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INFLUENCE OF PHYTOCHROME ON THE CONTENT OF ENDOGENOUS HORMONES IN LEMNA AEQUINOCTIALIS DURING THE LONG NIGHT PERIOD

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INTRODUCTION

It has already been shown by many authors that exogenously added hormones affect various aspects of development in *Lemnaceae,* particularly the flowering process. Hormones are effective in inducing flowering under unfavourable regime (Maheshwari and Venkataraman, 1966; Kandeler and Hügel, 1973) and in affecting the development of floral organs, stamen and pistil (Hügel, 1976). There is also a large number of data concerning the role of phytochrome in the growth and development of *Lemnaceae.* Red light action in photoperiodism in *L. perpusilla* was demonstrated by Hillman (1958). Purves (1961) showed that *L. perpusilla* had the features of short day plants: diurnal sensitivity to P_{fr} is such, that a high P_{fr} level stimulates flowering in the beginning of the inductive dark period, and becomes inhibitory in the middle of the night. It was also demonstrated that one inductive cycle is sufficient for floral induction in this species. Phytochrome was spectrophotometrically estimated in several species of *Lemnaceae,* including *L. perpusilla* (Rombach and Spruit, 1968; Rombach, 1978).

It can be assumed that changes leading to flowering could involve changes at the hormonal level. We have, therefore, tried to analyze the endogenous hormones in *L. aequinoctialis* and to find out the possible relationship between their content and the state of phytochrome during the inductive night period.

MATERIAL AND METHODS

The experimental material was a clone of *Lemna aequinoctialis*[1], grown aseptically in a modified Bonner–Devirian liquid medium (Gupta and Mahesh-

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[1] Formerly named *L. perpusilla* Torr. 6746, or *L. paucicostata*

w a r i, 1968), supplemented with 1% sucrose. pH was adjusted to 5.4–5.6 prior to autoclaving. The cultures were kept in 500 ml erlenmayer flasks, containing 250 ml of the medium. They were grown for 17 days in photoperiodic cycles consisting of 16 h white light and 8 h darkness. The temperature was regulated at 26°C during the day, and at 22°C during the night. The daylight was obtained from white fluorescent tubes and its intensity was 550 lx. For red irradiation (R), plants were exposed to a red fluorescent tube (Philips TL 15), equipped with a 3 mm thick plexiglass filter (Rohm and Haas, No. 501), with an intensity at the plant level of 1.4 $\mu W\ cm^{-2}\ nm^{-1}$ at 660 nm. Far red light (FR) was obtained from an incandescent bulb, installed over a 5 cm water layer and two 3 mm plexiglass filters (Rohm and Haas): red No. 501 and blue No. 627. The relationship between the intensity of red and far red was 1:4.

On the 18th day of cultivation plants were exposed to a single short day of 8 h and were devided in four groups. In the groups I and III the day was terminated by 10 min of red light and in the groups II and IV by 10 min of far red. After irradiation all plants were left in darkness. Groups I and II were extracted after 30 min, while the groups III and IV were given a long night of 16 h and extracted afterwards.

Plant material was frozen in liquid nitrogen prior to extraction, macerated in cold methanol and extracted for 12 h at 3°C. The methanol extract was shaken with petroleum ether and evaporated off in a rotary vacuum evaporator at 35°C. The preparative separation of the active substances was done using a 25 x 2 cm DEAE Sephadex A 25 column, eluted by step–wise increasing gradients of acetic acid in 80% methanol (G r ä b n e r *et al.*, 1976). Fractions of 10 ml were collected using a fraction collector and 1 ml of each fraction was tested in different bioassays. Fractions with biological activity were pooled together and used for silica gel thin layer (TLC), or paper (PC) chromatography. The following chromatography systems were used:

1. Silica gel H, developed in methylacetate – isopropanol – 7N NH_4OH, 45:35:20 (v:v:v), for auxins and inhibitors.
2. Whatmann 3 MM paper, developed in n–butanol –1.5N NH_4OH,3:1 (v:v), for auxins and inhibitors.
3. Silica gel G, developed in carbon tetrachloride – acetic acid – water, 8:3:5 (v:v:v), lower phase + ethylacetate 5:1 (v:v), for gibberellins.

The biological activity of the fractions obtained from the column, or by TLC and PC, was estimated using several bioassays. The gibberellin–like activity was measured by using the barley endosperm test (E–test), according to C o o m b e *et al.* (1967). For detecting auxins and inhibitors, oat first internode test (M–test) and wheat coleoptile test (C–test) were used, according to N i t s c h and N i t s c h (1956). In some cases, the inhibitors were also measured by a test with *Lemna* (L–test), according to T i l l b e r g (1975), except that *L. minor* was used.

RESULTS AND DISCUSSION

The activity of the fractions from DEAE Sephadex column, as assayed by three biological tests is shown in Fig. 1. As can be seen, each test revealed several zones of stimulation and inhibition. These eluates were further chromatographed and the results

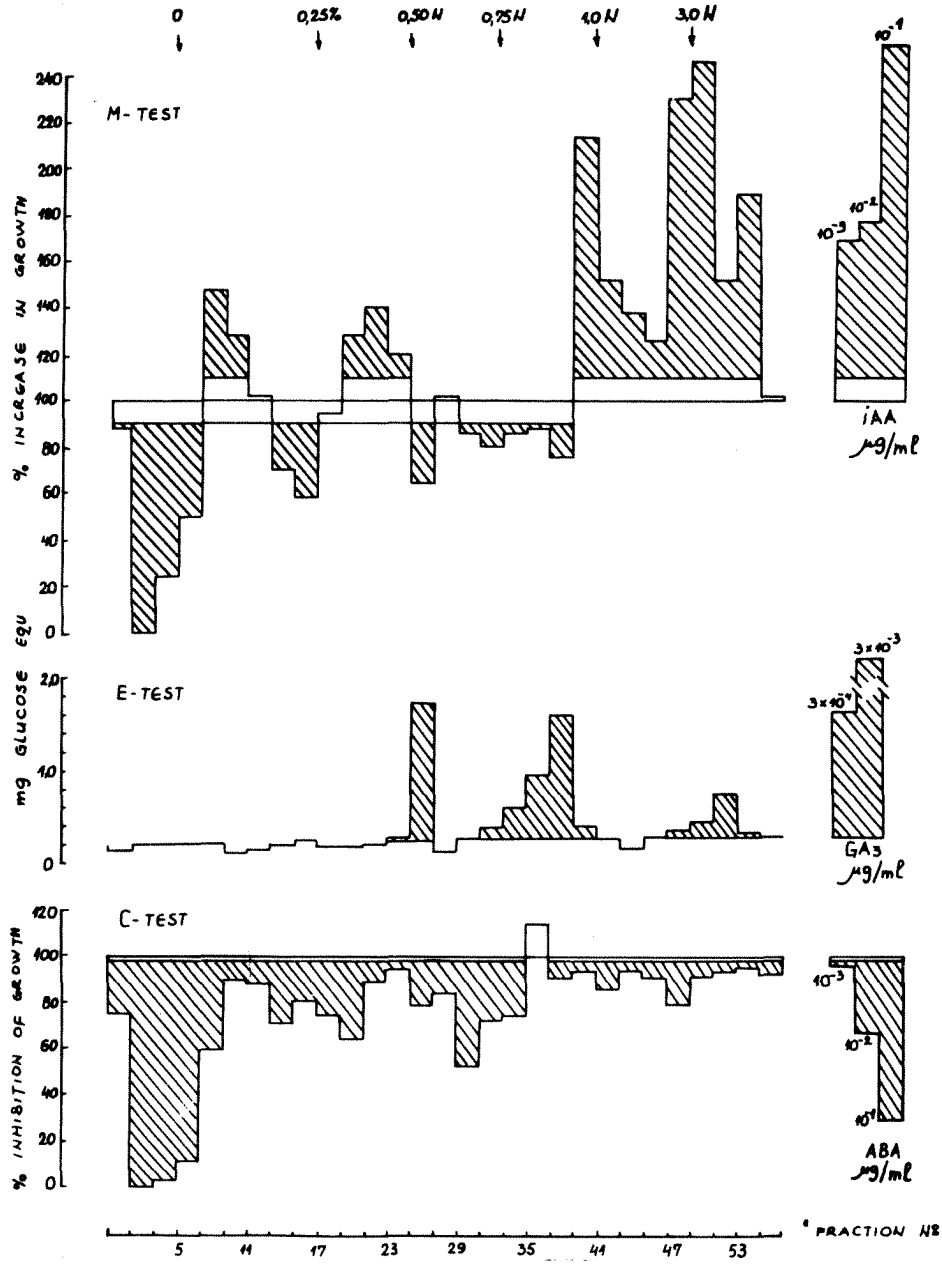

Fig. 1. — Example of the biological activity found in the extracts eluted from DEAE Sephadex column. M–test = oat first internode test; E–test = barley endosperm test; C–test = wheat coleoptile test; arrows indicate the concentration of acetic acid in 80% methanol.

of bioassays are presented in tables, indicating the Rf values of active substance in different solvent systems.

Table 1 shows the data on auxin–like stimulators. In the zone of neutral stimulation from the column (fractions 9–12) a substance was present, with Rf value similar to tryptophane, while in the zone of weak acidic stimulation (fractions 21–25) two stimulators were found. Fractions 36–55, in which IAA could be expected, contain perhaps four substances. One of them is similar to IAA in both systems.

Table 1. – Rf values of substances active as stimulators in M–test.

Pooled fractions from DEAE Sephadex column	Chromatography system	
	TLC (solvent 1)	PC (solvent 2)
2– 7	–	0.50–0.60
9–12	0.20–0.40	–
13–20	0.85–0.95	–
21–25	0.40–0.70	–
	0.90–1.00	–
36–55	0.20–0.30	0.10–0.20
	0.50–0.70	0.30–0.45
		0.55–0.60
		0.80–0.90
IAA	0.50	0.38
Tryptophane	0.40	–

Table 2. – Rf values of substances active in M–test and L–test as inhibitors (PC, solvent system 2).

Pooled fractions from DEAE Sephadex column	M–test	L–test
2– 7	0.30–0.40	0.10–0.40
	0.85–0.90	0.70–0.90
13–20	0.00–0.10	0.00–0.10
	0.30–0.40	0.30–0.50
	0.80–0.90	0,80–1.00
26–35	0.60–0.75	–
ABA	0.65–0.70	–

Substances with inhibitory activity are presented in Table 2. As can be seen, each zone from the column is resolved into 2–3 active substances. Fractions 26–35 are expected to contain ABA; a substance with corresponding Rf is detected in PC.

The separation of the gibberellin–like substances in TLC is shown in Fig. 2. Three active substances had the Rf values similar to the marker spots of GA_3, GA_5 and GA_{4+7}.

The content of biologically active substances in the beginning and at the end of a long night period is shown in Table 3. Although biological tests may not be adequate

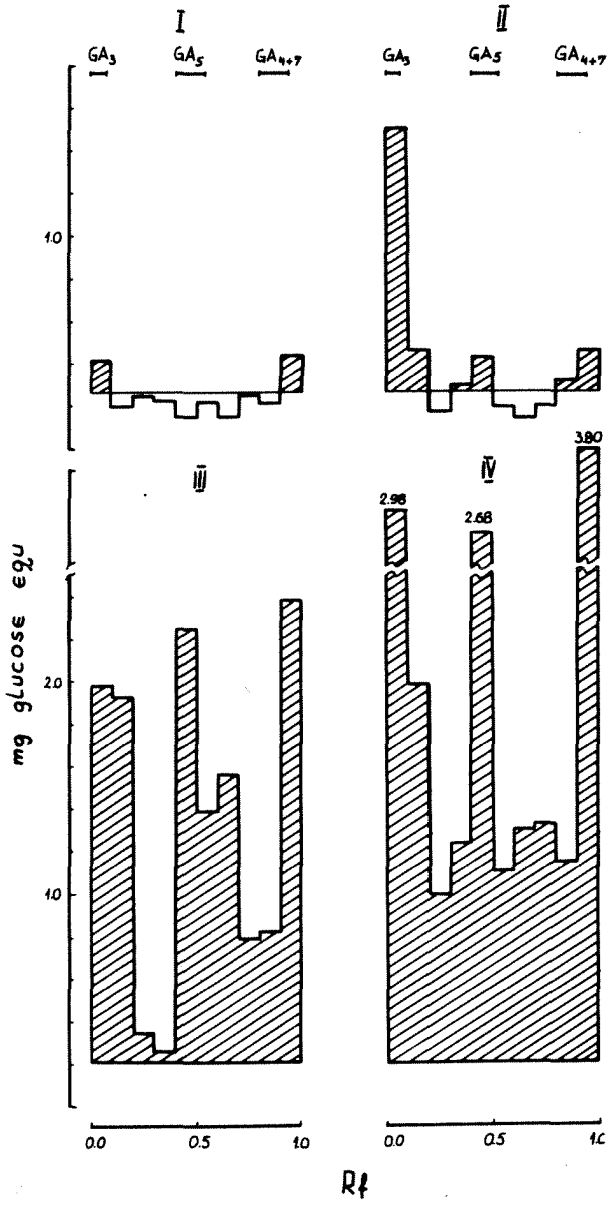

Fig. 2. – Histograms showing the gibberellin–like activity in the extracts, assayed by E–test. Acidic ethylacetate fraction, chromatographed in solvent system 3. End–of–day light treatment: I and III = 10 min red light; II and IV = 10 min far red light. Time of extraction: I and II = 30 min after irradiation; III nad IV = 16 h after irradiation. Bars represent Rf values of GA_3, GA_5 and GA_{4+7} marker spots.

enough to judge on the significance of small differences between the two samples, they can be relied on, when the differences are large. Consequently, it is not possible to state that the differences in growth substances' content between groups I and II, extracted in the beginning of the long night, were significant. But it is obvious that the content of all substances was significantly higher after 16 h of darkness. This seems to be a general trend for all auxin–like substances and ABA–like inhibitors, both in red and far red irradiated groups (III and IV). However, the state of phytochrome, established in the beginning of the night, seems to affect the gibberellins in a more specific manner. When total gibberellin–like activity was calculated from Sephadex column fractions, a considerable increase was found at the end of the dark period only in the group irradiated with far red light. This is also evident from the bioassays of acidic ethylacetate fractions (Fig. 2). The content of all three gibberellin–like substances is increased after the long night, but the increase is higher in far red irradiated, than in red irradiated plants. It appears, therefore, that the dark period favours the synthesis of the gibberellins. Their final content seems to be proportional to the length of the period in which the phytochrome was in its inactive form.

Table 3. The content of active substances in plants irradiated for 10 min with red (R) or far red (FR) light and extracted after different intervals in darkness.

Time in darkness following irradiation:	30 min		16 h	
End–of–day light treatment:	R (I)	FR (II)	R (III)	FR (IV)
Substances				
Auxin–like (μg IAA equ.|100 g^{-1} d.w.)	1.36	2.89	43.01	32.93
ABA–like (μg ABA equ.|100 g^{-1} d.w.)	32.20	23.20	96.40	94.50
Gibberellin–like (μg GA_3 equ. 100 g^{-1} d.w.)	2.39	2.20	2.95	7.81

There are also other examples of the end–of–day far red effect in *Lemnaceae*. Far red light, given in the beginning of the dark period induces senescence in *L. perpusilla* P 146 (Hügel *et al.*, 1979), and influences starch content in *L. gibba* (Kandeler *et al.*, 1980). The same treatment also affects the uptake of labelled GA_1 in *L. gibba* (Hartung and Kandeler, 1976). End–of–day far red inhibits (Hillman, 1961), or decreases (Hügel *et al.*, 1979) flowering in *L. perpusilla*. On the other hand, Hillman (1960) has reported that flowering in *L. perpusilla* is also inhibited by GA_3 application, both in long and in short days. Substances that interfere with gibberellin action, like CCC and ABA, induce the flowering in *L. paucicostata* under non–inductive long days (Kandeler and Hügel, 1973). Hügel (1976) has suggested that the photoperiods may affect the balance of endogenous hormones in flower meristems in *Lemnaceae*, which would lead either to promotion or to inhibition of flowering. Our results perhaps lend support to this suggestion, since they show that a non–inductive light treatment brings about an increased content of endogenous gibberellins, which may have the same inhibitory effect

on flowering, as the applied GA_3. Therefore, the effect of non–inductive photoperiodic conditions may be, at least partly, mediated by endogenous gibberellin metabolism.

SUMMARY

Endogenous hormones in *Lemna aequinoctialis* were studied during the long dark period, when the day was terminated either with red, or with far red light. Plants were grown in axenic culture for 17 days under non–inductive day–length of 16 h white light and 8 h darkness. The 18th day was shortened to 8 h and terminated with 10 min of red (max. 660 nm), or far red (max. 730 nm) light. Extractions were performed 30 min after irradiation, or at the end of the long night (16 h). The extracts of *L. aequinoctialis* contained in all cases substances with auxin–like and gibberellin–like activity, as well as neutral and acidic inhibitors. The content of all hormones studied was increased during the long night. A significant difference between red and far red action was found in respect to the gibberellins. Their content was markedly increased after far red light, when the phytochrome was left in its inactive form during the whole night.

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Rezime

SOFIJA PEKIĆ i MIRJANA NEŠKOVIĆ

UTICAJ FITOHROMA NA SADRŽAJ ENDOGENIH HORMONA TOKOM DUGAČKE NOĆI KOD LEMNA AEQUINOCTIALIS

Ispitivani su endogeni hormoni, ekstrahovani iz frondova *Lemna aequinoctialis,* na početku i na kraju dugačke noći, pošto je dan završen crvenom ili daleko crvenom svetlošću. Biljke su 17 dana rasle u kulturi pod neinduktivnim režimom, koji se sastojao od 16 h bele svetlosti i 8 h mraka. Osamnaesti dan je skraćen na 8 h i završen sa 10 min crvene (max. 660 nm), ili daleko crvene (max. 730 nm) svetlosti. Ekstrakcije su obavljene 30 min posle osvetljavanja, ili na kraju dugačke noći (16 h). Ekstrakti *L. aequinoctialis* sadrže supstance sa auksinskom i giberelinskom aktivnošću, kao i neutralne i kisele inhibitore. Sadržaj svih ispitivanih hormona se povećava u toku dugačke noći. Značajna razlika u dejstvu crvene i daleko crvene svetlosti utvrđena je pri merenju supstanci sličnih giberelinima. Njihov se sadržaj značajno povećava posle daleko crvene svetlosti, usled koje je fitohrom bio u neaktivnoj formi tokom cele noći.