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Report on Photoperiodic Responses in *Lemnaceae* from Slovenia

by

Božo KRAJNČIČ and Zvonimir DEVIDÉ

Contents

1. Introduction
 2. Materials and Methods
 3. Results
 - 3.1. *Spirodela polyrrhiza*
 - 3.2. *Lemna minor*
 - 3.3. *L. gibba*
 - 3.4. *L. trisulca*
 - 3.5. *Wolffia arrhiza*
 4. Discussion
- Summary - Zusammenfassung
- References

1. Introduction

Up to now photoperiodic responses were investigated in six species of *Lemnaceae* with the following results: *Lemna gibba* (KANDELER 1955, CLELAND 1967, CLELAND and BRIGGS 1967, KRAJNČIČ 1974b) and *L. minor* (BENNINK et al. 1970, KRAJNČIČ 1974b) are long day plants, *Spirodela polyrrhiza* (KRAJNČIČ 1974b) is a photoperiodically neutral plant, while *Lemna aequinoctialis*

(HILLMAN 1958*, YUKAWA and TAKIMOTO 1976, HILLMAN 1979), *Wolffia microscopica* (MAHESHWARI and CHAUHAN 1963) and *W. brasiliensis* (= *W. papulifera*) (MAHESHWARI and SETH 1966) are required short day photoperiod.

In a preliminary work the photoperiodic responses, the geographic distribution and other biological phenomena in *Lemnaceae* of northeastern Slovenia were studied (KRAJNČIČ 1974a, b, c). The present paper deals with the results of the further study, extended to the whole territory of Slovenia.

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2. Materials and Methods

Spirodela polyrrhiza (5 clones), *Lemna gibba* (4 clones), *L. minor* (7 clones), *L. trisulca* (4 clones), and *Wolffia arrhiza* (2 clones) were axenically isolated and cultured.

The plants were sterilized with mercuric chloride (corrosive sublimate) and ethanol (GORHAM 1945). After numerous attempts to sterilize several clones of *Lemnaceae* with 0,1 % HgCl₂ and 50 % C₂H₅OH, the authors have come to the conclusion that each species of *Lemnaceae* needs some modification of the sterilization method. Hitherto the following procedures have given the best results:

1. Washing with sterile tap water: 5 min.
2. Washing with sterile nutrient solution: 5 min.
3. 48 hours pre-culture in sterile nutrient solution at a temperature of 28°C.
4. Washing with sterile distilled water: 5 min.
5. Rinsing in a 0.1 % solution of mercuric chloride (sublimate - HgCl₂) for
60 sec.: *Spirodela polyrrhiza*,
50 sec.: *Lemna minor*, *L. gibba*, and *Wolffia arrhiza*, and
40 sec.: *L. trisulca*.
6. Washing with sterile distilled water: 30 sec.
7. Rinsing in 50 % ethanol for
55 sec.: *Spirodela polyrrhiza*
50 sec.: *Lemna minor*, *L. gibba*, and *Wolffia arrhiza*, and
40 sec. (not more!): *L. trisulca*.

* HILLMAN (1958) used - under the name of *L. perpusilla* - the clone 6746 of LANDOLT, which belongs in fact to *L. aequinoctialis* (= *L. paucicostata*) (KANDELER and HÜGEL 1974, LANDOLT 1980).

8. Washing with sterile distilled water: 30 sec.
9. Washing with sterile nutrient solution: 1 min.

A useful and simple general method is also sterilization with NaOCl (sodium hypochlorite) which was at first described by LANDOLT (1957) and later modified by HILLMAN (1961).

Each sterilized plant was transferred into a sterilized Erlenmayer flask of 500 ml volume containing 200 ml nutrient solution. A modified Pirson-Seidel solution (KRAJNČIČ 1974b) was used. The pH-values were adjusted to 4,55 and 4,8 by a 0.1 n solution of KOH before sterilization. The used modified Pirson-Seidel nutrient solution was adjusted to the relatively low pH-value because of sediment formation during the sterilization at values above 4.8

The experiments were carried out in two plant cabinets with controlled ecological conditions (KRAJNČIČ 1972) at the light intensity of 3000 ± 100 lx by means of 6 gro-lux fluorescent tubes Sylvania 40 W/220 V. The temperature ($28 \pm 1^\circ\text{C}$) was constant during day and night periods.

The percentage of flowering plants was determined in the same way as previously described (KRAJNČIČ 1974b).

3. Results

Out of 22 axenically isolated clones, flowering could be successfully induced in 5 clones of *Spirodela polyrrhiza*, 2 clones of *Lemna gibba*, 2 clones of *L. minor* and - for the first time under experimental conditions - in *Wolffia arrhiza* (1 clone). The experiments were carried out in 8 groups (Table 1-4). Each figure is the average of an analysis of plants from 5 - 10 Erlenmayer flasks.

The results may be summarized as follows:

1. *Spirodela polyrrhiza*: All five investigated clones were photoperiodically neutral. Two of them flowered from the right reproductive pouch, three from the left one. Turions were formed at any photoperiod.
2. *Lemna minor*: Out of seven isolated clones only two were able to develop flowers, one from the left reproductive pouch, the other from the right one. Both clones were flowering at long days stimulated herein by long day pre-culture.
3. *L. gibba*: Two out of the four investigated clones reached flowering. Both

Table 1. Photoperiod responses in *Spirodela polyrrhiza* from five stations (Petanjci, Zvirče, Lenart, Velika Polana, and Dobrnič) in Slovenia.

Group of experiments	Characteristics of the culture medium	Pre-culture		Culture											
		Duration in days		Duration in days		Percentage of flowering plants									
		Photo-period	in days	Petanjci	Zvirče	Velika Polana		Lenart		Dobrnič					
		LD	SD	LD	SD	LD	SD	LD	SD	LD	SD	LD	SD		
1	Fe-citrate EDTANa ₂ ·2H ₂ O pH = 4,55	30	LD	27	27	26	22	-	-	41	33	37	28	-	-
2	"	27	SD	27	27	29	25	-	-	45	44	48	44	-	-
3	Fe-EDTA pH = 4,55	27	LD	27	27	29	27	1	3	57	53	48	47	4	4
4	"	27	LD	27	25	29	28	1	3	57	55	50	48	4	4
5	"	25	SD	26	26	31	28	2	4	64	62	56	52	4	3
6	"	26	SD	27	27	30	28	2	4	65	63	56	54	4	4
7	Fe-EDTA pH = 4,8	27	LD	26	26	38	36	2	5	72	68	57	60	5	5
8	"	26	SD	27	27	46	42	3	5	77	70	61	60	6	5

LD = 16 hours light and 8 hours darkness, SD = 8 hours light and 16 hours darkness.

Table 2. Photoperiodic responses in *Lemna minor* from two stations (Barje and Dokležovje) in Slovenia.

Group of experiments	Characteristics of the culture medium	Pre-culture		Culture				
		Duration in days	Photo-period	Duration in days	Percentage of flowering plants			
					Barje		Dokležovje	
					LD	SD	LD	SD
1	Fe-citrate EDTANa ₂ .2H ₂ O pH = 4,55	30	LD	35	15	0	0	0
2	"	35	SD	33	3	0	0	0
3	Fe-EDTA pH = 4,55	33	LD	32	26	0	4	0
4	"	32	LD	31	25	0	3	0
5	"	31	SD	34	5	0	0	0
6	"	34	SD	34	5	0	0	0
7	Fe-EDTA pH = 4,8	34	LD	30	29	0	4	0
8	"	30	SD	31	8	0	0	0

LD = 16 hours light and 8 hours darkness, SD = 8 hours light and 16 hours darkness.

were long day plants and short day pre-culture further stimulated flowering.

4. *L. trisulca*: None of the four isolated clones flowered under the given experimental condition.
5. *Wolffia arrhiza*: In one of the two isolated clones it has been possible to induce flowering after a long day pre-culture (35 days, 16 hours light daily) at a short day photoperiod (2 - 4 weeks, 8 hours light daily). The nutrient solution and other ecological conditions were identical with those described in the group of experiments No. 7.

Table 3. Photoperiodic responses in *Lemna gibba* from two stations (Petanjci and Obrov) in Slovenia.

Group of experiments	Characteristics of the culture medium	Pre-culture		Culture				
		Duration in days	Photo-period	Duration in days	Percentage of flowering plants			
					Petanjci		Obrov	
					LD	SD	LD	SD
1	Fe-citrate EDTANa ₂ ·2H ₂ O pH = 4,55	30	LD	27	56	0		
2	"	27	SD	27	57	0		
3	Fe-EDTA pH = 4,55	27	LD	27	57	0	58	0
4	"	27	LD	25	58	0	58	0
5	"	25	SD	26	58	0	59	0
6	"	26	SD	27	61	0	63	0
7	Fe-EDTA pH = 4,8	27	LD	26	61	0	63	0
8	"	26	SD	27	63	0	65	0

LD = 16 hours light and 8 hours darkness, SD = 8 hours light and 16 hours darkness.

4. Discussion

The results obtained with the clones from Petanjci and Obrov confirm the previous data and further suggest that *L. gibba* is predominantly a long day plant (KANDELER 1955, 1960/61, 1968, CLELAND 1967; CLELAND and BRIGGS 1967; KRAJNČIČ 1974a, b, c).

In *L. minor* five of seven strains did not flower at all, and even in those which flowered the percentage of flowering plants was very low. Probably due to this fact, papers concerning flowering and photoperiodism in this species

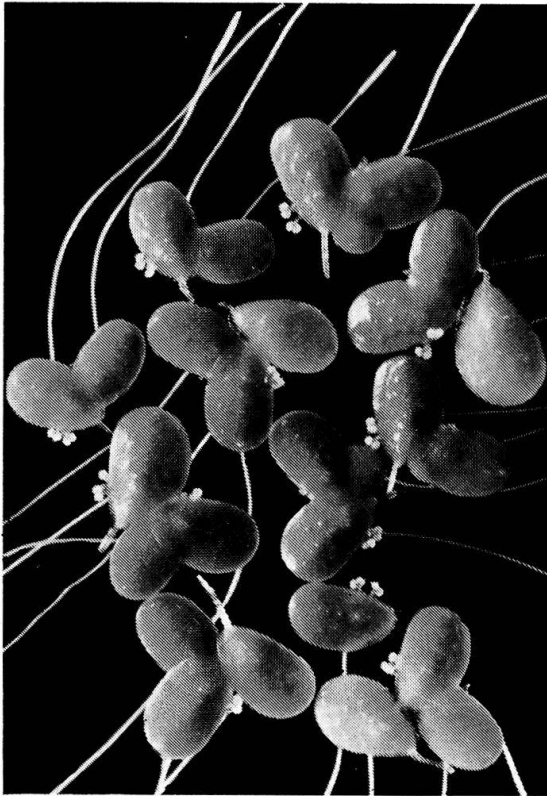
Table 4. Photoperiodic responses in *Wolffia arrhiza* from one station (Velika Polana) in Slovenia.

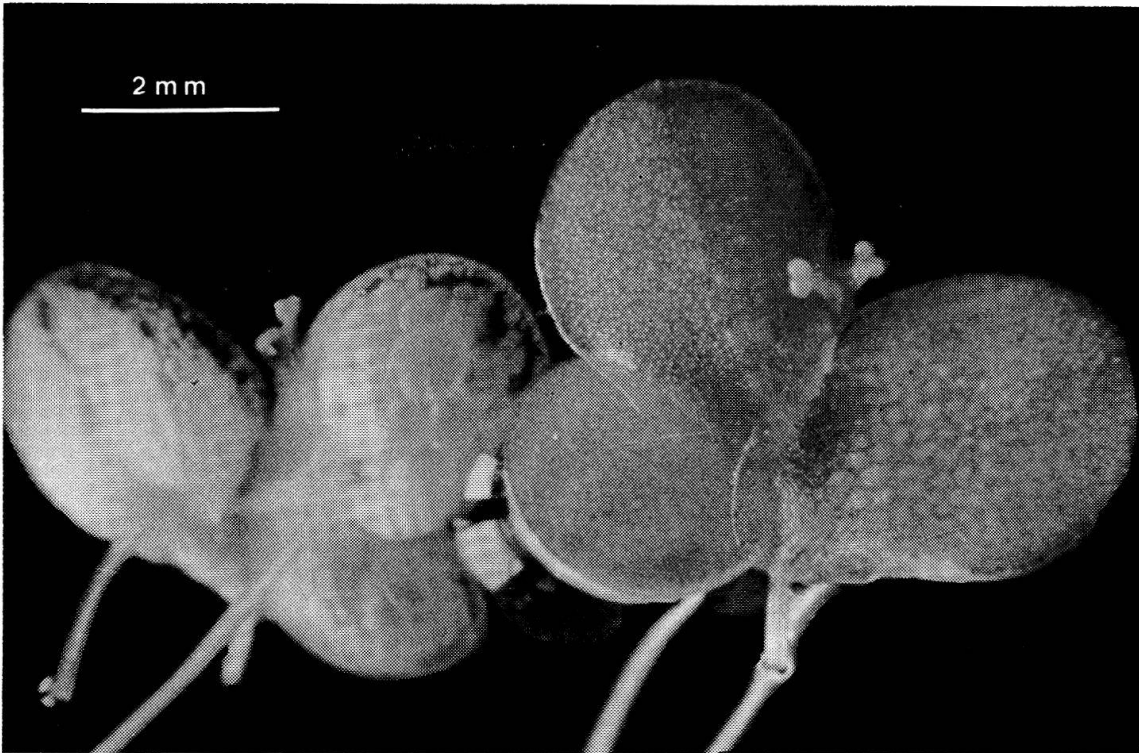
Group of experiments	Characteristics of the culture medium	Pre-culture		Culture		
		Duration in days	Photo-period	Duration in days	Percentage of flowering plants	
					Velika Polana	
					LD	SD
1	Fe-citrate EDTANa ₂ .2H ₂ O pH = 4,55	30	LD	27	0	0
2	"	27	SD	27	0	0
3	Fe-EDTA pH = 4,55	27	LD	27	0	0
4	"	27	LD	25	0	0
5	"	25	SD	26	0	0
6	"	26	SD	27	0	0
7	Fe-EDTA pH = 4,8	27	LD	26	0	0
8	"	26	SD	27	0	0
9	"	35 - 40	LD	14 - 28	0	0,5 - 1

LD = 16 hours light and 8 hours darkness, SD = 8 hours light and 16 hours darkness.

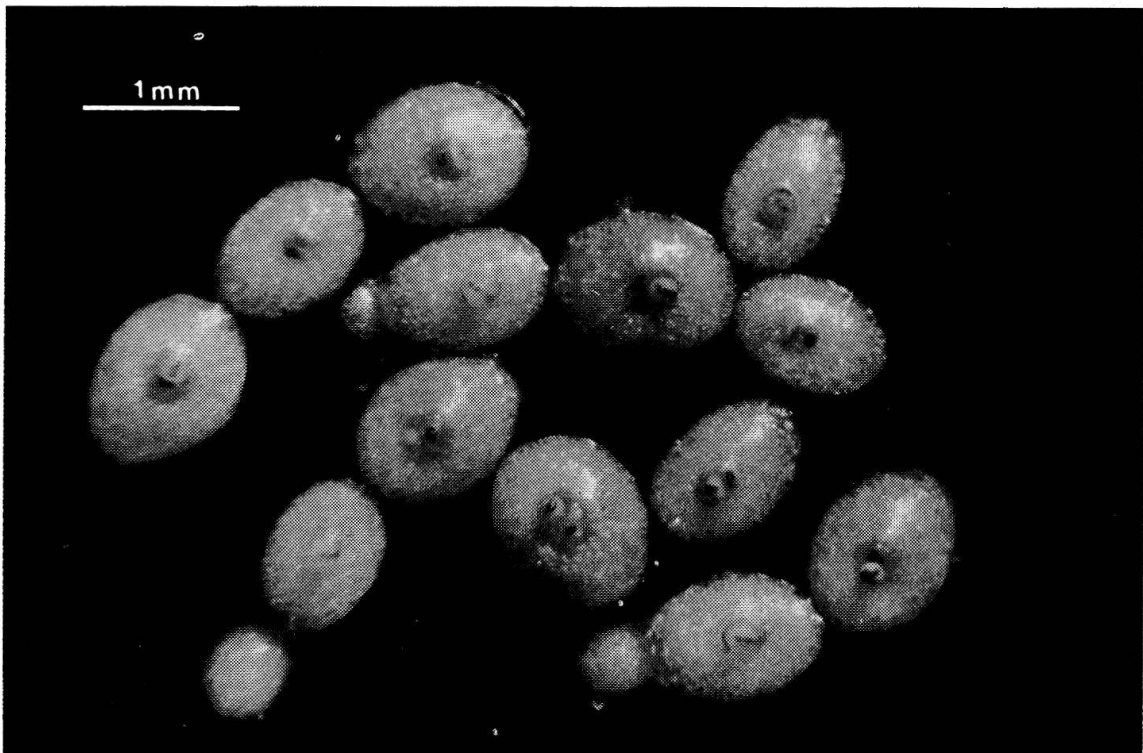
are so scarce. Data about experimentally induced flowering in *L. minor* were published by LANDOLT (1957, cit. after KANDELER 1968), BENNINK et al. (1970), BHALLA and SABHARWAL (1972), and KRAJNČIČ (1974b), only BENNINK et al. (1970) and KRAJNČIČ (1974b) referring to photoperiodic reactions. Both strains investigated in the present work, i.e. the strain from Barje as well as that from Dokležovje distinctly required a long day photoperiod. However, the strain of Dokležovje needed the highest number (= 70!) of long day cycles to reach flowering.

The flowering of *S. polyrrhiza* in the field conditions was reported by





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Fig. 1. *Spirodela polyrrhiza* (Petanjci) in flower. Fig. 2. A group of flowering plants of *S. polyrrhiza* (Petanjci). Fig. 3. *Lemna minor* (Barje) in flower. Fig. 4. A group of flowering plants of *L. minor* (Barje). Fig. 5. *Lemna gibba* (Petanjci) in flower. Fig. 6. *Wolffia arrhiza* (Velika Polana) in flower. In the centre of the fronds the reproductive pouch with the stigma and stamen.

HICKS (1932), SAEGER (1929), MAHESHWARI (1958), MAHESHWARI and MAHESHWARI (1963), and under experimental conditions by LACOR (1968), KRAJNČIČ (1974a, b), and WOLEK (1974).

To our knowledge only one paper on the photoperiodism in *S. polyrrhiza* was hitherto published (KRAJNČIČ 1974b). The results obtained in the present work confirm the previous results and suggest that under the given ecological conditions this species is photoperiodically neutral.

There are no data published about experimental induction of flowering in *W. arrhiza* so no comparison can be made.

Summary

Various *Lemnaceae* collected from the whole territory of Slovenia and studied under experimental conditions have shown the following photoperiodic responses:

Spirodela polyrrhiza (5 clones) was photoperiodically neutral. *Lemna minor* (2 clones) and *L. gibba* (2 clones) were long day taxa, while in *L. trisulca* (4 clones) no flowering could be obtained.

In *Wolffia arrhiza* (1 clone) flowering under experimental conditions was induced for the first time; after a long day pre-culture the plants flowered at a short day photoperiodic. The percentage of flowering plants was, however, very low (~0.5-1%).

Zusammenfassung

Untersuchungen der photoperiodischen Reaktionen bei Lemnaceen, die aus dem ganzen Gebiet Sloweniens gesammelt wurden, haben folgendes ergeben:

Spirodela polyrrhiza (5 Klone) ist eine photoperiodisch neutrale Pflanze. *Lemna minor* (2 Klone) und *L. gibba* (2 Klone) sind Langtagpflanzen, während bei *L. trisulca* keine blühfähigen Klone vorgefunden werden konnten.

Bei *Wolffia arrhiza* war es - zum ersten Mal - möglich, das Blühen unter experimentellen Bedingungen zu induzieren: Nach einer Langtag-Vorkultur blühten die Pflanzen bei kurzzeitiger Photoperiode. Der Anteil an blühenden Pflanzen war hingegen sehr niedrig (~0.5-1%).

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