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PHOTOPERIODIC CONTROL OF FLOWERING IN *WOLFFIA PAPULIFERA*

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The factors controlling flowering in duckweeds in nature or in vitro are not clearly understood. To date experimental control of flowering has been realised only in two genera, *Lemna* and *Wolffia*. KANDELER (*1, 2*) obtained flowering in *Lemna gibba* by long-day treatment, whereas HILLMAN (*3–5*) achieved the same result in *L. perpusilla*—grown on medium containing ethylenediaminetetraacetic acid (EDTA)—by subjecting plants to short-day conditions. In *L. minor* CZYGAN (*6*) induced flower formation by adding oestrogens to the medium. So far as *Wolffia* is concerned, flowering has been hitherto achieved only in *W. microscopica* under short-day conditions (*7*). More recently, we have been able to induce flowering in another species of *Wolffia, W. papulifera* Thomp. which is the subject of the present report.

Fronds of *W. papulifera* are slightly larger in size than those of *W. microscopica* and they do not have ventral projections. The frond is boat-shaped with a prominent papilla in the centre of the dorsal surface. Aseptic cultures were initiated by disinfecting the fronds with 0.1 per cent mercuric chloride and then thoroughly washing them with sterile water. As a rule only one plant was inoculated per test tube which contained 10 to 15 ml of either BONNER and DEVIRIAN's medium (Medium A) or HOAGLAND's modified liquid medium (Medium B). Medium A contained the following major salts (mg/liter): KNO_3 85, $Ca(NO_3)_2 \cdot 4H_2O$ 242, KH_2PO_4 20, KCl 60, $MgSO_4 \cdot 7H_2O$ 42, whereas Medium B contained the following (mg/liter): KNO_3 506, $Ca(NO_3)_2 \cdot 4H_2O$ 1180, KH_2PO_4 136, $MgSO_4 \cdot 7H_2O$ 493. Both the media contained an identical set of minor elements: $ZnSO_4$ 1 mg, H_3BO_3 1 mg, $MnSO_4$ 0.1 mg, $CuSO_4$ 0.03 mg, $NaMoO_4$ 0.025 mg and ferric citrate 4 mg per liter.

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Medium A with 1 per cent sucrose was found to be better than Medium B for the growth of the plant. Cultures were maintained under 16 hours of daily illumination from a mixed bank of tungsten and fluorescent lamps and the temperature was regulated at 26±1°. The light intensity ranged from 600 to 700 ft-c. The multiplication rate (MR) of *W. papulifera*—calculated from the formula of CLARK (*8*): $\mathrm{MR} = \frac{\log 10(Fd) - \log 10(Fo) \times 1000}{d}$ (where Fo is the original frond number, Fd is the frond number on day d) —approached a value of 180. Under long-day conditions the plants remained vegetative and seldom flowered.

Experiments were undertaken to determine the effect of different photoperiods on flowering. For this purpose, the plants were kept under continuous illumination and under photoperiods of 6, 12 and 16 hours duration for three days. The experiment was so arranged that all sets received 6 hours of high intensity light (600–700 ft-c) whereas the supplementary photoperiod was provided by about 40 ft-c of fluorescent illumination. Subsequently, the cultures were transferred to long-day conditions of 16 hours light + 8 hours darkness. Six days after the last photocycle, the plants were fixed in 90 per cent alcohol and the percentage of flowering was calculated (determined by dividing the number of flowering plants by the total number counted and multiplying this figure by 100). Average percentages of flowering under different treatments are presented in Table I.

TABLE I

The effect of different lengths of dark period on the flowering of Wolffia papulifera

	Percentage of plants flowering under different daily hours of darkness			
	0 hr	8 hr	12 hr	18 hr
1. Medium A	18	31	55	48
2. Medium B	10	26	34	41
3. Medium A+10^{-4} M EDTA	2	11	35	19
4. Medium B+5×10^{-5} M EDTA	12	21	35	23

The concentrations of EDTA were chosen on the basis of work done earlier on *W. microscopica* (*9*) where the combinations indicated bring about maximal stimulation of growth.

As evident from the table, the intensity of flowering in cultures increased with the lengthening of the dark period. In general, the optimum appears to be 12 hours of darkness. However, in HOAGLAND's medium the maximal flowering percentage was obtained under conditions of 18 hours darkness + 6 hours light.

In view of the previous reports regarding the effects of EDTA on photoperiodic sensitivity of *L. perpusilla* (see *5*) a comparison was also made of the flowering percentage obtained in media containing EDTA and no EDTA.

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Apparently, however, EDTA does not play a significant role in the growth and flowering of *W. papulifera* (Table I). In both the media tried the treatment which elicits maximum flowering is 12 hours daily dark period. In this respect *W. papulifera* shows striking differences also from *W. microscopica* where EDTA is essential for growth and flowering (*7*).

A few experiments have been done to test the effect on flowering of interruption of long night by red light. As a source of red light four 24 inches long 20 watt Philips fluorescent tube wrapped in red cellophane paper were used at a distance of 40 cm. It was noticed that flowering was inhibited almost completely by 15 minutes exposure to red light during the middle of the dark period.

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