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Experimental control of flowering in Spirodela polyrrhiza (L.) Schleid., strain 7401 – a preliminary report

by JERZY WOŁEK

Introduction

The flowering and seed formation in most of the *Lemnaceae* species appear to be infrequent, both in nature as well as in experimental conditions.

Reports of flowering and fruiting observed in nature have been summarrized by SAEGER (1929) and HICKS (1932); the latter author made a review of the known occurences and frequency of flower production in various species of *Spirodela*, *Lemna* and *Wolffia*. Flowers in several species of *Wolffiella* were reported for the first time by GIARDELLI (1935), MASON (1938), KURZ and CROWSON (1948) and OBERMEYER-MAUVE (1966). Some more detailed investigations on the flowering within the *Lemnaceae* have been carried out in Finland (LUTHER 1948), Poland (CZOPEK 1960) and USSR (IVANOVA 1970).

The species of the *Lemnaceae* notably differ as to the frequency of flowering. In certain taxa, particularly in *Lemna gibba* and *L. perpusilla*, flowers and fruits have been found in numerous stations; on the contrary, flowering in *Spirodela polyrrhiza* is extremely rare. *Wolffia arrhiza* in Central Europe fails to flower (KANDELER 1968).

Numerous attempts were made to explain the phenomenon of rarity of the flowering in experimental way; influence of temperature, light intensity, photoperiodism, chemical effects etc. were investigated. Many recent studies, however, do not explain the nature of the flowering in the *Lemnaceae*. The very first experiments with the *Lemnaceae* have been performed by HICKS (1932); he reported that an ultraviolet rays treatment caused the flowering in *Lemna trisulca*, *L. valdiviana*, *L. minor*, *L. minima* and *Wolffia columbiana*. However, the results of HICKS have not been confirmed hitherto (see also LANDOLT 1957 and HILLMAN 1959). Further experiments were started by KANDELER (1955, 1956) who investigated the influence of photoperiodism upon the flowering in *Lemna gibba* that was a

long-day plant; out of this material, the strains G1 and G3 become a classical object for later experiments. As far as *Lemna perpusilla* is concerned, the first report of experimentally obtained flowering was that of LANDOLT (1957) who found that his strain 6746 flowered under almost all conditions. Later on, HILLMAN (1958) reported a short-day response in the same material. Experimental control of flowering has been achieved in *Wolffia microscopica* and *W. papulifera* by MAHESHWARI and his collaborators (MAHESHWARI and CLUHAN 1963, MAHESHWARI and SETH 1966, MAHESHWARI and VENKATARAMAN 1966); according to these authors, photoperiod and chelating agents represent the factors which induce the flowering.

The aim of the present experiments was to induce the flowering in *Spirodela polyrrhiza*, strain 7401 originating from Poland. Flowers in *S. polyrrhiza* are extremely rare; HICKS (1937) estimated that during 200 years, the flowering individuals of this species have been found in about 20 cases (cit.acc. to IVANOVA 1970). Several attempts to induce the flowering of *S. polyrrhiza* in experimental way have been unsuccessful; in view of this, further investigations seemed advisable.

Quite recently, when the present manuscript was being prepared, a report by KRAJNČIĆ (1974) has been published. The Yugoslavian author induced experimentally the flowering in some *Spirodela polyrrhiza*; by coincidence, the conditions of his experiment corresponded to those used in the course of the present investigations.

The present study has been carried out in June-August 1974, at the Geobotanical Department, Swiss Federal Institute of Technology, Zurich. The strain 7401, belonging to the collection of the Institute, has been kindly put at my disposal by Prof. Dr. E. LANDOLT. I am very much indebted to the authorities of Swiss Federal Institute of Technology who enabled me to carry out these studies. My sincere thanks are due especially to Professor Dr. E. LANDOLT, Head of the Geobotanical Department, by whom both the facilities and the laboratory equipment of the Department were put kindly at my disposal. I wish alto to express my gratitude to Doc. Dr. K. URBANSKA-WORYTKIEWICZ for stimulating discussions, suggestions and criticism during the preparation of the manuscript.

Results

Out of a series of pilot experiments, only one proved to be successful; the resp. set of conditions as well as the preparation of the cultures are described below.

All the cultures of *Spirodela polyrrhiza*, strain 7401 from Poland, have been inoculated on June 30, 1974; they were started with two four-fronds colonies taken from a two-weeks-old stock. The plants were grown aseptically in 500 ml Erlenmeyer flasks, each of them containing 200 ml of 1/5 strength Hutner's medium prepared according to HILLMAN (1969), without sucrose. Eight flasks contained the fresh medium with pH adjusted to 5.3. Four other flasks were filled with an old solution, already used for cultures of *S. polyrrhiza* with *Wolffia arrhiza* during 15 days and subsequently cleared of plants. Prior to the inoculations, this nutrient solution was mixed with active carbon (500 mg/200 ml of medium) and filtered through a blotting paper; the pH of the filtrate was 6.1.

The twelve flasks described above were kept in a controlled environment room where 16 hr photoperiod was applied. During 19 days the light intensity ranged from 15'500 Lux to 18'400 Lux, its average value being about 16'840 Lux (= c. 195'894 erg $cm^{-2}s^{-1}$). The light source was provided by 215 W Philips daylight fluorescent lamps. Red light was emitted by four Philinea Philips lamps, each of 120 W. The air temperature was kept at $20^{o}C \pm 0.5$. during day and night. With the light being at full intensity, the temperature in the culture medium reached $27^{o}C$. It should be noted that the position of the culture flasks was changed every day in order to ensure relatively uniform illumination and temperature.

After 19 days the plants covered the whole surface of the nutrient solution; the vegetative growth of the cultures was nearly checked. From this day on, the air temperature was raised to $28^{o}C \pm 0.5$ and the light intensity was reduced to about 8'444 lux (= c. 98'418 erg $cm^{-2}s^{-1}$), ranging from 8'000 to 9'000 Lux. Red light was emitted by two lamps. During the daytime, the temperature in the culture medium reached $31^{o}C$.

The flowers in all cultures were found for the first time on August 8, 1974; it seems, however, that the very beginning of the flowering has taken place earlier, for quite a few overblown flowers were also observed that day. After about one week the flowering was over. The fruiting has not been ovserved. The fronds that flowered were smaller than the others; their upper surface was green-yellow, the lower one exhibited large amounts of anthocyanin pigments. No turions were observed.

Many *Lemnaceae* species seem to fail to flower in nature and numerous attempts to explain this phenomenon were made. Some of the environmental factors were believed to be associated with the flowering. SAEGER (1929) collected field evidence that flowering might be at least partly controlled by the composition of the pond water. HICKS (1932) suggested that alterations of mineral content of the water medium, increased temperatures of water and air as well as the light factor might be of utmost importance. LANDOLT's observations (1957) showed that high temperature and photoperiodism might be implicated in the flower production in some species. Landolt found that the pH values of the water (varying between 4.7 and 8.2) and the mineral salts concentration corresponding to 0.06 - 0.0005 M KCl do not influence the flowering. According to OBERMEYER-MAUVE (1966), conditions that are unfavourable for a vegetative growth might lead to the production of flowers. SCULTHROPE (1971) referred to paleobotanical evidence; he pointed out that BEASTO (1955) as well as WALKER and LAMBERT (1955) have found conspicously high frequencies of pollen and seeds resembling those of *Lemna minor* in the British material collected from various deposits of the Boreal and Atlantic periods of post glacial time, when the climate was warmer and milder than it is now.

Among numerous authors that have studied experimentally the problem of flowering in the *Lemnaceae*, KANDELER (1955,1956) and LANDOLT (1957) emphasized importance of light and temerature; they reported as well a stimulating influence of old media. The results obtained in the course of the present work corroborate the assumption of KANDELER and LANDOLT: alterations in the temperature and the light intensity seem to induce, in a general way the flowering of *Spirodela polyrrhiza*. The recent report of KRAJNČIČ (1974) appears to present a further argument in favour of this opinion. It should be added that *S. polyrrhiza* proved to be photoperiodically neutral in the experiments ot the Yugoslavian author; however, the results obtained hitherto permit to assume that various species of the *Lemnaceae* may respond to photoperiodism in rather a differentiated way (KANDELER 1968). It seems therefore that this factor does not play itself a decisive rôle in the stimulation of the flowering.

A stimulating effect of aged medium was explained by HILLMAN (1969) who assumed that chelating agents, produced by plants themselves, get

accumulated after some time. On the other hand, numerous authors stressed the important rôle of EDTA added to the medium (HILLMAN 1961, KANDELER 1968, KRAJNČIĆ 1974). It seems, however, that chelating agents do not always represent factors of an immediate effect, for the present author observed the flowering *Spirodela polyrrhiza* grown on media filtrated with active carbon. The results of our experiment suggest that the flowering can only appear in crowded cultures, being probably a result of the inhibition of vegetative growth. In this respect, the present investigations correspond to the observations of KANDELER (1955) and LANDOLT (1957).

According to some authors, reduced rate of flowering in some aquatic vascular plants and in particular in the *Lemnaceae*, might be caused by tendencies towards replacement of sexual reproduction by vegetative propagation (SAEGER 1929, HICKS 1932, JACOBS 1948, SCULTHROPE 1971). The present author suggests the following working hypothesis to explain the mechanism of the flowering in the *Lemnaceae:*

The plants flower under the conditions that are unfavourable for the vegetative growth; thus, inhibition of vegetative growth represents the factor directly responsible for induction of the flowering in the *Lemnaceae*. Crowding, exhaustion of mineral salts in water, allelopathy and other factors which inhibit vegetative growth, induce the flowering in an indirect way. The stage of disposition for flower production reached, the plants may bloom under the conditions that promote flowering (light, temperature, perhaps also photoperiodism); if these conditions are not fulfilled, the plants grow turions or other resting forms. This "awaiting" of conditions that are favourable for the flowering might be considered as a result of replacement of sexual reproduction by vegetative propagation; the latter mechanism is extremely well developed and entirely adequate to maintain the *Lemnaceae*. Normally developed fronds of *S. polyrrhiza* do not endure cold and dryness, but its turions support well low temperatures. On the other hand, fruits of the *Lemnaceae* seem to stand well dry conditions; in *L. perpusilla*, fruits were seen for a long time in harvested rice field (personal communication of Prof. Dr. E. Landolt). It seems therefore that alternative formation of turions or fruits might largely depend upon ecological factors.

In view of the aforementioned, the results obtained by CZOPEK (1963)

deserve a special mention. The Polish author investigated the formation of turions in *Spirodela polyrrhiza;* he was of the opinion that the inhibition of vegetative growth was a factor directly responsible for the induction of the turions formation in his material and all the external factors influenced this process only in an indirect way. The opinion of CZOPEK presents an argument in favour of our hypothesis; inhibition of vegetative growth in the *Lemnaceae* may bring about either a turn towards sexuality, or a shift towards the formation of propagules. It seems that these various life functions are precariously balanced. Further investigations on this problem are continued.

The choice of the material was a matter of chance; however, it seems probable that the presented formula may be a good starting point for further investiagtions on the flower production in other strains of *S. polyrrhiza.*

Summary

The flowering of *Spirodela polyrrhiza* has been obtained in the following conditions: Aseptic cultures were grown in 500 ml Erlenmeyer flasks, each of them containing 200 ml of 1/5 strength Hutner's medium prepared without sucrose. Eight flasks contained the fresh medium with pH adjusted to 5.3; four others were filled with an aged solution, used before for cultures of *S. polyrrhiza* with *Wolffia arrhiza* during 15 days and subsequently cleared of plants. Prior to the inoculations, this nutrient was mixed with active carbon (500 mg/200 ml of medium) and filtered through a blotting paper; the pH of the filtrate was 6.1.

The flasks were kept in a controlled environment room where 16 h photoperiod was applied. During 19 days, the light intensity of an average value about 17'000 lux and the air temperature of 20°C were programmed. From the 19th day on, when the vegetative growth of the cultures was nearly checked, the air temperature was raised to 28°C and the light intensity was reduced to about 8'500 Lux(= c. 100'000 erg $cm^{-2} s^{-1}$). The flowers were observed in all cultures for the first time on the 39th day of the experiment.

The present author assumes that the inhibition of the vegetative growth represents the factor directly responsible for the induction of the flowering of the *Lemnaceae;* it may bring about either a turn towards sexuality, or a shift towards the formation of propagules.

Zusammenfassung

Spirodela polyrrhiza wurde unter den folgenden Bedingungen zum Blühen gebracht:

Die Pflanzen wurden in 500 ml Erlenmeyerkolben steril kultiviert. Als Nährlösung diente 1/5 Hutner-Lösung (je 200 ml) ohne Zucker. 8 Kolben er-

hielten die frische Nährlösung mit pH 5.3. Vier andere Kolben enthielten alte Lösungen, in denen vorher während 15 Tagen *S. polyrrhiza* und *Wolffia arrhiza* gewachsen waren und die anschliessend mit Aktivkohle behandelt (500 mg/200 ml) und filtriert wurden. Das pH betrug 6.1.

Die Pflanzen wurden in einer Klimakammer unter 16-stündigen Lichtperioden kultiviert. Während 19 Tagen betrug die Lichtintensität ungefähr 17'000 Lux und die Lufttemperature 20°C. Vom 19. Tag an, nachdem die Oberfläche der Lösung fast ganz mit Sprossen bedeckt war, wurde die Lufttemperatur auf 28°C erhöht und die Lichtintensität auf 8'500 Lux (ca. 100'000 erg cm^{-2} s^{-1}) erniedrigt. Die Blüten wurden in allen Kulturen erstmals am 39. Tag des Experimentes beobachtet.

Es wird angenommen, dass die Hemmung des Wachstums direkt verantwortlich ist für die Auslösung der Blütenbildung bei *Lemnaceae*; diese Hemmung bewirkt unter bestimmten Bedingungen die Bildung von Blüten (sexuelle Fortpflanzung) und unter anderen die Bildung von vegetativen Ueberdauerungsorganen.

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