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Short Communication

Induction of Flowering in *Lemna paucicostata*, a Short-Day Plant, by Chelating Agents and Iron

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Summary. *Lemna paucicostata* is a short-day plant which normally flowers only in a medium supplemented with EDTA or EDDHA. On a molar basis EDDHA is more effective for induction of flowering. The chelating agent can be replaced by high concentrations of ferric citrate in the medium. Simultaneous supply of both EDDHA and a high level of ferric citrate results in flowering even under long days.

Recently it was found in our laboratory that substitution of the usual iron source in the medium by an iron chelate, namely, the iron salt of ethylenediamine-di-o-hydroxyphenylacetic acid (Fe-EDDHA), results in a change of the photoperiodic behaviour of *Wolffia microscopica* from short-day to day-neutral (MAHESHWARI and SETH, 1966). This finding raises the question whether initiation of flowering by this compound under non-inductive photoperiods is common to members of the *Lemnaceae* or is something unique to *W. microscopica*. In the present investigation we have tried to answer this question with respect to *Lemna paucicostata* Hegelm., a local duckweed.

Fronds of *L. paucicostata* were cultured under aseptic conditions in a medium consisting of the following (in mg/l): KNO_3 85, $Ca(NO_3)_2 \cdot 4H_2O$ 242, KH_2PO_4 20, KCl 61, $MgSO_4 \cdot 7H_2O$ 42, $ZnSO_4 \cdot 7H_2O$ 1, $CuSO_4 \cdot 5H_2O$ 0.03, H_3BO_3 1, $Na_2MoO_4 \cdot 2H_2O$ 0.025, $MnSO_4 \cdot H_2O$ 0.1, ferric citrate 4.0 (ca. 10^{-5} M), and sucrose 10 g/l. Chelating agents, namely ethylenediaminetetraacetic acid (EDTA) or ethylenediamine-di-o-hydroxyphenylacetic acid (EDDHA), were included when required. The cultures were maintained at $25 \pm 1°$ under illumination from a mixed bank of fluorescent and incandescent lamps, 650 ft.-c. intensity, under long-day conditions (18 hours light, 6 hours darkness).

It was found that the fronds grew reasonably satisfactorily in the medium even in the absence of EDTA or EDDHA (Fig. 1)[1]. Flowering occurred only when the plants were subjected to a minimum of one short-day treatment (16 hours darkness, 8 hours light) and even then only in the presence of a chelating agent. No flowering took place in medium devoid of EDTA or EDDHA except under special circumstances

[1] Growth has been expressed in terms of the multiplication rate calculated by the formula given by CLARK (1925).

as described below. It may be observed that the optimal concentration of EDDHA for flowering is as low as 5×10^{-6} M, compared to 10^{-4} M for EDTA (Fig. 1 A and B).

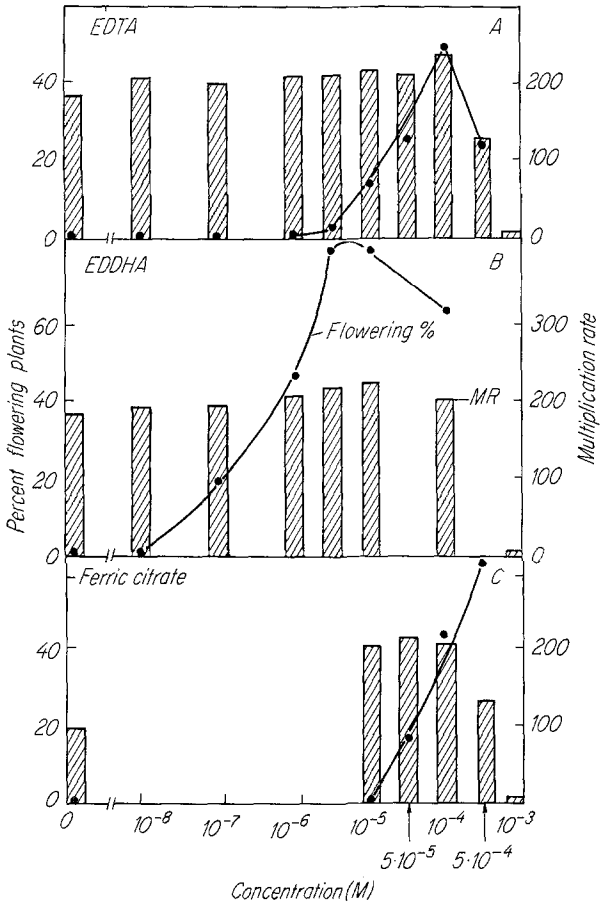

Fig. 1 A—C. Effect of EDTA (A), EDDHA (B) and ferric citrate (C) on multiplication rate and flowering in *Lemna paucicostata* (pH of medium 5.5). The plants were subjected to 4 short days and analyzed for flowering on the 4th day after induction. Iron in A and B was kept constant at the normal level (10^{-5} M ferric citrate). Note that flowering in these cases was obtained under short days only. Multiplication rate determined according to CLARK (1925)

The flower-inducing effect of EDTA and EDDHA at rather low concentrations implied the chelation of some trace element(s). Hence, experiments were undertaken to identify the ion involved, and to determine a suitable concentration which could dispense with the

requirement of a chelating agent for flowering. Of the chemicals ($MnSO_4$, $CuSO_4$, $ZnSO_4$, Na_2MoO_4, H_3BO_3 and ferric citrate) tried, only high concentrations of ferric citrate were effective in replacing the requirement for EDTA or EDDHA. Fig. 1 C illustrates an experiment where 5×10^{-4} M ferric citrate brought about approximately 60% flowering even though this concentration resulted in a decline in the multiplication rate of the plants.

Interestingly, when both EDDHA and excess iron (5×10^{-4} M ferric citrate) were provided simultaneously, our strain of *L. paucicostata*, which has been cultured in this laboratory for over 4 years and had been known to us as a strict short-day plant, flowered under long-day conditions. Addition of EDDHA at a concentration as low as 5×10^{-6} M (optimum 5×10^{-5} M) to the excess-iron medium was sufficient to bring about this change in the flowering response (Table).

Table. *Effect of EDDHA in excess-iron medium (5×10^{-4} M ferric citrate) on flowering in Lemna paucicostata under long and short days*

Figures are percentage of flowering plants, determined according to HILLMAN (1959).

Photoperiod	EDDHA (molar)						
	0	10^{-6}	5×10^{-6}	10^{-5}	5×10^{-5}	10^{-4}	5×10^{-4}
Long days (18 hrs. light)	0	0	5.6	31.5	57.5	51.8	2.8
Short days (8 hrs. light)	74.0	78.7	79.9	79.6	82.0	87.6	80.1

The present findings in *L. paucicostata* are in general agreement with our previous reports about the role of iron and chelating agents for flowering in *W. microscopica* (MAHESHWARI and SETH, 1966; also MAHESHWARI and CHAUHAN, 1963). At this stage, we are unable to provide any convincing explanation of the mechanism by which the effects of these chemicals on flowering in *Lemnaceae* are brought about. It may be that iron in some way is specifically involved in flowering and the very high affinity of EDTA and particularly EDDHA (KROLL, 1957) to this metal makes it more rapidly available for this process.

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