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Influence of night-breaks on flowering and phytohormones content in *Hyoscyamus niger* L.

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Abstract

Night-breaks caused both stimulated shoot growth and caused formation of flowers as well as a general increase in the content of phytohormones in leaves of the long-day plant *Hyoscyamus niger* L. At the time of flower formation in night-break treated plants, new gibberellin-like substances also appear. The results show that night-breaks cause similar changes in the phytohormones content as a long inductive photoperiod. It may be assumed that independently of the way of induction, the generative differentiation of long-day plants is always accompanied by a general increase in the amount of endogenous hormones and the appearance of new gibberellins. These results suggest the possibility of a morphogenetic role of hormones, especially gibberellins, in the phenomena of flower formation and differentiation.

INTRODUCTION

The photoreactions which control the induction of flowering in long (LDP) and short (SDP) day plants are remarkably similar. Both LDP and SDP respond to night-breaks because the presence of P_{FR} phytochrome exceeding a certain threshold level in light treatment during the night break inhibits induction in SDP, but is required for photoperiodic induction in LDP (V i n c e 1972). It has been recognized, however, that LDP are usually less sensitive to a night-break than SDP (V i n c e 1975). LDP also require longer exposure and/or higher intensity of light. Flowering is completely prevented in many species of SDP by night-breaks of 15-30 minutes, but for LDP, such treatments frequently have little or no effect. Night-breaks of 1-3 hours are usually sufficient to induce flowering in LDP growing under short-day conditions, but may not saturate the response (V i n c e 1975). Neither is single conversion of phytochrome P_{FR} frequently sufficient to induce

maximal flowering in LDP. Cyclic exposure to light, which is thought to depend for its effect on the fact that P_{FR} remains above some threshold value in the intervening periods of darkness, is often highly effective for LDP, indicating that phytochrome is involved in these responses to long duration of light (V i n c e 1975).

Black henbane (*Hyoscyamus niger* L.) is a classical qualitative long-day plant and the inductive photoperiod causes in it a general increase in the amount of phytohormones and the production of flowers (K o p c e w i c z et al. 1979). The problem remains open for discussion, however, whether the increase in the level of phytohormones is the cause or the consequence of flower formation. Since the flowering of long-day plants may be also evoked in a different way, by application of night-breaks, it was decided to check whether such treatment also leads to similar changes in the metabolism of hormones, as in the case of a long inductive photoperiod. Thus, investigations were undertaken in order to learn, whether, independently of the way of generative induction, this phenomenon is always accompanied by similar changes in the content of hormones.

MATERIAL AND METHODS

Seeds of black henbane (*Hyoscyamus niger* L. f. *annuus*) 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 grown in growth chambers at a temperature of 23°C in light and 18°C in darkness, and a light intensity of 6500 lux from a mixture of cool white fluorescent tubes and incandescent bulbs. Then, the plants were divided into two groups: 1) control plants — kept under a short photoperiod (L:D=10:14 hours); 2) interrupted night (IN) plants (INP) — plants treated with white light night breaks (L:D:L:D=7:7:3:7 hours). The controls as well as the INP were on the whole treated in the same manner, L:D=10:14.

The material for hormonal investigation was collected during the fourth hour of the light phase on the last day before changing light conditions (0 on the diagrams) and 5, 10, 15, 20, 30 and 35 days later. The morphological observations were carried out on the 25th, 30th, 35th, 40th and 50th day of the experiment after changing the photoperiod.

In order to examine the content of hormonal substances 50 g of well formed leaves were collected each time. The collected material was kept at —15°C. Plant growth hormones (auxins, gibberellins (GAs) and the abscisic acid (ABA)-like inhibitor) were isolated from the same samples (50 g of leaves) with 80% methanol according to the methods described previously (K o p c e w i c z et al. 1979).

Gibberellins were partitioned chromatographically on Whatmann 3 MM paper with distilled water as solvent. The place of localization of GAs (zone R_f 0.7-1.0) was eluted and rechromatographed using TLC (silica gel G, solvent system — chloroform:ethyl acetate:acetic acid 90:10:5 v/v). The lettuce hypocotyl test (Frankland and Wareing 1960) was used for quantitative determination.

Auxins were chromatographed on Whatmann 3 MM paper with the solvent system — isopropanol: ammonia: water 10 : 1 : 1 v/v and bioassayed by the *Avena* section straight growth test (Nitsch 1956).

The abscisic acid-like inhibitor was chromatographed on Whatmann 3 MM paper with distilled water and bioassayed by the wheat coleoptile test (Bentley and Housley 1954).

Statistical analysis of data obtained from the bioassays was based on three analytical replications (extractions) × two replicates (chromatograms) within each extraction — a total of six replicates. These data were subjected to analysis of variance.

RESULTS AND DISCUSSION

The results of morphological observations (Table 1) show that interruption of a long night with white light in the middle of a dark period leads to induction of both shoot growth and formation of flower buds and development of flowers. Control plants remain in a vegetative stage (Fig. 1). Thus, interruption of the period of darkness causes, qualitatively, the same effect as the application of a long inductive photoperiod (Kopcewicz et al. 1979).

Table 1

Effect of night-breaks on the growth of shoot and flower formation in *Hyoscyamus niger* L.

Day of experiment	Mean shoot height, cm	Plants with elongated shoot[x], %	Plants with flower buds[x], %	Plants with fully developed flowers[x], %
25	13.5	94.3	64.1	0
30	18.2	100.0	79.6	3.7
35	24.7	100.0	88.9	13.0
40	33.5	100.0	96.3	59.2
50	39.4	100.0	96.3	96.3

[x] total 54 plants

The results concerning changes in the content of GAs (Fig. 2) show that control plants grown on a short photoperiod contain only one group of GAs localized on the chromatograms at R_f 0.0-0.2. In INP

Fig. 1. Effect of night-breaks (fifty interrupted nights) on the growth and development of *Hyoscyamus niger* L. A_1 — **INP**, B_1 — Controls

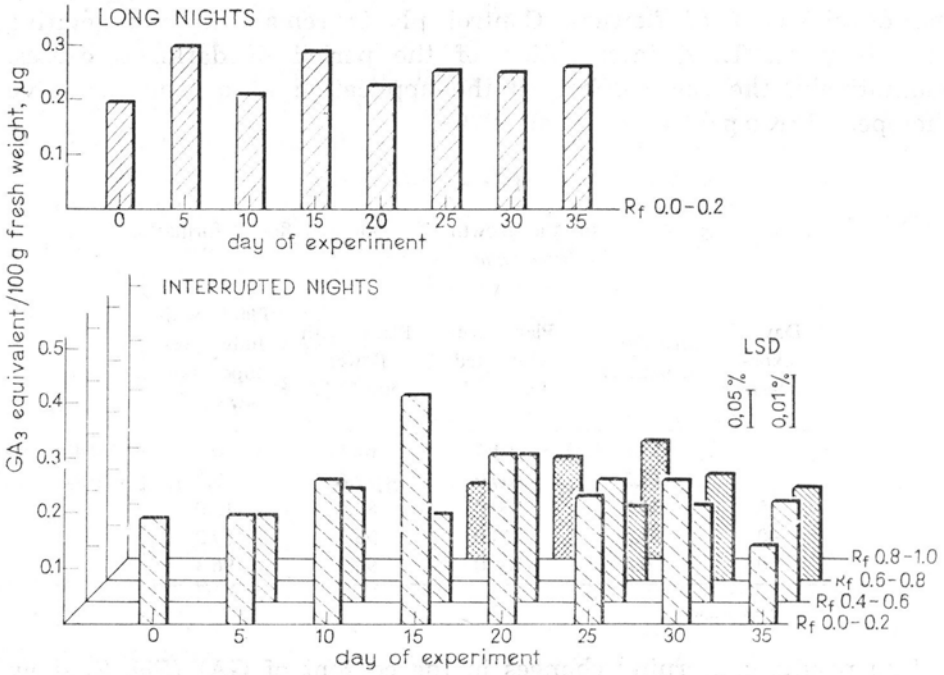

Fig. 2. Effect of night-breaks on the content of various gibberellin-like substances in the leaves of *Hyoscyamus niger* L.

there appear three additional groups of these substances (R_f 0.4-0.6, R_f 0.6-0.8 and R_f 0.8-1.0). Similar groups of more polar GAs appear in black henbane plants at the period of flower initiation under the influence of a long photoperiod (K o p c e w i c z et al. 1979). The total contents of GAs (Fig. 3) shows that the amount of these substances in the leaves of *Hyoscyamus* increases during the successive dark intervals interrupted by short photoperiods. The maximal content of GAs was found between the 15th and 25th photoperiod.

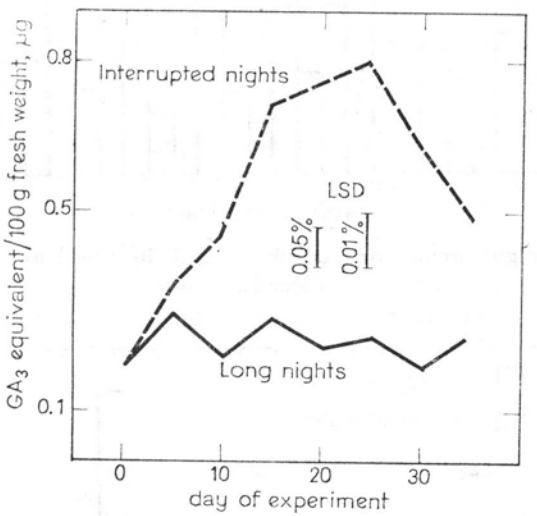

Fig. 3. Effect of night-breaks on the total content of gibberellins in the leaves of *Hyoscyamus niger* L.

Auxins content in the leaves of INP was higher than in the control ones (Fig. 4) and increases during the successive days. Thus, the content of auxins in night-break treated plants increases similarly as in black henbane exposed to a long inductive photoperiod (K o p c e w i c z et al. 1979). Detailed identification of auxin-like substances has not been carried out yet; however, preliminary tests have shown that the main auxin in the extracts is indole-3-acetic acid (IAA).

Night-breaks caused an increase in the content of ABA-like inhibitor in the leaves of black henbane (Fig. 5). The content of this compound also increases under long day conditions (K o p c e w i c z et al. 1979).

The obtained results show that in a long-day plant night-breaks caused the formation of flowers and very pronounced changes in the level of hormonal substances. It seems that the mechanism of this phenomenon is connected with the effect of light on the photostationary state of phytochrome. Breaking the dark period with white light increa-

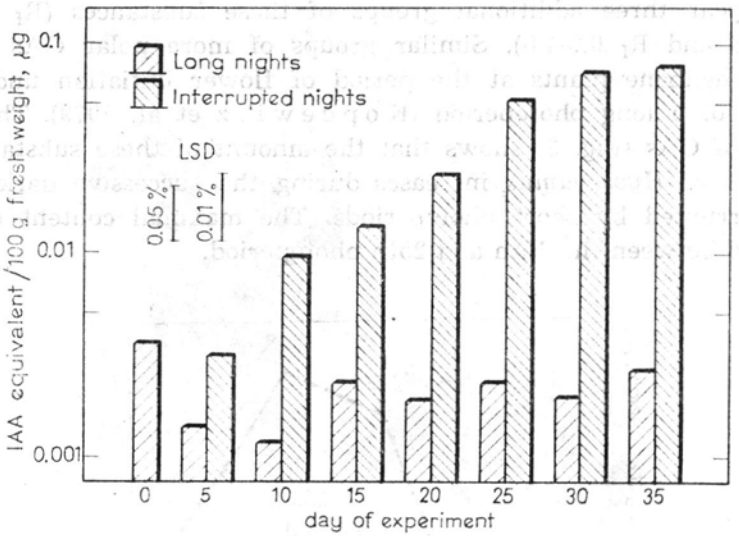

Fig. 4. Effect of night-breaks on auxins content in the leaves of *Hyoscyamus niger* L.

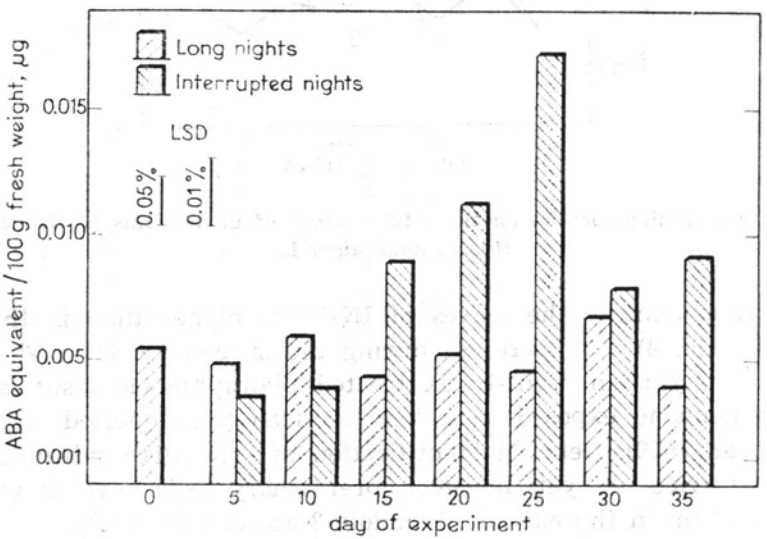

Fig. 5. Effect of night-breaks on abscisic acid-like inhibitor content in the leaves of *Hyoscyamus niger* L.

ses the level of phytochrome P_{FR} (V i n c e 1972). This results in a situation similar to long day conditions (high level of P_{FR}) and the consequence of this is the possibility of general induction of plants and gradual flower formation and differentiation. We could also presume that a high level of phytochrome P_{FR} in the tissue could change the metabolism of plant hormones (K o p c e w i c z 1979). The influence of

light, through phytochrome, on the hormones level could be due to the effects on biosynthesis, transport, release from bound form or degradation (Black and Vlitos 1972).

The results obtained show that night-breaks cause similar changes in the metabolism of hormones as a long inductive photoperiod (Kopcewicz et al. 1979). Also Chailakhyan and Lozhnikova (1966) found that short day followed by a night-break was the equivalent of a long day for the production of gibberellin-like substances in *Nicotiana silvestris* and *Rudbeckia bicolor*. These results suggest that the generative differentiation of long-day plants, independently of the way of induction, is connected with the increased content of endogenous hormones. Of course, it is difficult to conclude whether the increased amount of hormones is the cause or the consequence of generative induction. However, the appearance, during flower initiation, of new gibberellins which do not occur in vegetative plants, as well as the influence of some gibberellins on the flowering of black henbane plants under noninductive conditions (Lang 1965), suggest the possibility of a morphogenetic role of hormones in the phenomena of flower formation and differentiation. The results obtained may help to understand better the mechanism of generative photoinduction in long-day plants.

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Wpływ przerywania długiej nocy okresami świetlnymi na kwitnienie i zawartość fitohormonów w Hyoscyamus niger L.

Streszczenie

Przerwanie nocy białym światłem powodowało zarówno stymulację wzrostu pędu i formowanie się kwiatów, jak również i ogólne podwyższenie się zawartości fitohormonów w liściach lulka czarnego. W okresie formowania się kwiatów pojawiły się również nowe, nie występujące w roślinach wegetatywnych, substancje giberelinopodobne. Wyniki wskazują, że przerwy świetlne powodują podobne zmiany w metabolizmie hormonów jak długi indukcyjny fotoperiod. Przypuszczać więc można, że niezależnie od sposobu indukcji, dyferencjacji generatywnej roślin dnia długiego towarzyszy zawsze podwyższenie ogólnej zawartości endogennych hormonów i pojawienie się nowych giberelin. Świadczyć to może o morfogenetycznej roli hormonów, zwłaszcza giberelin, w zjawiskach tworzenia i różnicowania się kwiatów.