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Phytohormones level in the leaves of *Hyoscyamus niger* L. during variable photoperiods at the time of flower initiation and differentiation

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Abstract

The investigations concern changes in the content of endogenous phytohormones in the leaves of the long-day plant *Hyoscyamus niger* L. during variable photoperiods applied before and after flower initiation. The results show that alternation of inductive photoperiods with short days leads to quantitative changes in the content of phytohormones. The changeable photoperiod leads to a general decrease in the contents of gibberellins, cytokinins and auxins during the short noninductive days both before and after flower initiation. Alternation of the inductive photoperiod with short days does not influence the content of abscisic acid-like substances before flower initiation and causes an increase of the amount of inhibitors in the postinductive period. The content of hormonal substances is subjected to rhytmical changes related to the periods of light and darkness in the twenty-four hours' cycle.

INTRODUCTION

In our previous investigations (Kopcewicz et al., 1979) it was established that 2.5 months old seedlings of black henbane grown under short-day conditions reached a state of generative induction during the first five long photoperiods, whereas the post-inductive period was characterized by a gradual differentiation of flower elements. The inductive photoperiod also caused a general increase in the amount of phytohormones, with intensive accumulation of cytokinins and gibberellins in the leaves in the period of flower initiation (3-5 long days). The postinductive period was characterized by variable contents of gibberellins, cytokinins and abscisic acid and a successive increase in the level of auxins. It was also observed that the content of the phytohormones is

subjected to rhytmical changes related to the periods of light and darkness in the twenty-four hours' cycle.

In the present work the influence of a variable photoperiod on the phytohormones level was investigated by interrupting the inductive photoperiod before the appearance of the first anatomical and morphological signs of generative differentiation (after three inductive photoperiods) and in the later period, related to the differentiation of previously formed flower elements (after seven long photoperiods).

Thus, the aim of our investigations was to study the changes in the content of phytohormones caused by alternation of the inductive photoperiod with short day. It is generally known that such manipulation leads to the inhibition of flower formation and the appearance of specific flowering inhibitors in the tissues of long-day plants (Evans, 1971; Wellensiek, 1977). The aim of the present work was to find an answer to the question whether, under such conditions, the directional changes in the content of known groups of phytohormones also take place.

MATERIAL AND METHODS

The experiments were carried out under controlled conditions in growth chambers (8 or 16 hours of daylight, cool-white fluorescent tubes, light intensity about 6500 lux, 23° C in light and 18° C in darkness). Seeds of black henbane (*Hyoscyamus niger* L. *f. annus*) were germinated in containers with garden soil in a greenhouse. After 10 days the seedlings were selected and exposed in growth chambers to a non-inductive short (8 hours of daylight and 16 hours of darkness) photoperiod during 75 days. After this time light conditions were changed by introducing an inductive long 16-hour photoperiod (16 hours of daylight and 8 hours of darkness) for three (variant 1) or seven (variant 2) days. The inductive long photoperiods were alternated with non-inductive short days and the material for investigations was collected at the following periods:

A. Variant 1

I. The third day of a long (L) photoperiod (D) — (3LD)
   a. after 8 hours of light
   b. „ 16 „ „ „
   c. „ 4 „ „ darkness
   d. „ 8 „ „ „

II. The second day of a short (S) photoperiod (D) — (3LD + 2SD)
   e. after 8 hours of light
   f. after 8 hours of darkness
   g. „ 16 „ „ „

III. The fifth day of a short photoperiod — (3LD + 5SD)
h. after 8 hours of light
i. after 8 hours of darkness
j. „ 16 „ „ „

IV. The second day of an alternate long photoperiod — (3LD + 5SD + 2LD)
k. after 8 hours of light
l. „ 16 „ „ „
m. after 4 hours of darkness
n. „ 8 „ „ „

V. Five days of an uninterrupted long photoperiod — (5LD)
o. after 8 hours of light
p. „ 16 „ „ „
r. after 4 hours of darkness
s. „ 8 „ „ „

B. Variant 2

I The seventh day of a long photoperiod — (7LD)
A. after 8 hours of light
B. „ 16 „ „ „
C. after 4 hours of darkness
D. „ 8 „ „ „

II. The second day of a short photoperiod — (7LD + 2SD)
E. after 8 hours of light
F. after 8 hours of darkness
G. „ 16 „ „ „

III. The fifth day of a short photoperiod — (7LD + 5SD)
H. after 8 hours of light
I. after 8 hours of darkness
J. „ 16 „ „ „

IV. The third day of an alternate long photoperiod — (7LD+5SD+3LD)
K. after 8 hours of light
L. „ 16 „ „ „
M. after 4 hours of darkness
N. „ 8 „ „ „

V. Ten days of an uninterrupted long photoperiod — (10LD)
O. after 8 hours of light
P. „ 16 „ „ „
R. after 4 hour of darkness
S. „ 8 „ „ „

In order to examine the content of hormonal substances 50 g of well formed leaves were gathered each time. Collection of the material in the period of darkness was done at low intensity of green-safe light. The collected material was kept at —15°C. Plant growth hormones (gibberellins, cytokinins, auxins and abscisic acid-like inhibitor) were isolated from the same samples (50 g of leaves) with 80% methanol according to the methods described previously (Kopcewicz et al., 1979).

RESULTS AND DISCUSSION

The investigations on the influence of a variable photoperiod on the content of endogenous growth substances were conducted on a qualitatively long-day plant *Hyoscyamus niger* L. very often used in photoperiodic investigations. The plants were grown for 2.5 months under short day conditions and were developing vegetatively. After this time the photoperiod was changed to the inductive one and this caused the plants to pass to the phase of generative differentiation. From the previous data (Kopcewicz et al., 1979) it is known that during the inductive long photoperiods induction of flowering and successive differentiation of generative elements in black henbane take place. The moment of flower induction occurs during the first four long days, since the first anatomical symptoms of generative differentiation were observed during the fifth long photoperiod (Kopcewicz et al., 1979). Moreover, at this very time a gradual increase of the length of the shoot could be observed. During the first five inductive photoperiods the accumulation of cytokinins and gibberellins was also observed in the leaves of black henbane (Kopcewicz et al., 1979). As the number of long days was extended (a post-inductive period), the gradual differentiation of the axillary meristems forming axillary and then flower buds was noted. This period was characterized at the same time by the changing content of phytohormones (Kopcewicz et al., 1979).

The present investigations dealt with the changes in the content of hormonal substances in black henbane leaves, caused by a variable photoperiod before (variant 1) and after flower initiation (variant 2). In both the variants long days were alternated with five short non-inductive cycles. The content of hormonal substances in plants growing under the uninterrupted inductive photoperiod for five (variant 1) and ten (variant 2) days was also examined. Since during the twenty-four hours' changes of light and darkness there occur in the plants some changes of the photostationary state of phytochrome (Vince, 1972), and since there is a probability that the changes in the content of hormones take places as the consequence of phytochrome conversion

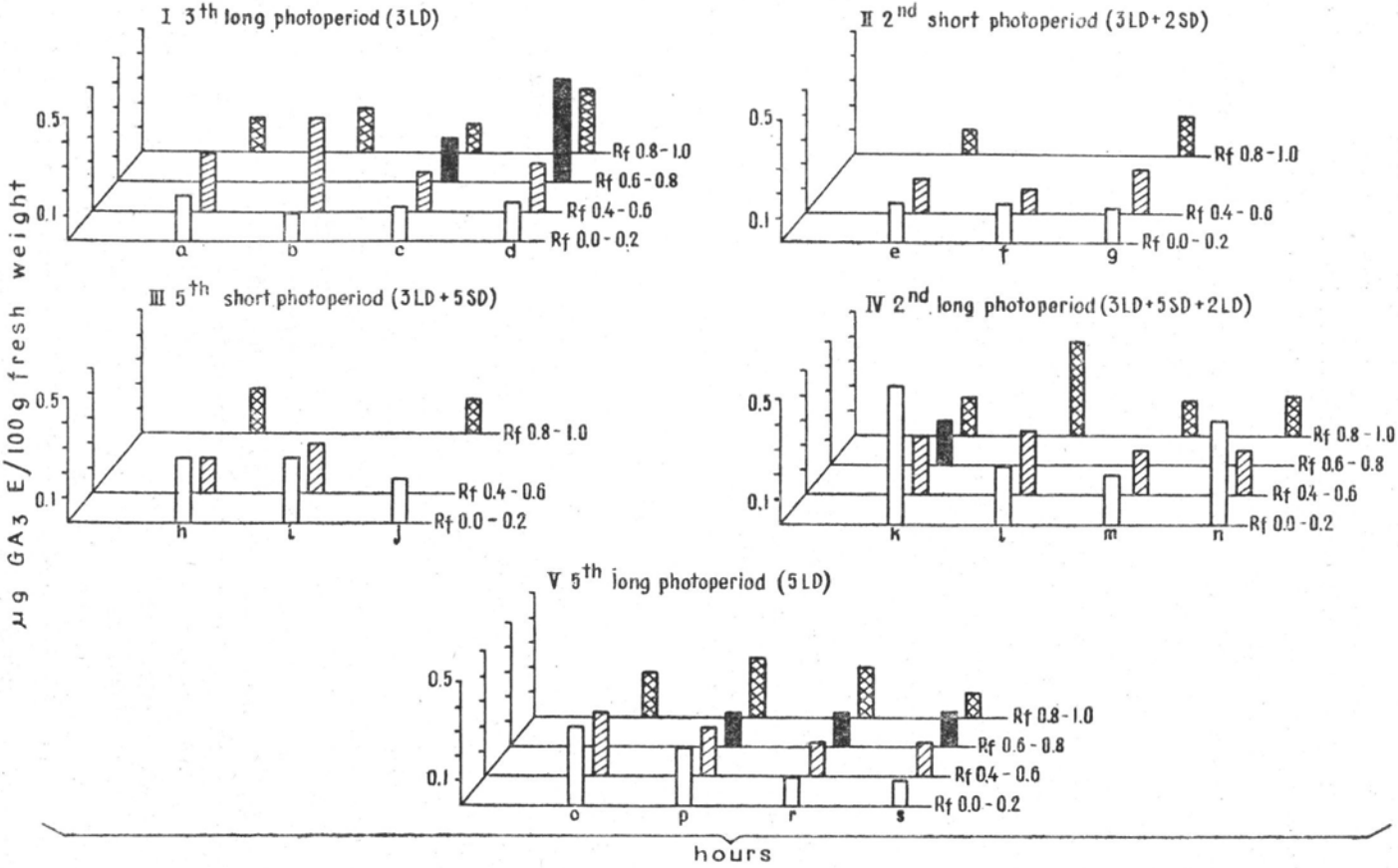

Fig. 1. Changes in the levels of various gibberellins in leaves of *Hyoscyamus niger* L. during variable photoperiods at the time of flower initiation.

(Black, Vlitos, 1972), the investigations were conducted at various periods of day and night. The material was collected after 8 and 16 hours of light and 4 and 8 hours of darkness in the case of a long photoperiod and after 8 hours of light and 8 and 16 hours of darkness in the case of a short photoperiod.

The results concerning the changes in the content of gibberellin-like substance (Figs. 1, 2, 3 and 4) show that in the leaves of black henbane there are four groups of gibberellins localized on the chromatograms at R_F 0.0-0.2, R_F 0.4-0.6, R_F 0.6-0.8 and R_F 0.8-1.0. The levels of particular gibberellins undergo quantitative changes depending on the applied photoperiod and twenty four hours' changes of light and darkness (Figs 1 and 2). Gibberellins in the zones R_F 0.4-0.6 and R_F 0.0-0.2 occur independently of the applied photoperiod, but the content of substances localized at R_F 0.4-0.6 decreases under short-day conditions. Gibberellins in the zone R_F 0.6-0.8, during the first three long photoperiods are present only in darkness, reaching a relatively high level (Fig. 1-I). After five long uninterrupted cycles these gibberellins appear also under light (Fig. 1-V), whereas they disappear completely under non-inductive short-day conditions (Fig. 1-II, III). Gibberellins of this zone are also present after seven as well as after ten long photoperiods (Fig. 2-I, V). Similarly as in the period before flower initiation, a decline of these substances during short-day cycles has been observed (Fig. 2-II, III). These data confirm the earlier results (Kopcewicz et al., 1979) and suggest that gibberellins localized on the chromatograms at R_F 0.6-0.8 may play an important role in the induction and development of flowers in black henbane. Thus, the interruption of inductive cycles by a short photoperiod leads both to the inhibition of flower formation and the disappearance of this group of gibberellins.

The total content of gibberellins (Figs. 3 and 4) shows that introduction of a short photoperiod between inductive cycles, both in the period before as well as after flower initiation, causes a general decrease in the content of gibberellins. The obtained results also point to the existance of some rhytmical changes in the content of gibberellin-like substances in the twenty-four hours' cycle connected with the occurrence of light and darkness. Both in the period before as well as after flower initiation, independently of the applied photoperiod, increased amounts of gibberellins occur in the light phase (Figs 3 and 4). During the first hours of darkness a decrease in the content of these substances take place. It seems possible that twenty-four hours' changes in the content of endogenous gibberellin-like substances are correlated with the state of phytochrome and in the period when a high level of phytochrome P_{FR} occurs, increased amounts of endogenous gibberellins appear in the leaves of black henbane. The mechanism of these phenomena

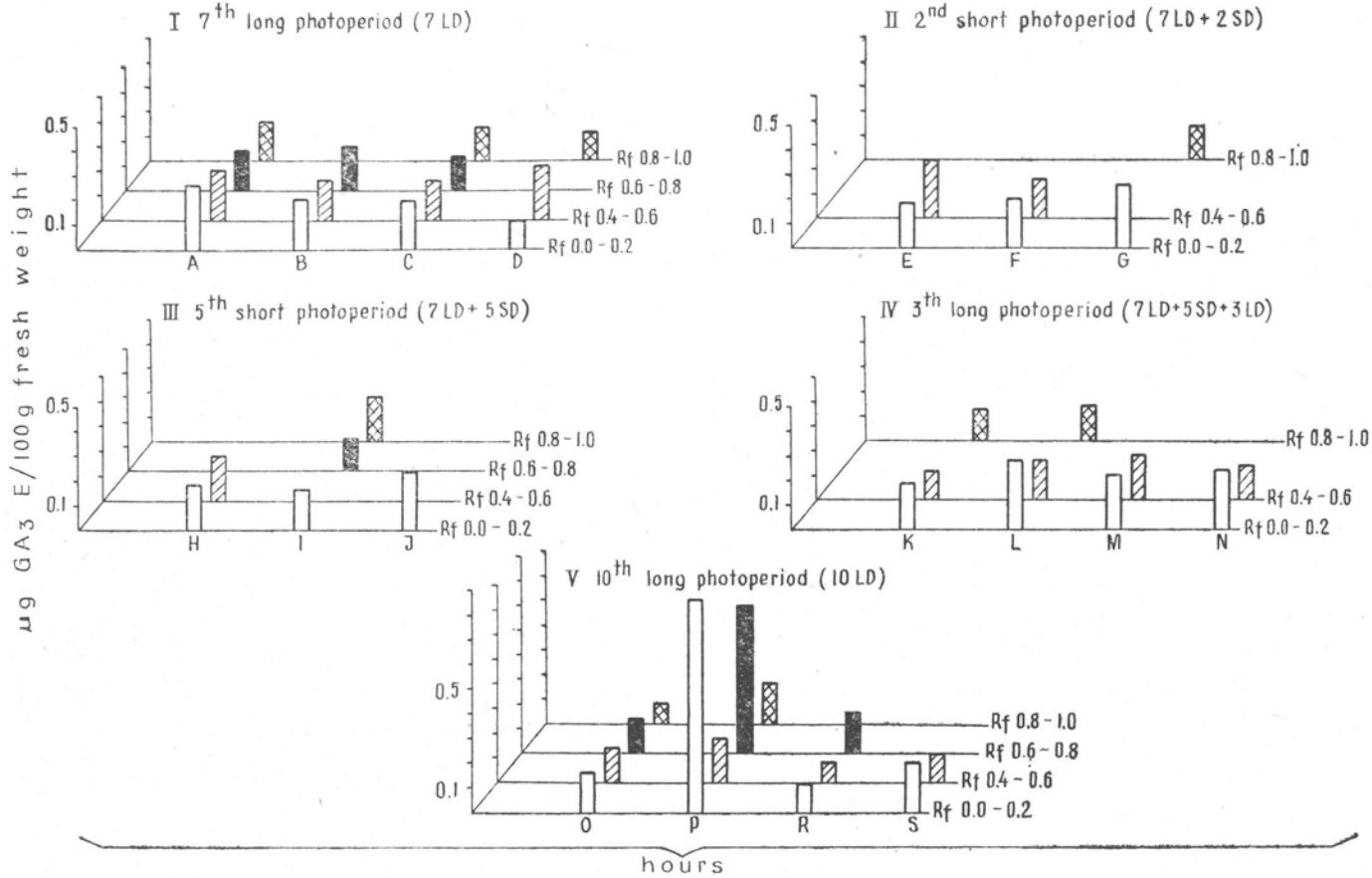

Fig. 2. Changes in the content of various gibberellins in leaves of *Hyoscyamus niger* L. during variable photoperiods at the time of flower differentiation.

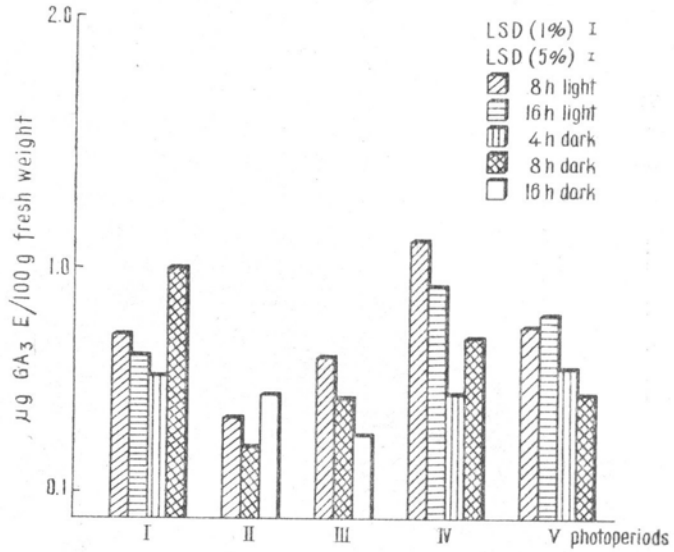

Fig. 3. Total amount of gibberellins in leaves of *Hyoscyamus niger* L. during variable photoperiods at the time of flower initiation.

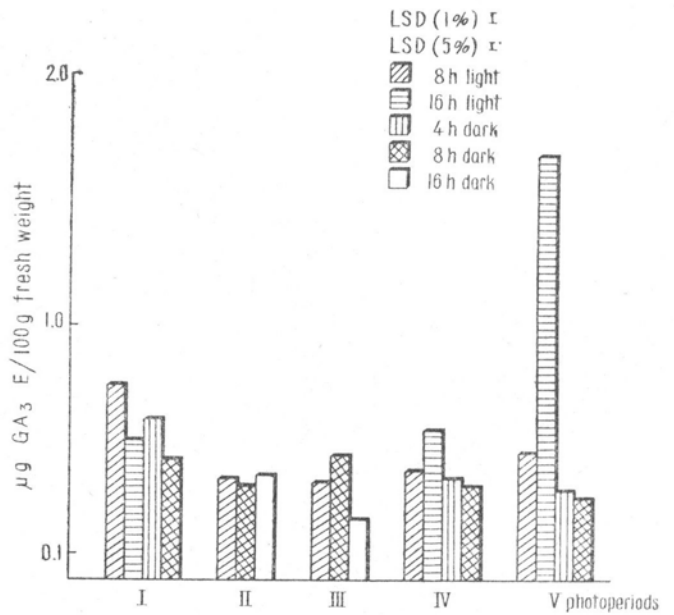

Fig. 4. Total amount of gibberellins in leaves of *Hyoscyamus niger* L. during variable photoperiods at the time of flower differentiation.

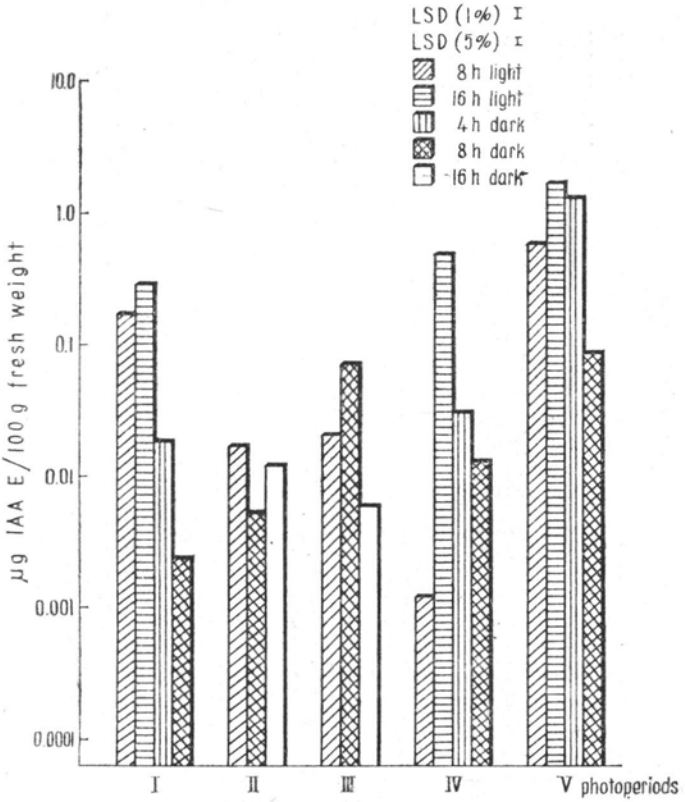

Fig. 5. Auxins in leaves of *Hyoscyamus niger* L. during variable photoperiods at the time of flower initiation.

is unknown but it is most probably related to the mode of action of phytochrome in the plant photoperiodic processes.

The changes in the content of auxins (Figs 5 and 6) show that the application of short photoperiods, both before (Fig. 5) and after (Fig. 6) flower initiation leads to a decrease in the content of these substances. The plants under five or ten uninterrupted inductive photoperiods are characterized by higher levels of auxins than the plants treated with a changeable photoperiod. Previous investigations (K o p c e w i c z et al., 1979) showed that inductive photoperiods caused a successive increase in the level of auxins. When comparing in our present investigations the contents of auxins in the leaves of plants treated with three (variant 1) and seven (variant 2) inductive photoperiods, one may draw a misleading conclusion that after seven uninterrupted photoperiods there is a decrease in the amount of these substances. However, this was caused by the fact that investigations within variant 1 and variant 2 were conducted as completely separate experiments with a time interval between them, therefore comparison of absolute amounts of active

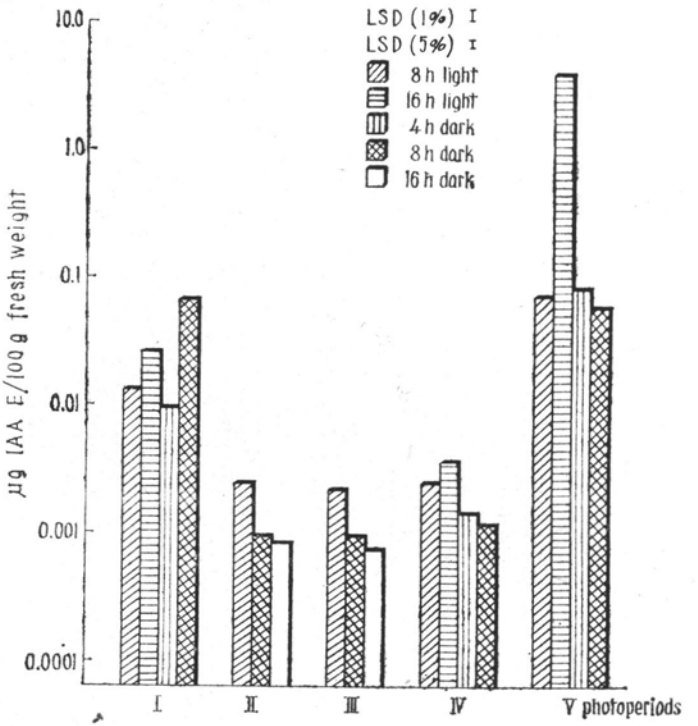

Fig. 6. Auxins in leaves of *Hyoscyamus niger* L. during variable photoperiods at the time of flower differentiation.

substances is not possible. In particular photoperiods the amount of auxins increased during the light phase and decreased during darkness, both under long and short photoperiods (Figs 5 and 6). Detailed identification of auxin-like substances has not been carried out yet; however, the preliminary experiments showed that the main auxin in the examined extracts was indole-3-acetic acid (IAA).

The changes concerning the content of cytokinins before (Fig. 7) and after (Fig. 8) flower initiation show that alternation of an inductive photoperiod with short days leads to a decrease in the level of cytokinins in the leaves of black henbane. The renewed application of long photoperiods causes an increase in the content of these substances. During long photoperiods accumulation of cytokinins was observed in the light phase of the twenty-four hours' cycle. During short photoperiods the highest amounts of cytokinins were found in the first hours of darkness (Figs 7 and 8). In the extracts from black henbane the presence of two groups of cytokinin-like substances, localized on the chromatograms at R_F 0.1-0.4 and R_F 0.6-0.9 was noted. The second zone displayed greater activity and its localization corresponded to zeatine riboside.

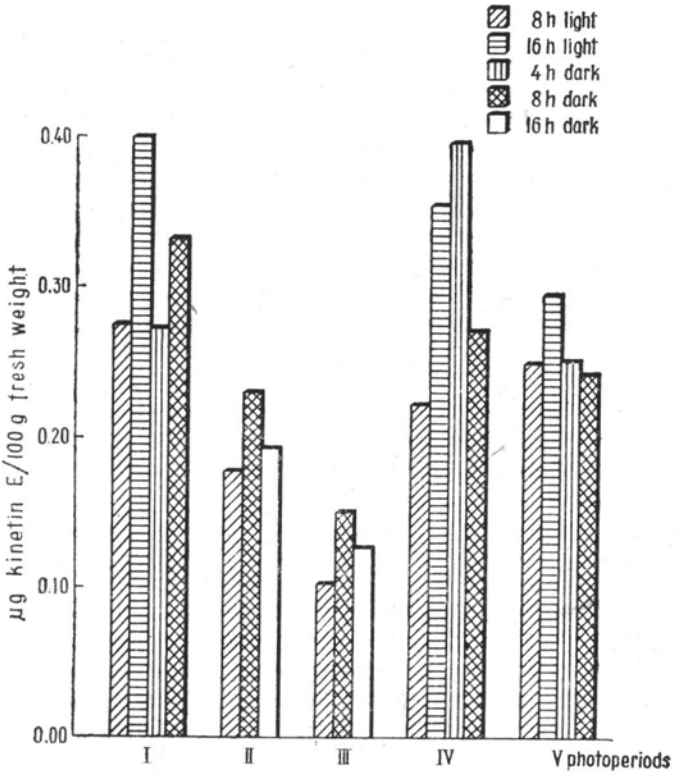

Fig. 7. Cytokinins in leaves of *Hyoscyamus niger* L. during variable photoperiods at the time of flower initiation.

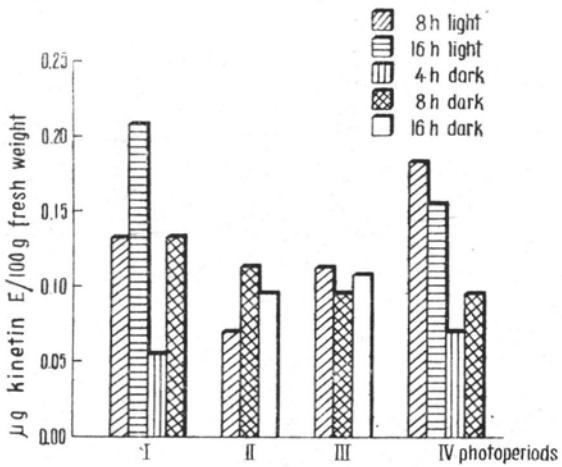

Fig. 8. Cytokinins in leaves of *Hyoscyamus niger* L. during variable photoperiods at the time of flower differentiation.

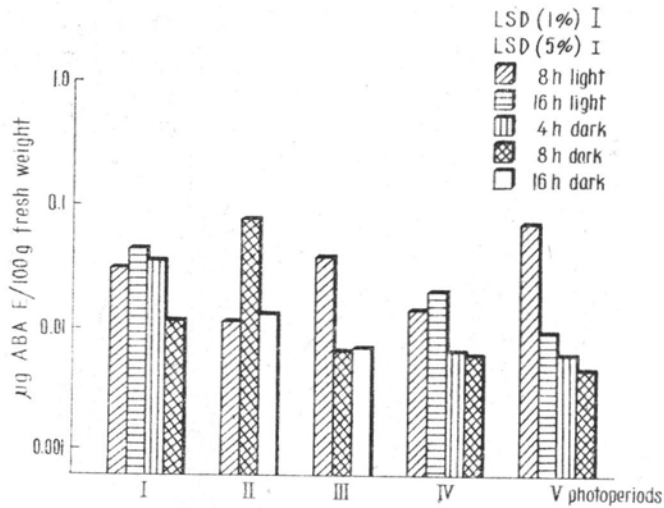

Fig. 9. Abscisic acid-like inhibitors in leaves of *Hyoscyamus niger* L. during variable photoperiods at the time of flower initiation.

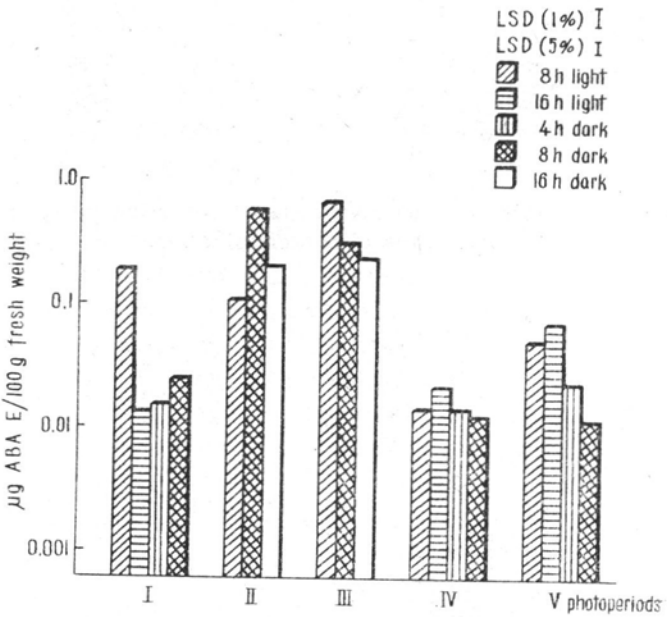

Fig. 10. Abscisic acid-like inhibitors in leaves of *Hyoscyamus niger* L. during variable photoperiods at the time of flower differentiation.

The influence of changeable photoperiod on the level of abscisic acid-like inhibitors was also studied. The results (Figs 9 and 10) show that interruption of the inductive photoperiod before flower initiation does not result in any basic changes in the level of ABA as compared with the plants growing on five uninterrupted long photoperiods (Fig. 9).

On the other hand, the application of short photoperiods after flower induction results in a distinct increase in the content of endogenous inhibitors (Fig. 10). In the twenty-four hours' cycle of changes of light and darkness a higher level of abscisic acid-like substances was observed in the light phase (Figs 9 and 10).

Generally speaking, the obtained results show that in a long-day plant alternation of an inductive photoperiod with short days leads to quantitative and qualitative changes in the content of phytohormones. The problem remains open for discussion, however, whether these changes are the cause or the consequence of the observed morphogenetic reactions. Although interruption of an inductive photoperiod causes a decrease in the content of gibberellins, cytokinins and auxins, it is known, however, that under a long photoperiod the plants, as a rule, contain more hormonal substances, independently of their photoperiodic requirements and flower formation (Chailakhyan, 1968). On the other hand, interruption of the inductive photoperiod in black henbane results in the inhibition of formation and development of flowers, and this in connection with the decline of occurrence of some gibberellins and a general decrease in the content of stimulators suggests causal links between these processes. In the literature there are data suggesting an important role of gibberellins and cytokinins in the phenomena of plant flowering (Lang, 1965; Michniewicz, Kamieńska, 1967; Chailakhyan, 1968; Zeevaart, 1976). Such data are seldom as far as auxins and abscisic acid are concerned (Lang, 1965; Addicot, Lyon, 1969; Zeevaart, 1976); it cannot be excluded, however, that in regulation of generative differentiation in plants the absolute amount of particular hormonal substances is not as important as their proportional participation in relation to other groups of hormones, as it is e.g. in the phenomenon of sex expression (Atsmon et al., 1968). Such a situation occurs in many morphogenetic processes and there are no reasons to think that morphogenesis of flowers is an exception. The explanation of the mechanisms of generative morphogenesis is a future problem; special attention, however, should be paid to the dependences between the phytochrome and the hormonal system, since these two control systems participate directly in the regulation of generative differentiation in plants.

Acknowledgments

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Zawartość fitohormonów w liściach Hyoscyamus niger L. podczas zmiennego fotoperiodu w okresie inicjacji i różnicowania kwiatów

Streszczenie

Badano zmiany w zawartości endogennych fitohormonów w liściach rośliny długodniowej *Hyoscyamus niger* L. w trakcie przemiennego fotoperiodu stosowanego w okresie inicjacji i różnicowania kwiatów. Wyniki wskazują, że przerwanie indukcyjnych fotoperiodów dniami krótkimi prowadzi do jakościowych i ilościowych zmian w zawartości fitohormonów. Zmienny fotoperiod prowadzi do ogólnego spadku zawartości giberelin, cytokinin i auksyn w czasie krótkiego, nieindukcyjnego dnia zarówno w okresie inicjacji jak i różnicowania. Przerwanie indukcyjnego fotoperiodu dniem krótkim nie wpływa na zawartość substancji abscysynopodobnych w okresie inicjacji i powoduje podwyższenie ilości inhibitorów w okresie różnicowania. Zawartość wszystkich grup fitohormonów podlega rytmicznym zmianom związanym z występowaniem okresów światła i ciemności w cyklu dobowym.