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PHOTOPERIODIC RESPONSES OF *LEMNACEAE* FROM NORTHEASTERN SLOVENIA

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Introduction

Fundamental investigations of photoperiodic responses in Lemnaceae have been carried out by Hillmann, Kandeler, Maheshwari and others. They have shown that different races are characterized by specific mechanisms of photoperiodism which are in general very much dependent on metabolic factors and nutrient substances. From 28 species 7 have been investigated successfully for the mechanism of flower induction. For further details see reviews by Kandeler (1968) and Bopp (1971).

An attempt was made to examine in details the mechanism of flower induction in Lemnaceae of Slovenia, which, to the author's best knowledge, have not been investigated yet. The results of these investigations of photoperiodic responses of some isolated clones obtained at given standard conditions are briefly reported here. (See also Krajnčič 1972 a, b, 1974.)

Material and Methods

All species of *Lemnaceae* found in northeastern Slovenia were collected for examination. The following clones were obtained in axenic culture:

Spirodela polyrrhiza (Odranci)
Lemna gibba (Odranci)
Lemna minor (Trije ribniki — Maribor)
Lemna trisulca (Muretinci)
Wolffia arrhiza (Petanjci)

The plants were sterilized by rinsing in the following way:

1. Sterilized distilled water — 1 minute
2. 0.1% solution of sublimate ($HgCl_2$) in distilled water — 40 seconds
3. Sterilized distilled water — 30 seconds
4. 50% ethanol — 30 seconds
5. Sterilized distilled water — 30 seconds
6. Sterilized nutrient solution — 1 minute

Each sterilized plant was transferred into a sterilized 500 ml-Erlenmayer flask with 200 ml of nutrient solution. The following modified Pirson-Seidel nutrient solution with EDTA-$Na_2 \cdot 2H_2O$ or iron salt of EDTA was used:

KH_2PO_4	200 mg
KNO_3	400 „
$MgSO_4 \cdot 7H_2O$	300 „
$CaCl_2 \cdot 6H_2O$	1 198 „
$MnCl_2 \cdot 4H_2O$	0.3 „
H_3BO_3	0.5 „
EDTA-$Na_2 \cdot 2H_2O$	18.6 „
(or $FeNa_2$-EDTA	10 „)
Iron citrate	5 „
Sucrose	10 g
Asparagine	0.1 „
Bidistilled water	1 000 „

The pH-value was adjusted by means of 1/10 n KOH to 4.55 and 4.8. The pH-measurements were carried out by a pH-meter Radiometer Kopenhagen. The experiments were carried out in two plant cabinets (K r a j n č i č 1972 a) with controled ecological conditions. The illuminating intensity of 3 000 lx was achieved by 6 gro-lux fluorescent tubes Sylvania 40 W/220 V. The temperature was constant during day and night (28 ± 1° C). The percentage of the flowering plants was determined from at least two Erlenmayer-flasks by taking at least 100 plants at random from each and counting them under a stereomicroscope. All fronds above a certain minimal size were considered for scoring. The presence of the primordia of inflorescences was established by dissecting the fronds with flattened needles.

Experiments and Results

Eight series of experiments were carried out with each of the five isolated clones but only three of them developed flowers. The results are shown in Table 1—3.

Table 1. *SPIRODELA POLYRRHIZA* (Odranci)

No. of experiment	Characteristics of nutrient solution used	Preculture		Final experiment		
		Duration in days	Photoperiod	Duration in days	Percentage of flowering plants in LD	Percentage of flowering plants in SD
1.	Iron citrate, EDTA-$Na_2 \cdot 2H_2O$, pH = 4.55	30	LD	27	58	46
2.	„	27	SD	27	78	68
3.	Iror salt of EDTA, pH = 4.55	27	LD	27	85	82
4.	„	27	LD	25	69	68
5.	„	25	SD	26	86	84
6.	„	26	SD	27	86	83
7.	Iron salt of EDTA, pH = 4.8	27	LD	26	86	84
8.	„	26	SD	27	88	85

Abbreviations: LD = long day (16 hours light daily)
SD = short day (8 hours light daily)

Table 2. *LEMNA GIBBA* (Odranci)

No. of experiment	Characteristics of nutrient solution used	Preculture		Final experiment		
		Duration in days	Photoperiod	Duration in days	Percentage of flowering plants in LD	Percentage of flowering plants in SD
1.	Iron citrate, EDTA-Na_2 · · $2H_2O$, pH = 4.55	30	LD	27	86	0
2.	„	27	SD	27	88	0
3.	Iron salt of EDTA, pH = 4.55	27	LD	27	88	0
4.	„	27	LD	25	90	0
5.	„	25	SD	26	90	0
6.	„	26	SD	27	92	0
7.	Iron salt of EDTA, pH = 4.8	27	LD	26	92	0
8.	„	26	SD	27	93	0

(For abbreviation see Table 1.)

Table 3. *LEMNA MINOR* (Trije ribniki, Maribor)

No. of experiment	Characteristics of nutrient solution used	Preculture		Final experiment		
		Duration in days	Photoperiod	Duration in days	Percentage of flowering plants in LD	Percentage of flowering plants in SD
1.	Iron citrate, EDTA-$Na_2 \cdot 2H_2O$, pH = 4.55	30	LD	35	18	0
2.	„	35	SD	33	22	0
3.	Iron salt of EDTA, pH = 4.55	33	LD	32	30	0
4.	„	32	LD	31	27	0
5.	„	31	SD	34	31	0
6.	„	34	SD	34	30	0
7.	Iron salt of EDTA, pH = 4.8	34	LD	30	30	0
8.	„	30	SD	31	32	0

(For abbreviation see Table 1.)

Discussion

The results obtained with *Lemna gibba* (Odranci) are in good agreement with the results of Kandeler who has shown that *L. gibba* is a typical long-day plant.

In spite of the great number of data on *L. minor* and *Spirodela polyrrhiza* little is known on their flowering and photoperiodism. In *L. minor* the main reason for that may be the fact that many clones do not flower at all, and in those which flower the percentage of flowering plants is very low. Only Landolt (1957) and Czygan (1962, quoted after Kandeler 1968) have published data on flowering of L. minor under experimental conditions. Landolt achieved the flowering during a 16-hour-long-day, while Czygan succeeded in inducing the flowers by means of oestrogens.

The flowering of *S. polyrrhiza* was stated by Hicks (1932), Saeger (1929) and Maneshwari (1958, 1963) in the field, but data on experimental work have been lacking till now. So the results obtained in this work may be of some interest. The results are consistently reproducible as the repetition of the experiments has clearly shown.

Little is known on experimental work with *Wolffia arrhiza* and *L. trisulca,* with the exception of Zurzycka and Zurzycki (1950) and Zurzycka (1951) who investigated the intracellular phototaxy of chloroplasts (in *L. trisulca*). It is not likely that there have been no studies done on these species but they seem to be a much more difficult material for the study of flower induction than *L. gibba.*

The favourable effect of a short-day preculture on the development of *L. gibba* G_1 has been stated by Kandeler (1955). The *Lemnaceae* of northeastern Slovenia show this phenomenon in a higher degree, since a short-day preculture seems to increase even the percentage of flowering plants.

Fig. 1. A young frond of *Spirodela polyrrhiza* (Odranci) in cross section showing the vegetative and the reproductive pouch. The latter shows the characteristic formation of inflorescencences with microsporangia in which pollen mother cells are visible.

Sl. 1. Mladi kormusni članak klona *Spirodela polyrrhiza* (Odranci) u poprečnom presjeku s vegetativnim i reproduktivnim džepom. Posljednji pokazuje karakteristično stvaranje cvatova s mikrosporangijima u kojima se vide polenove matične stanice.

Fig. 2. A group of flowering plants of *Spirodela polyrrhiza* (Odranci).

Sl. 2. Skupina cvatućih biljaka klona *Spirodela polyrrhiza* (Odranci).

Fig. 3. *Spirodela polyrrhiza* (Odranci) in flower.

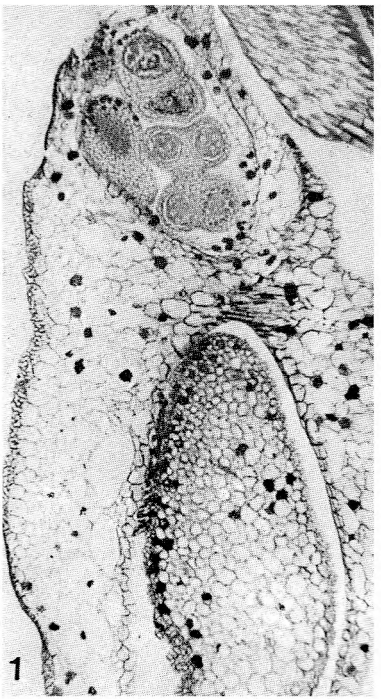
Sl. 3. *Spirodela polyrrhiza* (Odranci) u cvatnji.

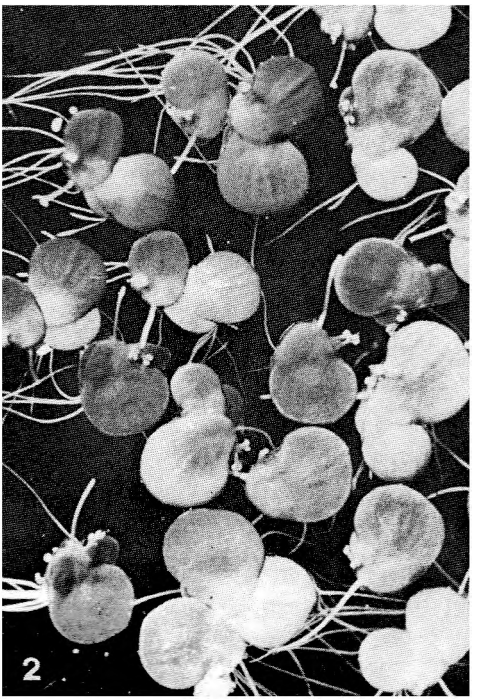
Fig. 4. *Lemna gibba* (Odranci) in flower.

Sl. 4. *Lemna gibba* (Odranci) u cvatnji.

Fig. 5. *Lemna minor* (Trije ribniki, Maribor) in flower.

Sl. 5. *Lemna minor* (Trije ribniki, Maribor) u cvatnji.





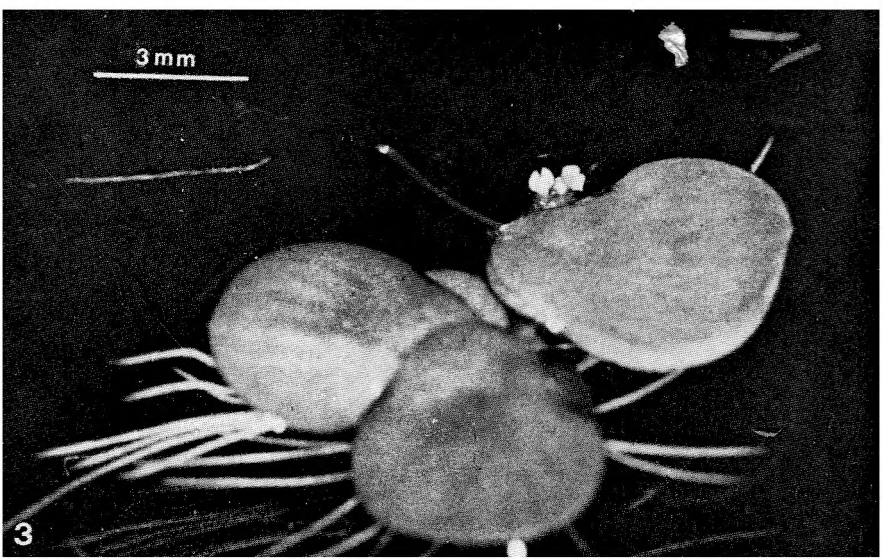
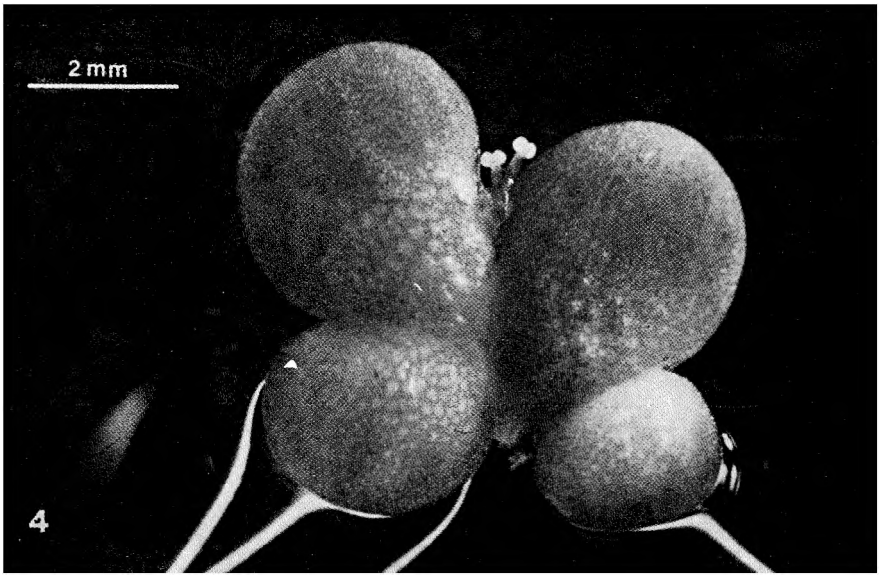


Fig. 1—3. — Sl. 1—3.



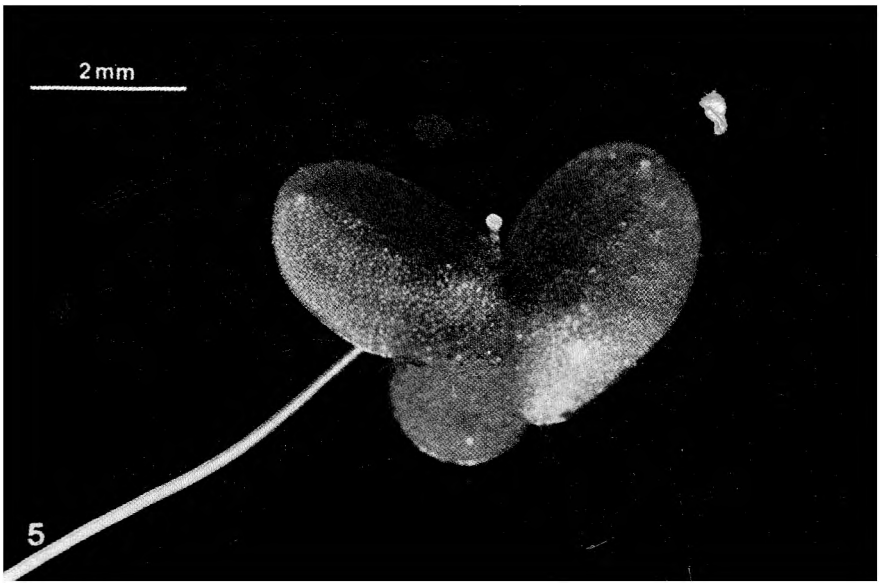


Fig. 4—5. — Sl. 4—5.

The stimulative influence of iron salts of EDTA on the growth and flowering of *W. microscopica* was pointed out by Maheshwari (1966). This stimulative action of EDTA has been confirmed by the results of all experiments carried out in this work.

*

I wish to express my gratitude to Professor Z. Devidé for his untiring help and interest he took in my work.

Summary

Photoperiodic responses of *Lemnaceae*, collected from the region of northeastern Slovenia, have been investigated under experimental conditions. The axenic cultures were grown in a modified Pirson-Seidel solution at an illuminating intensity of 3 000 lx and a temperature of 28 ± 1° C in plant cabinets under controlled conditions. The investigations have given the following results.

1. *Spirodela polyrrhiza* (Odranci) is a photoperiodically neutral plant, which produces inflorescences in the left reproductive pouch. Its flowering is considerably stimulated by the addition of iron salt of EDTA (10 m./1) at pH = 4.8.

2. *Lemna gibba* (Odranci) is under the given conditions a long-day plant, which produces inflorescences in the right pouch only. The convex fronds are developed during long days only. Under short dav conditions the fronds are flat and similar to those of *L. minor*.

3. *Lemna minor* (Trije ribniki — Maribor) is also a long-day plant, which develops its inflorescences in the right pouch.

4. A short-day preculture (of at least 14 days) stimulates flowering in all three investigated species, especially in *Spirodela*.

5. At the given experimentals conditions in *Lemna trisulca* (Muretinci) and *Wolffia arrhiza* (Petanjci), grown also in axenic cultures, it has not been possible to induce flowering up to now.

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SADRŽAJ

FOTOPERIODIČKE REAKCIJE VODENIH LEĆA SJEVEROISTOČNE SLOVENIJE

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Kao objekt istraživanja izabrane su vodene leće sjeveroistočne Slovenije. S tog područja sabran je materijal (s devet nalazišta), od kojeg su izolirane aksenične kulture te istražene njihove fotoperiodičke reakcije. Biljke su uzgajane u modificiranoj Pirson-Seidelovoj otopini pri osvjetljenju od 3000 luksa i temperaturi 28° C. Istraživanja su dala ove rezultate:

1. *Spirodela polyrrhiza* (Odranci) je fotoperiodički neutralna biljka, koja stvara cvatove samo u lijevom reproduktivnom džepu. Njezinu cvatnju znatno pospješuje dodatak željezne soli EDTA (10 mg/1) te pH 4.8. Njezini turioni podjednako se razvijaju u uvjetima kako kratkog tako i dugog dana.

2. *Lemna gibba* (Odranci) je biljka dugog dana koja razvija cvatove samo u desnom reproduktivnom džepu. Trbušasti kormusni članci razviju se samo u uvjetima dugog dana. Kod kratkog dana kormusni su članci plosnati te slični onima u vrste *L. minor*.

3. *Lemna minor* (Trije ribniki, Maribor) je biljka dugog dana koja stvara cvatove također samo u desnom reproduktivnom džepu.

4. Pretkultura na kratkom danu (najmanje u trajanju od 14 dana) pospješuje cvatnju kod svih triju proučavanih vrsta, a osobito u spirodele.

5. U navedenim eksperimentalnim uvjetima u akseničkoj kulturi također uspjevaju *Lemna trisulca* (Muretinci) i *Wollfia arrhiza* (Petanjci) kod kojih međutim zasad još nije uspjela indukcija stvaranja cvjetova.

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