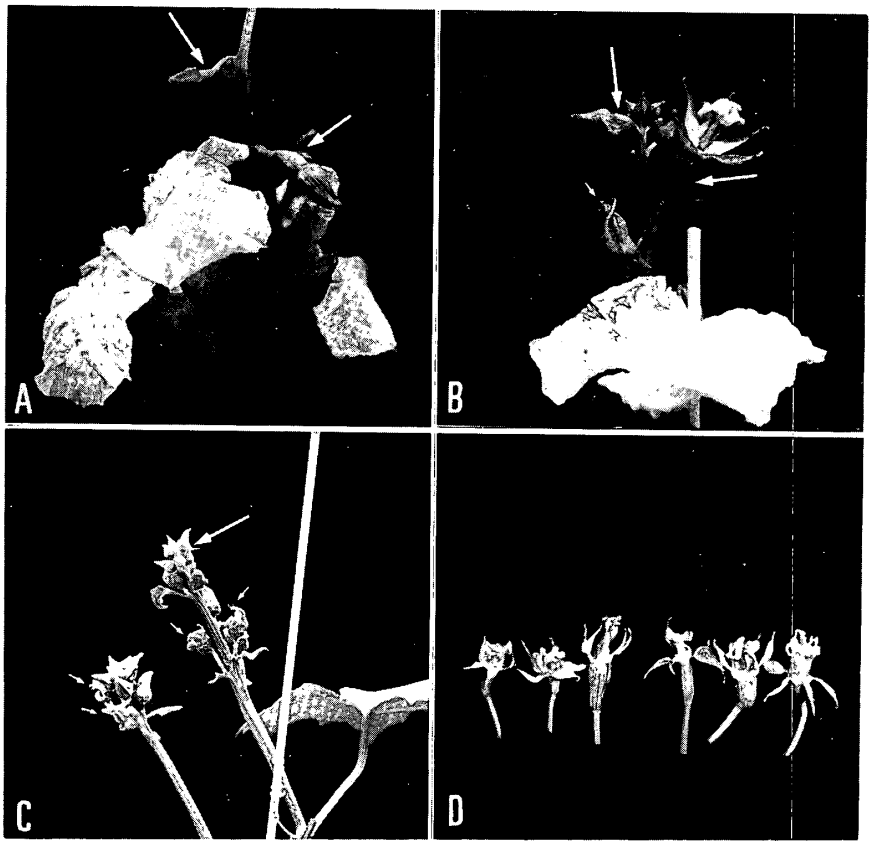

Fig. 2 Sex modification of flowers in the staminate inflorescences of *Luffa cylindrica* cv. Futo-hechima as influenced by BR. A–C: Pistillate and bisexual flowers induced by BR treatment are shown by large arrow. In one of them (B) petal was removed for the convenience of the photograph. Small arrows indicate abnormally developed sepals. D: Examples of different steps of sex expression under the influence of BR. All petals were removed for the convenience of the photograph.

Table 2 Flowering and growth of *Raphanus sativus* as influenced by BR and GA_3

Vernalization of seed (Days)	Chemicals applied [a]	Days to first anthesis	Plant height at the time of first anthesis (cm)	Final number of leaves	Final plant height (cm)
0	Control	155.9±5.3	70.1± 8.7	32.1±1.7	116.4±11.2
	GA_3	131.7±4.3*	127.0± 7.5*	31.3±1.4	152.8±13.0*
	BR	146.0±3.3*	61.5± 3.7	32.7±1.6	107.6± 9.0
5	Control	142.6±4.2	60.7± 6.8	32.5±3.7	106.1±17.2
	GA_3	126.9±3.7*	124.4±10.8*	27.8±1.4	154.3± 8.4*
	BR	142.9±3.5	69.3± 5.8	29.9±1.4	114.0±10.7
10	Control	47.3±1.3	36.5± 6.1	10.7±0.6	97.3±16.3
	GA_3	44.5±1.3*	40.9± 2.9	10.9±0.6	83.0± 6.9*
	BR	44.6±2.1	38.8± 4.7	10.7±0.6	81.6±11.1*

* Significantly different from control at $p<0.05$.
[a] GA_3 at 0.1 μg/ml and BR at 1 μg/ml.

shoot alone, as indicated in the controls of Table 1 and Fig. 1. Combined application of BA and BR did not give an additive effect on the sex modification.

Flowering of vernalized and nonvernalized radish plants—Under the present experimental conditions, even nonvernalized plants flowered eventually, although vernalization treatment of seeds clearly promoted flowering.

As shown in Table 2, GA_3 decreased the number of days to the first anthesis in all vernalized

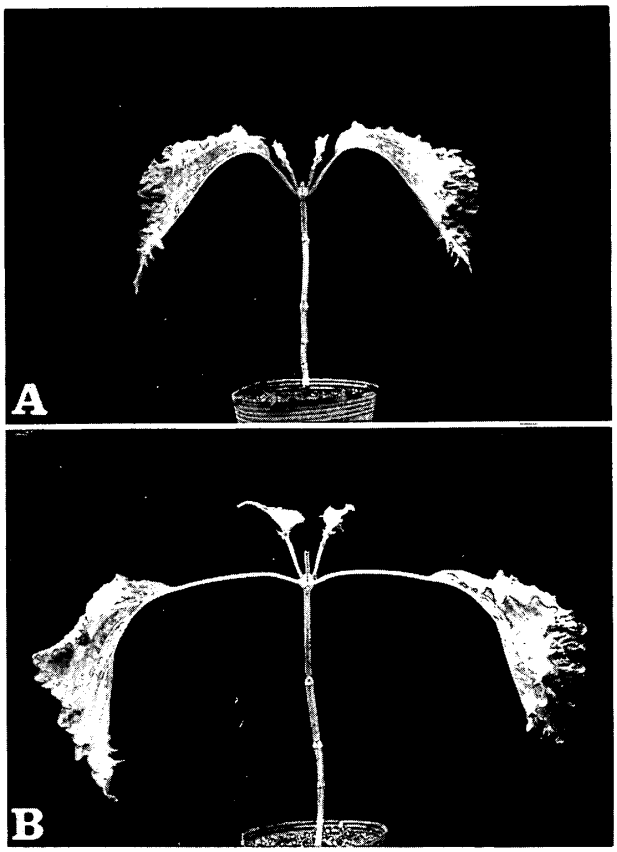

Fig. 3 Growth of *Perilla* plant as influenced by BR. Increase in leaf blade size, petiole length, internode length and lateral bud length is evident in BR treated plant. A: nontreated control plant, B: BR-treated plant.

and nonvernalized plants, although the degree of promotion was greater in nonvernalized plants than in vernalized ones. Plant height at the time of the first anthesis was also increased by GA_3 treatment. However, no significant change was detected in the final number of leaves (Table 2).

BR also hastened the first anthesis in nonvernalized plants, but the effect was slight and no such effect was seen in vernalized plants. BR did not induce increase of plant height. Evident leaf epinasty was observed during the period of BR application, but normal leaf orientation was recovered when the BR treatment was discontinued. Final stem height was decreased by treatment either with BR or GA_3 in plants vernalized for 10 days (Table 2).

Flowering of Perilla frutescence ver crispa *under noninductive conditions*—BR did not induce flowering in *Perilla,* a short day plant, under noninductive conditions, although it promoted the growth of leaf and lateral shoot as well as that of internodes (Fig. 3). Petiole length of control and BR-treated plants were 3.43 ± 0.08 and 7.13 ± 0.28 cm, respectively.

Discussion

In *Luffa cylindrica,* sex expression of flowers in the proximal to distal parts of the BA-treated staminate inflorescence changed according to the order of flowers, from staminate to bisexual and pistillate flowers and finally to foliage leaves (Takahashi et al. 1980). BR was also found to induce sex modification in the staminate inflorescence, as does BA, but the activity of BR was lower than that of BA. Sex modification by BR treatment was induced only in the upper most node located just below the cutting site of the main stem, whereas BA treatment induced sex modifications in all three nodes (Table 1), as described elsewhere (Takahashi et al. 1980). Combined application of BA and BR gave no additive effect on the sex modification and induced abnormal development in the flower organs such as the sepals.

BR promoted flowering only slightly in nonvernalized radish plants, and this promotion was not seen when plants were seed-vernalized. On the other hand, GA_3 promoted flowering not only in nonvernalized but also in vernalized plants. As the final number of leaves were not decreased significantly even in GA_3-treated plants, the promotion of flowering in this experiment might be due to the hastening of growth and not to the effect on the development of flowers.

Kopcewicz (1970) reported that flowering in the cold-requiring long day plant *Cichorium intybus* L. grown under noninductive conditions was induced by estradiol-17 and estrone. GA-treated plants started flowering earlier and were characterized by elongated stems, whereas plants treated with estrogens started flowering 10 days later and had less elongated stems.

As nonvernalized plants of the same radish variety did not flower in other experiments (Suge and Rappaport 1968, Suge 1977), the present materials might have been partially vernalized under the present growing conditions.

BR did not show any activity in inducing flowering in a short day plant *Perilla* under noninductive conditions, although it promoted the growth of leaf, lateral shoot and internode (Fig. 4). The reason why BR had a positive effect on the flowering of a long day plant, *Raphanus sativus,* whereas it gave no such effect on that of a short day plant, *Perilla frutescence,* remains unclear.

Flowering of short day plants was reported to be inhibited by an inhibitor of steroid biosynthesis, although attempts to remove the inhibition by normal steroidal metabolites have been unsuccessful (Bonner et al. 1963). Increase of steroidal metabolites was reported at the time of onset of flowering in several plants including *Perilla* (Kopcewicz 1971, 1972a, b). It is interesting to note that BR was isolated from pollen and has been found to have a steroidal structure. The present study failed to show any significant relationships to the hypothetical flowering hormone. More detailed experiments are needed before the role of BR in the reproductive development can be established.

BR has been known to interact with auxin (Yopp et al. 1981, Katsumi 1985), so the possi-

bility that BR exerts its effect on flowering through an interaction with endogenous auxin cannot be neglected.

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