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Influence of gibberellins on flower formation in *Hyoscyamus niger* L.

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Abstract

Gibberellins (GA_{4+7}) and gibberellin-like substances isolated from generatively induced black henbane (*Hyoscyamus niger* L.) bring about the growth of shoots and a partial differentiation of axillary meristem in black henbane plants grown under non-inductive light conditions. Long-lasting application of gibberellins, however, did not result in full development of flowers in the majority of the plants investigated. Thus, it seems, that gibberellins are not specific flowering hormones in black henbane — a long-day plant.

INTRODUCTION

It is known that in some long-day plants, among them biennal black henbane not treated with cold, gibberellins can initiate flowering under non-inductive conditions (Lang 1957, Vince 1975). In annual black henbane, however, GA_1, GA_3 and GA_{13} stimulate only the growth of shoots under non-inductive light conditions. They do not induce formation of flower buds and flower development (Kopcewicz and Centkowska 1983).

The aim of our present investigations was to study the effect of GA_{4+7}, which are relatively more active than GA_1, GA_3 and GA_{13} in some long-day plants (Vince 1975), as well as the effect of gibberellin-like substances isolated from generatively induced black henbane (Ga^s fraction) on flower formation in black henbane grown under non-inductive light conditions.

MATERIAL AND METHODS

The experiments were carried out with a classical long-day plant *Hyoscyamus niger* L. f. *annuus*. Seeds of black henbane were germinated in containers with garden soil in a greenhouse. After 10 days the

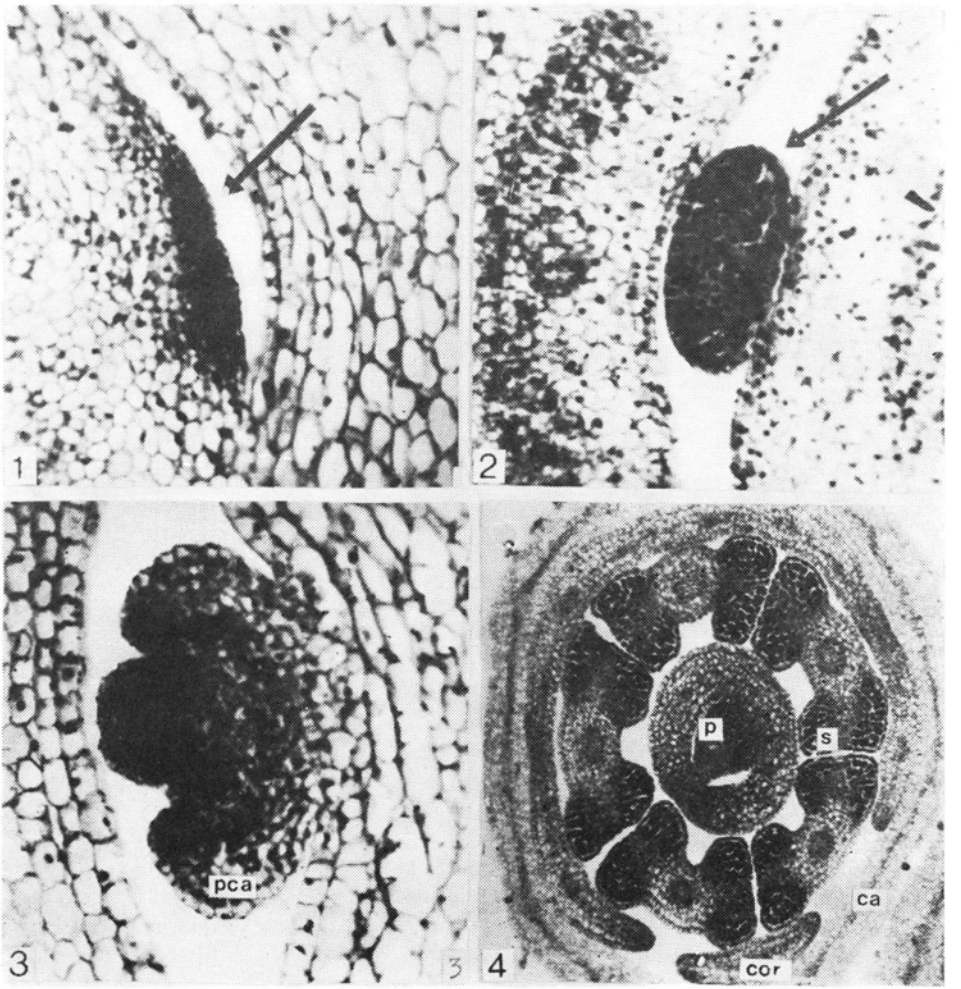
seedlings were selected and exposed to short day conditions (L:D=8:16 hours) for 75 days. The plants were kept in growth chambers at a temperature of 23°C in light and 18°C in darkness, and light intensity of 26 $W \times m^{-2}$ applied simultaneously from white fluorescent tubes and incandescent bulbs. The treatment started on the 76th day. Gibberelins (GA_{4+7} and a gibberellin fraction isolated from leaves of generatively induced black henbane plants) were applied in a 0.05% water solution of Tween 80 to the leaves of the plants once daily during 75 successive days, in the amounts of 10μg (GA_{4+7}) or the equivalent of 300 mg of fresh weight of extracted tissue (GA^s fraction) per plant, per application. The control plants were treated with 0.05% water solution of Tween 80 alone. Seventy plants were used in each experiment (controls, GA_{4+7}, GA^s fraction). Anatomical and morphological observations were carried out on the 5th, 7th, 10th, 15th, 25th, 35th and 75th day from the start of hormone application. Ten plants were used for each observation.

Apical parts (0.5-1.5 cm) and five successive upper nodes were fixed with gluteraldehyde and formalin-propionic acid-ethanol (Gerlach 1972) and embedded in paraffin (after dehydratation in a graded series of ethanol and infiltration with toluene). Serial horizontal 6 or 8 μm sections were stained with Ehrlich hematoxylin (Johansen 1940).

The gibberellin fraction was isolated from leaves of plants which, at first, were grown for 75 days under short day conditions and then transferred for five days to a long inductive photoperiod. Gibberellins were extracted from 1 kg of leaves according to the methods described previously (Kopcewicz et al. 1979). Gibberellins were partitioned chromatographically using Whatmann 3MM paper with distilled water as solvent. The place of localization of gibberellins (zone R_f 0.7-1.0) was eluted with methanol which was then evaporated. The residue was redissolved in 0.05% water solution of Tween 80 and used for application. The total amount of active substances in a gibberellin fraction was about 2 μg of GA_3 equivalent to 100 g of fresh weight of the tissue.

RESULTS AND DISCUSSION

The increase of stem elongation and the gradual differentiation of axillary meristems were taken into account for characterization of the degree of flower differentiation. The increase of the stem elongation is commonly known as an early symptom of flower formation in rosette-forming plants (Lang 1965). The choise of the axillary meristem for anatomical observation followed from the fact that the individual flowers or flower-bearing lateral shoots of black henbane are produced directly by the differentiation of the axillary meristems. In order to establish a possible correlation between gibberellin application and the



Figs. 1-4. Stages of axillary meristem differentiation in *Hyoscyamus niger* L.
Figs. 1 and 2. Tranverse section through shoot, arrows indicate axillary meristem — stage A and B, respectively. × 1230
Fig. 3. Longitudinal section through young flower bud — stage C. × 1230
Fig. 4. Transverse section of flower bud — stage D. × 310
pca — calyx primordium, ca — calyx, cor — corolla, s — stamen, p — pistil

Fig. 5. Effect of GA_{4+7} and gibberellin-like substances isolated from generatively induced *Hyoscyamus niger* L. plants (E) on the growth and development of black henbane; C — control. × 00

degree of flower bud development, the following stages of axillary meristem differentiation were considered:

A. Axillary meristem composed of few cells rich in RNA. No morphological differentiation noticeable (Fig. 1).

B. Protuberant axillary meristem (Fig. 2).

C. Axillary bud with initiated calyx primordium (Fig. 3).

D. Axillary bud with all flower parts initiated (Fig. 4).

During the whole period of the experiment the control plants remained at the vegetative stage (Table 1, Fig. 5). The number of nodes did not exceed 50 and no change in the length of the internodes was noticed. The axillary meristem of these plants remained small and poorly differentiated. Black henbane treated with gibberellins did not show any noticeable difference in comparison with the control plants during five (GA_{4+7}) or seven (GA^s fraction) days. After this time a gradual increase in both the number of nodes (up to 70) and the length of the shoots could be observed. A particularly fast increase in the length of the internodes was observed between the 35th and 75th day of gibberellin application (Table 1). The axillary meristem also began to differentiate. During 35 days, however, only a part of the plants reached the stage of an axillary bud with initiated calyx primordia (Fig. 3, stage C). Also after 75 days of treatment only a few plants reached the stage of an axillary bud with all flower parts initiated (Fig. 4, stage D). Such a situation occurred both in the GA_{4+7} and the GA^s fraction treated plants (Table 1).

The obtained results show that GA_{4+7} are more effective in promoting flowering in black henbane than GA_1, GA_3 and GA_{13} (Kopcewicz and Centkowska 1983). However, even they are not able to substitute completely the inductive influence of a long photoperiod on flower formation in *Hyoscyamus*. It must be taken under consideration that the application lasted for 75 days and every day as much as 10 μg of GA_{4+7} was applied. It may, therefore, be assumed that under natural conditions these gibberellins are not endogenous specific flowering factor. In the case of the GA^s fraction, the total amount of applied gibberellins was undoubtedly lower than in the case of GA_{4+7}; in this fraction, however, it was a mixture of different gibberellins. Earlier investigations indicate the presence of four groups of gibberellin-like substances in this fraction (Kopcewicz et al. 1979). It seems that only some of them may play role in the process of flowering (Kopcewicz et al. 1979).

Thus, gibberellins promote, first of all, elongation growth of the generative shoot. In the course of shoot growth, with continuous excess of applied gibberellins, a partial differentiation of axillary meristems in some plants is reached. Long-lasting application of gibberellins, how-

Table 1

Influence of gibberellins on stem elongation and axillary meristem differentiation in *Hyoscyamus niger* L. grown under non-inductive light conditions

Day of experiment	Mean shoot length, cm			Stage of axillary meristem differentiation**			Per cent of plants at particular stages of axillary meristem differentiation		
	control	GA_{4+7}	GA*	control	GA_{4+7}	GA*	control	GA_{4+7}	GA*
5	0.4	0.5	0.5	A	A	A	100	100	100
7	0.5	1.4	0.7	A	B	A	100	100	100
10	0.5	3.3	2.6	A	C	B	100	50	100
15	0.6	8.7	5.5	A	C	C	100	50	50
25	0.6	26.2	18.4	A	C	C	100	50	50
35	0.6	43.8	31.6	A	C	C	100	50	50
75	0.6	101.8	91.2	A	D	D	100	20	20

* gibberellin-like substances isolated from generatively induced black henbane plants.
** see text and Figs. 1-4.

ever, did not result in a full development of flowers in the majority of plants. Thus, it seems possible, that gibberellins do not play the role of specific flowering hormones in the generative differentiation of black henbane plants.

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Wpływ giberelin na tworzenie kwiatów u Hyoscyamus niger L.

Streszczenie

Gibereliny (GA_{4+7}) oraz substancje giberelinopodobne wyizolowane z poddanych fotoindukcji roślin lulka czarnego (*Hyoscyamus niger* L.), powodują wzrost pędu oraz częściowe różnicowanie merystemów pachwinowych roślin lulka czarnego rosnących w warunkach nieindukcyjnego fotoperiodu. Długotrwała aplikacja giberelin nie doprowadziła jednak do pełnego rozwoju kwiatów u większości roślin. Tak więc, gibereliny nie wydają się być specyficznymi hormonami kwitnienia u lulka czarnego — rośliny długiego dnia.