Difference in Effectiveness of Various Gibberellins on Shoot Elongation and Flower Formation in *Pharbitis nil* Chois.

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Summary

Various C₂₀-gibberellins, A₁₈, A₃₈, A₂₃, A₂₈ and A₂₇, and various C₁₉-gibberellins, A₁, A₂, A₃, A₄, A₅, A₇, A₈, A₉, A₂₀, A₂₁ and A₂₂ were applied to the cotyledonary plumule of *Pharbitis nil* Chois., cv. Kidachi which was subjected subsequently to an inductive dark period of relatively short duration. Their promotive activities were examined in shoot elongation as well as flower formation. The order in the promoting activity of these C₂₀-gibberellins except GA₂₇ and GA₃ at 0.05 μg dosage on both the shoot elongation and the flower formation was observed as follows: A₃ > A₃₈ ≥ A₂₃ ≥ A₁₈ > A₂₈ = water control. At 0.5 μg dosage the same order in the shoot elongation was observed, but the order in the activity on the flower formation was observed as follows: A₃₈ > A₃ = A₂₃ = A₁₈ > A₂₈ = water control. The order in the promotive activity of various C₁₉-gibberellins and GA₂₇ at 0.05 μg dosage on both of the responses was observed as follows: A₃ > A₁ = A₇ > A₅ > A₂ = A₄ ≥ A₂₀ > A₂₂ > A₉ ≥ A₈ = A₂₁ = A₂₇ = water control. At 0.5 μg dosage the same order in growth activity on the shoot elongation was also observed, but the order in the activity on the flower formation was observed as follows: A₂ = A₄ = A₅ > A₁ = A₇ ≥ A₃ = A₂₀ > A₂₁ > A₉ = A₈ = A₂₇ = water control. The gibberellins which have growth promotive activity were always active in flowering, and the opposite was also true. The discrepancy in the order of highly active gibberellins at high dosage between the growth promotive activity and the flower promotive activity may be due to the fact that flower promotive activity at high dosage somewhat decreases, irrespective of highly promotive activity in shoot elongation.

Introduction

The extracts obtained from the seeds of *Lupinus*, *Pharbitis* or *Phaseolus*, which were applied to cotyledonary plumule of *Pharbitis* plants preceding an inductive dark period, have promoted flower formation as well as shoot elongation (10, 12). Similar promotion has been obtained also by the application of GA₃ (11, 13). Therefore, the promotive activity of the seed extract is possibly caused by some gibberellins present in the extract. On the other hand, C₂₀-gibberellins, A₁₈, A₂₃ and A₂₈ were isolated from the extract of *Lupinus* seeds (2, 4, 5, 14) and the same carbon gibberellin, A₃₈ from the extract of *Phaseolus* seeds (3). The various C₁₉-gibberellins, A₁, A₂, A₃, A₄, A₅, A₇, A₈, A₉, A₂₀, A₂₁ and A₂₂, and C₂₀-gibberellin, A₂₇ have been isolated from the seed extract of *Pharbitis*, *Phaseolus* and others

(3, 6, 7, 8, 9, 19). The present paper will report comparative effectiveness of these gibberellins on both the shoot elongation and the flower formation in *Pharbitis nil* Chois., which was exposed to an inductive dark period of relatively short duration, preceded by the external application of the gibberellins.

Materials and Methods

As the experimental plant, the seedling of *Pharbitis nil* Chois., cv. Kidachi was used. The seeds which treated with conc. H₂SO₄ for 30 minutes were washed thoroughly in running water for one day, and spread on moistened sand in a petri dish. Next days, the seeds which begin to germinate were sown in six parallel lines in 35×45cm wooden box filled with garden soil. Each of the lines is consisted of 10 seeds. They were placed under sun light during the day and a fluorescent lamp of 2000 lux intensity at plant level during the night in the glasshouse controlled at

$28 \pm 2^\circ\text{C}$. Two days after sowing, the seedling emerged from the soil and expanded the cotyledon, and five days after sowing the aqueous solution of gibberellin was dropped onto the cotyledonary plumule of plant by means of glass capillary, line to line. First, C_{20} -gibberellins, A_{18} , A_{38} , A_{23} and A_{28} , which had been isolated from *Lupinus* seeds with exception of GA_{38} , were tested in their activity by comparing with the activity of GA_3 . Second, C_{19} -gibberellins, A_1 , A_2 , A_3 , A_4 , A_5 , A_7 , A_8 , A_9 , A_{20} , A_{21} and A_{22} and GA_{27} , most of which had been isolated from *Pharbitis* seeds, were tested. Each plant was treated with about 0.05 ml of test solution. Many boxes with the plants were used so that 16 to 20 plants receive a test solution. Thereafter, the plants were subjected to an inductive dark period of 11 hour duration at $26 \pm 1^\circ\text{C}$ and then they were transferred again to the same glasshouse. About two or three weeks after the dark period the plants were harvested and the shoot length was measured in mm by a ruler and the flower bud on the stem was examined under binocular microscope. The formation of flower bud was indicated as the number of flower bud per one plant.

Results

The activities of A_{18} , A_{38} , A_{23} , A_{28} and A_3 on both shoot elongation and flower formation are shown in Figs. 1 and 2. All the gibberellins except A_{28} promoted strongly shoot elongation as well as flower formation. The order in the promotive activity at $0.05 \mu\text{g}$ dosage was the same on both of the responses and was shown as follows: $A_3 \gg A_{38} \geq A_{23} \geq A_{18} \gg A_{28}$ = water control. At $0.5 \mu\text{g}$ dosage these gibberellins except GA_{28} promoted much more shoot elongation as well as flower formation. The order in the promotive activity on the shoot elongation was almost the same as that mentioned above, but the order in the activity on the flower formation was observed as follows: $A_{38} > A_3 = A_{23} = A_{18} \gg A_{28}$ = water control. Thus, the order of the active gibberellins on the flower formation was different from the order on the shoot elongation.

The activities of A_1 , A_2 , A_3 , A_4 , A_5 , A_7 , A_8 , A_9 , A_{20} , A_{21} , A_{22} and A_{27} on shoot elongation and flower formation are shown in Figs. 3 and 4.

Fig.1. Effects of C_{20} -gibberellins, A_{18} , A_{38} , A_{23} and A_{28} , and GA_3 at $0.05 \mu\text{g}$ dosage on shoot elongation and flower formation in *Pharbitis nil* Chois. Each bar represents the mean of 16-20 plants. Standard error is given by vertical line.

Fig.2. Effects of C_{20} -gibberellins, A_{18} , A_{38} , A_{23} and A_{28} , and GA_3 at $0.5 \mu\text{g}$ dosage on shoot elongation and flower formation in *Pharbitis nil* Chois. Each bar represents the mean of 16-20 plants. Standard error is given by vertical line.

Fig.3. Effects of C₁₉-gibberellins, A₁, A₂, A₃, A₄, A₅, A₇, A₈, A₉, A₂₀, A₂₁ and A₂₂, and GA₂₇ at 0.05 µg dosage on shoot elongation and flower formation in *Pharbitis nil* Chois. Each bar represents the mean of 16-20 plants. Standard error is given by vertical line.

Fig.4. Effects of C₁₉-gibberellins, A₁, A₂, A₃, A₄, A₅, A₇, A₈, A₉, A₂₀, A₂₁ and A₂₂, and GA₂₇ at 0.5 µg dosage on shoot elongation and flower formation in *Pharbitis nil* Chois. Each bar represents the mean of 16-20 plants. Standard error is given by vertical line.

GA₈, GA₉, GA₂₁ and GA₂₇ were slightly active or almost inactive in both shoot elongation

and flower formation. The order in gibberellin activity at 0.05 µg dosage was much the same in both of the responses, and was shown as follows: A₃ > A₁ = A₇ > A₅ > A₂ = A₄ ≥ A₂₀ > A₂₂ > A₉ ≥ A₈ = A₂₁ = A₂₇ = water control. At 0.5 µg dosage, the active gibberellins promoted much more shoot elongation as well as flower formation. The same order was observed also on the shoot elongation at 0.5 µg dosage. However, the order of the active gibberellins was differently observed in flower formation, that is, A₂ = A₄ = A₅ > A₁ = A₇ ≥ A₃ = A₂₀ > A₂₁ > A₉ = A₈ = A₂₇ = water control.

The activity of GA₃ at various dosages on flower formation was examined by comparing with the activity on shoot elongation. The results are shown in Fig. 5. Even a small dosage as 0.005 µg was effective on the flower formation, although no significant

Fig.5. Effects of GA₃ at various dosages on shoot elongation and flower formation in *Pharbitis nil* Chois. Each point represents the mean of 16-20 plants. Standard error is given by vertical line.

promotion was obtained in shoot elongation. The increase of dosage brought about higher promotion in both shoot elongation and flower formation. However, the extent of the promotive activity in flowering caused by 0.5 µg dosage decreased to some degree, and promotion in flowering became much less at 5 µg dosage, even though much higher promotion in growth was obtained.

Discussion

It has been reported previously that the seed extract from *Lupinus*, *Phaseolus* or *Pharbitis* brought about promotion in flower formation as well as shoot elongation in *Phar-*

bitis nil Chois. (10,12). On the other hand, the other previous papers have shown that the extract from *Lupinus* seed contains GA₁₈, GA₂₃ and GA₂₈, and probably does not contain other gibberellin (2,4,5,14). The extract from *Phaseolus* seed has been found to contain GA₃₈ besides some C₁₉-gibberellins (3). Thus, it is concluded that the *Lupinus* extract is promotive in flowering by the activity of GA₁₈ and GA₂₃, and the *Phaseolus* extract is promotive by the activity of GA₃₈ and some other active gibberellins. These C₂₀-gibberellins are assumed to be precursors of C₁₉-gibberellins, A₁ and A₃ which are very highly active (1). However, it is difficult to establish whether these C₂₀-gibberellins are physiologically active *per se* in the plumule of *Pharbitis*, or whether their promotive activities occur after conversion to GA₁ and GA₃. In the present study, GA₃₈ is somewhat more active than GA₂₃ irrespective of the idea that GA₃₈ is the precursor of GA₂₃, suggesting that GA₃₈ is active *per se* in *Pharbitis*. Incidentally, the other experiments carried out with barley endosperm support the former viewpoint that GA₃₈ and GA₂₃ are active *per se* (15,17). In *Pharbitis* seed the occurrence of gibberellins, A₃, A₅, A₈, A₂₀, A₂₆, A₂₇ and A₂₉, and that of their glucosides except A₅ has been reported (6,19,20,21). Although GA₂₆ and GA₂₉ have not been examined in the present study, they are not thought to have relation to the response of *Pharbitis*, judging from their very weak activities in other bioassays (21). In the other report, it is also noted that GA₃-glucoside is also very weakly promotive in flower formation and almost inactive in shoot elongation of *Pharbitis* (16). Thus, the flower promotive activity of the extract from *Pharbitis* seed is assumed to be due mainly to GA₃, GA₅ and GA₂₀, and rarely to their glucosides except GA₅. Speaking generally with *Pharbitis* seedling, the gibberellins which have growth promotive activity are always active in flowering, and the opposite is also true. The promotion in mitotic activity of GA₃ in the apical meristem of *Pharbitis* seedling has been reported by OKUDA (18). Therefore, the flower promotive activity of various gibberellins must also depend upon their mitotic activities in the stem apex. It has been noted that the order in grow-

th promotive activity caused by highly active gibberellins at high dosage is not always the same as the order in flower promotive activity. The difference in both of the orders could result in the fact that at high dosage the flower promotive activity decreases to low irrespective of much highly promotive activity in shoot elongation. It might be suggestion that the difference is related to some different function of gibberellin between the flower formation and the shoot elongation.

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アサガオの茎の伸長ならびに花芽形成に及ぼす各種のジベレリンの作用の比較

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摘 要

誘導暗期の直前に、短日植物、アサガオの幼芽にアサガオ、ルーピンあるいはインゲンなどの種子のアセトン抽出物を与えると、対照区に比して茎の伸長ならびに花芽の形成が促進される。同じような促進作用がジベレリン A₃ を与えた場合でも得られることが知られている。本報文では、上記の各種の種子から分離された炭素-20個のジベレリン (C₂₀) A₁₈, A₃₈, A₂₃, A₂₈ と A₂₇, また炭素-19個のジベレリン (C₁₉) A₁, A₂, A₃, A₄, A₅, A₇, A₈, A₉, A₂₀, A₂₁ と A₂₂ の作用を、アサガオの茎の伸長

ならびに花芽形成において比較調査した。

アサガオの園芸品種、キダチをガラス室内で (28 ± 2 °C), 昼間は太陽光、夜間は蛍光灯の連続照明下で育成し、その幼芽に上記の各種のジベレリン溶液を滴下し、直ちに1回の11時間の誘導暗期を与えた。

0.05 μg の供試量の各種 C₂₀-ジベレリンと GA₃ の茎の伸長における促進順位は、A₃ ≫ A₃₈ ≧ A₂₃ ≧ A₁₈ ≧ A₂₈ = 対照区である。同じ促進順位が花成においてもみられる。0.5 μg の供試量の茎の伸長における促進順位は、0.05 μg

の場合とほとんど同じである。花成においては、 $A_{38} > A_3 = A_{23} = A_{18} \gg A_{28} = \text{対照区}$ の順位を示し、茎の伸長順位と異なる。

0.05 μg の供試量の各種 C_{19} -ジベレリンと GA_{27} の茎の伸長における促進順位は、 $A_3 > A_1 = A_7 > A_5 > A_2 = A_4 \geq A_{20} > A_{22} > A_9 \geq A_8 = A_{21} = A_{27} = \text{対照区}$ を示し、花成においても同じ促進順位である。0.5 μg の供試量の茎の伸長における促進順位は、0.05 μg の場合とほとんど同じであるが、花成においては、 $A_2 = A_4 = A_5 > A_1 = A_7 \geq A_3 = A_{20} > A_{21} > A_9 = A_8 = A_{27} = \text{対照区}$ の順位を示し、茎の伸長順位と異なる。

各種の供試量の GA_3 の作用は、0.005 μg のような少量では茎の伸長の促進はみられないが、花成に対し明らかな促進がみられる。そしてその供試量が増すにつれ

て茎の伸長や花成の促進は両者ともに平行して強まる。しかし 5 μg のような多量では茎の伸長は著しく促進されるにもかかわらず花成に対する促進は減少する。

アサガオの茎の伸長と花成において各種ジベレリンの作用を一般的にみて、茎の伸長を促進するジベレリンは花芽形成をも促進する。あるいは逆に、花芽形成を促進するジベレリンは茎の伸長をも促進する。しかし供試量の多い場合には、各種ジベレリンの茎の伸長における促進順位と花芽形成における順位とは異なる。この両者の相異は、活性の高いジベレリンは茎の伸長を著しく促進するにもかかわらず、花芽形成をあまり促進しないためと思われる。これらの事は、ジベレリンの生理的機能が茎の伸長と花芽形成との間で、その濃度によって、似ている場合と異なる場合があることを示唆している。