Acta Bot. Croat. 41, 57—63, 1982.

CODEN: ABCRA2
YU ISSN 0365—0588

UDC 582.547.2:581.035.2=20

PHOTOPERIODIC RESPONSES IN *LEMNACEAE* FROM NORTH CROATIA

BOŽO KRAJNČIČ and ZVONIMIR DEVIDÉ

(College of Agriculture, University of Maribor, Slovenia and Department of Botany, Biology Division, University of Zagreb, Croatia, Yugoslavia)

Received January 15, 1982.

Photoperiodical responses of *Lemnaceae* from the North Croatian area, Yugoslavia, are investigated. The experiments, conducted in axenical conditions in thermostatized plant cabinets in a modified Pirson-Seidel nutrient solution at the temperature of 28 ± 1° C and illumination of 3000 lx, have showed the following results: Among the five examined clones of the species *Spirodela polyrrhiza*, four clones are photoperiodically neutral plants, while one clone represents quantitative long day plants. In this species, the above said fact has been determined for the first time. From the two investigated clones of the species *Lemna gibba*, both are long day plants. Among the six clones of the species *Wolffia arrhiza*, flowering has been reached only in one clone, grown in short-day conditions after long-day pre-culture. In none of the clones of the species *Lemna minor* and *L. trisulca*, at the given experimental conditions, could flowering be obtained.

Introduction

Hitherto, photoperiodical responses have been examined in eight species of the *Lemnaceae* with the following results:

Lemna gibba (Kandeler 1955, Cleland 1967, Cleland and Briggs 1967, Krajnčič 1974b, Krajnčič and Devidé 1980) and *Lemna minor* (Bennink et al. 1970, Krajnčič 1974b, Krajnčič and Devidé 1980) are long day plants, while *Spirodela punctata* reacts (with EDDHA respectively SA*) as a quantitative long day plant (Scharfetter, Rottenburg und Kandeler 1978), and

* Abbreviations: EDDHA, ethylenediamine-di-o-hydroxy-phenylacetic acid; SA, Salicylic acid.

Spirodela polyrrhiza is a photoperiodically neutral plant (Krajnčič 1974 b, Krajnčič and Devidé 1980).

Wolffia arrhiza is a long-short day plant (Krajnčič and Devidé 1980), while *Lemna aequinoctialis* (Hillman 1958**, Yukawa and Takimoto 1976, Hillman 1979), *Wolffia microscopica* (Maheshwari and Chauhan 1963) and *Wolffia brasiliensis* (= *W. papulifera*) (Maheshwari and Seth 1966) require a short day photoperiod.

The present publication gives the results of a study of photoperiodical responses of the *Lemnaceae* species collected from various growing places in North Croatia.

Materials and Methods

Spirodela polyrrhiza (5 clones: Đelekovec, D. Miholjac, Sunja, Slavonski Brod and Vinkovci), *Lemna gibba* (2 clones: Bročice and Stupna), *L. minor* (2 clones: D. Miholjac and Krapje), *L. trisulca* (2 clones: Stupna and Vukovar) and *Wolffia arrhiza* (6 clones: Zagreb, Bročice, Krapje, Sunja, Novo selo and Đelekovec) were axenically isolated and cultured.

The plants of the clone Zagreb of the species W. *arrhiza* were collected in the Zagreb Botanical Garden, while all other enumerated clones of all species of *Lemnaceae* treated, were gathered from growing places described, throughout North Croatia (Krajnčič and Devidé, 1982). The plants were sterilized with 0.1% $HgCl_2$ and 50% C_2H_5OH (Krajnčič and Devidé 1980).

Each sterilized plant was put into a sterilized Erlenmayer flask of 500 ml volume containing 200 ml nutrient solution. We used a modified Pirson — Seidel solution (Krajnčič 1974 b). Before sterilization, the pH-values were adjusted to 4.55 and 4.8 by a 0.1 solution of KOH. The modified Pirson-Seidel nutrient was adapted to the relatively low pH-value, because during sterilization, sediments are formed at pH-values above 4.8. The experiments were performed in two separate 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 ± 1° C) was kept constant day and night.

The percentage of flowering plants was determined as shown in the previous description (Krajnčič 1974 b).

Results

From the total of 17 axenically isolated clones, flowering could be obtained in 5 clones of *Spirodela polyrrhiza*, in 2 clones of *Lemna gibba* and — in experimental conditions for the second time — in *Wolffia arrhiza* (1 clone). The experiments were carried out in 8 groups (Table 1—3). Each figure is an average of the plant analysis. The plants were taken each time from 5—10 Erlenmayer flasks.

** Hillman (1958) used — under the name of *L. perpusilla* — the clone 1746 of Landolt, which in fact belongs to the *L. aequinoctialis* (= *L. paucicostata*) (Kandeler and Hügel 1974, Landolt 1980).

Results may be summarized as follows:

1. *Spirodela polyrrhiza:* Out of the five clones, four are photoperiodically neutral plants, while one clone (from Vinkovci) comprises quantitative long-day plants. Four of them flowered from the left reproductive pouch (Vinkovci, Slavonski Brod, Sunja, Đelekovec), one of them from the right one (D. Miholjac). Turions were formed at any photoperiod.

2. *Lemna gibba:* Two out of the two clones investigated reached flowering. Both were long day plants, and a short day preculture stimulated further flowering.

3. *L. minor* and *L. trisulca:* None of the four isolated clones flowered under the conditions experimental given.

4. *Wolffia arrhiza:* In one of the six isolated clones it has been possible to induce flowering, after a long day preculture of 35—40 days (16 hours light daily), at a short day photoperiod (2—4 weeks, 8 hours light daily).

Discussion

To the best of our knowledge, only two reports on photoperiodism in *Spirodela polyrrhiza* have been hitherto published (Krajnčič 1974 b, Krajnčič and Devidé 1980). The results obtained in the present work confirm the previous results and suggest that, under the given ecological conditions, this species is predominantly a photoperiodically neutral one.

On the other hand, the results of the clone from Vinkovci of the species *S. polyrrhiza* — those results have been experimentally confirmed five times up till now — give valid evidence that the clone from Vinkovci is a quantitative long day plant.

In the literature available on the photoperiodical responses of the species *S. polyrrhiza,* no other reports could have been found but those written by the authors of this work. Therefore, no comparative results by other investigators can be given here.

After 12 years of research into the photoperiodical responses in the species *S. polyrrhiza,* we can only state that, within this species, beside the photoperiodically neutral clones, there exists a quantitative long day clone, which is probably not the only one.

In the plant family of *Lemnaceae,* photoperiodically different clones have been found also in the species *Lemna paucicostata* (Yukawa and Takimoto, 1976; Hillman, 1979).

The results obtained with the clones from Bročice and Stupna confirm the previous data and suggest that *L. gibba* is predominantly a long day plant (Kandeler 1955, 1960/61, 1968; Cleland, 1967; Cleland and Briggs, 1967; Krajnčič, 1974 a, b, c; Krajnčič and Devidé 1980).

The results obtained with the clone Zagreb of the species *Wolffia arrhiza* confirm the only report available (Krajnčič and Devidé 1980) stating that this species is a long-short-day plant.

The negative results with two clones of the species *Lemna minor,* with two clones of the species *L. trisulca,* and with five clones of the species *W. arrhiza,* agree with the reports (Landolt, 1957; Krajnčič in Devidé, 1980) stating that numerous clones of those species do not flower.

Table 1. Photoperiodic responses in *Spirodela polyrrhiza* from five stations (Đelekovec, D. Miholjac, Slavonski Brod, Bijelo Brdo and Vinkovci) in North Croatia.

Group of experiments	Characteristics of the culture medium	Pre-culture		Culture									
		Duration in days	Photoperiod	Duration in days	Percentage of flowering plants								
					Đelekovec		D. Miholjac		Slavonski Brod		Bijelo Brdo		Vinkovci	
					LD	SD	LD	SD	LD	SD	LD	SD	LD	SD
1.	Fe-citrate $EDTANa_2 \cdot 2H_2O$ pH = 4.55	30	LD	27	9	8	8	8	40	40	14	12	—	—
2.	"	27	SD	27	11	10	12	12	48	47	16	15	—	—
3.	Fe-EDTA pH = 4.55	27	LD	27	13	12	15	14	48	47	20	18	—	—
4.	"	27	LD	25	13	13	15	15	49	49	25	25	—	—
5.	"	25	SD	26	14	12	17	15	52	49	27	25	—	—
6.	"	26	SD	27	14	12	19	17	52	52	28	28	—	—
7.	Fe-EDTA pH = 4.8	27	LD	26	17	15	21	20	56	51	36	30	16	—
8.	"	26	SD	27	20	16	25	22	58	52	37	30	18	2

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

Table 2. Photoperiodic responses in *Lemna gibba* from two stations (Bročice and Stupna) in North Croatia.

Group of experiments	Characteristics of the culture medium	Pre-culture		Culture				
		Duration in days	Photo period	Duration in days	Percentage of flowering plants			
					Bročice		Stupna	
					LD	SD	LD	SD
1.	Fe-citrate $EDTANa_2 \cdot 2H_2O$ pH = 4.55	30	LD	27	—	—	7	—
2.	"	27	SD	27	1	—	8	—
3.	Fe-EDTA pH = 4.55	27	LD	27	1	—	12	—
4.	"	27	LD	25	1	—	12	—
5.	"	25	SD	26	3	—	14	—
6.	"	26	SD	27	3	—	14	—
7.	Fe-EDTA pH = 4.8	27	LD	26	2	—	15	—
8.	"	26	SD	27	5	—	20	—

Table 3. Photoperiodic responses in *Wolffia arrhiza* from one station (Zagreb) in North Croatia.

Group of experiments	Characteristics of the culture medium	Pre-culture		Culture		
		Duration in days	Photo-period	Duration in days	Percentage of flowering plants	
					LD	SD
1.	Fe-citrate $EDTANa_2 \cdot 2H_2O$ 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.

References

Bennink G. J. H., R. Van den Berg, H. J. Kool, D. Stegwee, 1970: Flowering in *Lemna minor.* Acta Bot. Neerl. *19,* 385—392.

Cleland, C. F., 1967: The physiology of flowering of *Lemna gibba* L. strain G_3. Ph. D. thesis, Stanford Univ.

Cleland C. F., W. R. Briggs, 1967: Flowering responses of the long day plant *Lemna gibba* G_3. Plant Physiol. *42,* 1553—1561.

Hillman W. S., 1958: Photoperiodic control of flowering in *Lemna perpusilla.* Nature *181,* 1275.

Hillman W. S., 1979: Temporal compartmentation in *Lemna paucicostata:* photoperiodism, respiration, nitrogen nutrition, and heterotrophic growth of different strains. Amer. J. Bot. *66,* 1021—1028.

Kandeler, R., 1955: Über die Blütenbildung bei *Lemna gibba* L. I. Kulturbedingungen und Tageslängenbhängigkeit. Z. Bot. *43,* 61—71.

Kandeler, R., 1960/61: Lemnaceen als Forschungsobjekt. Ber. Phys. Med. Ges. Würzburg *70,* 81—86.

Kandeler, R., 1968: Blühinduktion bei Lemnaceen. Biol. Rsch. *6,* 49—57.

Kandeler, R., B. Hügel, 1974: Wiederentdeckung der echten *Lemna perpusilla* Torr. und Vergleich mit *L. paucicostata* Hegelm. Plant Syst. Evol. *123,* 83—96.

Krajnčič, B., 1972: Fotoperiodične reakcije lemnacej severovzhodne Slovenije. (Photoperiodic reactions in *Lemnaceae* of northeastern Slovenia.) M. Sc. thesis, Faculty of Science, Univ. of Zagreb, 1—106.

Krajnčič, B., 1974a: Studij fotoperiodičkih reakcija lemnaceja Slovenije. (Study of photoperiodic reactions in *Lemnaceae* of Slovenia), IV kongres biologa Jugoslavije, Sarajevo, 25.—28. 6. 1974, Rezimei referata p. 84.

Krajnčič, B., 1974b: Photoperiodic responses of *Lemnaceae* from northeastern Slovenia. Acta Bot. Croat. *33,* 81—88.

Krajnčič, B., 1974c: Prispevek poznavanju lemnacej severovzhodne Slovenije. (Contribution to the knowledge of *Lemnaceae* from northeastern Slovenia.) Biol. vestn. (Ljubljana) 22, 21—28.

Krajnčič, B., Z. Devidé, 1980: Report on photoperiodic responses in *Lemnaceae* from Slovenija, Ber. Geobot. Inst. ETH, Stiftung Rübel, Zürich *47,* 75—86.

Krajnčič, B., Z. Devidé, 1982: Rasprostranjenost vodenih leća *(Lemnaceae)* u sjevernoj Hrvatskoj. (Distribution of *Lemnaceae* in North Croatia). Acta Bot. Croat., Zagreb *41,* 175—180.

Landolt, E., 1957: Physiologische und ökologische Untersuchungen an Lemnaceen. Ber. Schweiz. Bot. Ges. *67,* 171—407.

Landolt, E., 1980: Biosystematische Untersuchungen in der Familie der Wasserlinsen *(Lemnaceae).* Veröff. Geobot. Inst. ETH, Stiftung Rübel, Zürich 1980 (70 Heft).

Maheshwari, S. C., O. S. Chauhan, 1963: In vitro control of flowering in *Wolffia microscopica. Nature 6,* 99—100.

Maheshwari. S. C., P. N. Seth, 1966: Photoperiodic control of flowering in *Wolffia papulifera.* Plant Cell Physiol. *7,* 163—165.

Scharfetter, E., Th. Rottenburg, R. Kandeler, 1978: Die Wirkung von EDDHA und Salicylsäure auf Blütenbildung und vegetative Entwicklung von *Spirodela punctata.* Z. Pflanzenphysiol. *87,* 445—454.

Yukawa, I., A. Takimoto, 1976: Flowering response of *Lemna paucicostata* in Japan. Bot. Mag. (Tokyo) *89,* 241—250.

The authors express their thanks to the Research Found of Slovenia for cofinancing the present research.

POVZETEK

FOTOPERIODIČNE REAKCIJE LEMNACEJ (*LEMNACEAE*) S PODROČJA SEVERNE HRVATSKE

Božo Krajnčič in Zvonimir Devidé

(Višja agronomska šola Univerze v Mariboru in Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu)

Proučevane so fotoperiodične reakcije lemnacej s področja severne Hrvatske.

Eksperimenti, ki so potekali v akseničnih pogojih v termostatiranih rastlinjakih v modificirani Pirson-Seidelovi hranilni raztopini, pri temperaturi 28 ± 1° C in osvetlitvi 3000 lx, so dali naslednje rezultate:

Izmed petih proučevanih klonov vrste *Spirodela polyrrhiza* so štirje fotoperiodično nevtralne rastline, eden pa je kvantitativna dolgodnevnica, kar je pri tej vrsti prvič ugotovljeno. Oba izmed proučevanih klonov vrste *Lemna gibba* sta dolgodnevni rastlini.

Izmed šestih klonov vrste *Wolffia arrhiza* je dosežno cvetenje le pri enem v kratkodnevnih pogojih po dolgodnevni predkulturi.

Pri nobenem klonu vrst *Lemna minor* in *L. trisulca* v danih eksperimentalnih pogojih ni doseženo cvetenje.

SADRŽAJ

FOTOPERIODIČKE REAKCIJE VODENIH LEĆA (*LEMNACEAE*) S PODRUČJA SJEVERNE HRVATSKE

Božo Krajnčič i Zvonimir Devidé

(Višja agronomska šola Univerze v Mariboru i Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu)

Proučavane su fotoperiodičke reakcije vodenih leća (*Lemnaceae*) s područja sjeverne Hrvatske.

Eksperimenti, koji su provedeni u akseničkim uvjetima u termostatiranim komorama u modificiranoj Pirson-Seidelovoj hranidbenoj otopini, pri temperaturi od 28 ± 1° C i osvjetljenju od 3000 lx, dali su ove rezultate:

Od pet proučavanih klonova vrste *Spirodela polyrrhiza* četiri su fotoperiodički neutralne biljke, a jedan je kvantitativna biljka dugoga dana, što je prvi put utvrđeno kod te vrste.

Oba istraživana klona vrste *Lemna gibba* biljke su dugoga dana.

Od šest klonova vrste *Wolffia arrhiza* u uvjetima kratkog dana bilo je moguće postići cvatnju samo kod jednoga, nakon prethodnog kultiviranja na dugom danu.

U danim eksperimentalnim uvjetima nije bilo moguće postići cvatnju ni kod jednog klona vrsta *Lemna minor* i *Lemna trisulca*.

Dr. Božo Krajnčič
Višja agronomska šola
Vrbanska 30
62000 Maribor (Jugoslavija)

Prof. dr. Zvonimir Devidé
Botanički zavod (IV) PMF
Rooseveltov trg 6/III, p.p. 933
41001 Zagreb (Jugoslavija)