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INDUCTION OF FLOWERING IN LEMNA MINOR BY EDDHA

P. R. BHALLA and P. S. SABHARWAL

Department of Botany, University of Kentucky, Lexington, Kentucky 40506, U.S.A.

SUMMARY

The effect of EDDHA (ethylenediamine-di-o-hydroxyphenylacetic acid) on growth and flowering of *Lemna minor* cultured in M-medium was investigated. It was observed that inclusion of this compound induced flowering in this plant. The maximum flowering was observed in a medium supplemented with 15 ppm of this metal chelate. Endogenous copper analysis of flowering and non-flowering plants demonstrated 45–65% less copper in flowering plants, thereby indicating the role of copper in the flowering of *L. minor*.

1. INTRODUCTION

Recently it was found in our laboratory that ethylenediamine-di-o-hydroxyphenylacetic acid (EDDHA) has a marked effect on the growth and flowering of *Lemna gibba* G3 (PIETERSE *et al.* 1970a, b, c). It induced profuse flowering when plants were cultured in modified Hutner's medium. Prior to this observation, GUPTA & MAHESHWARI (1970) found that *L. paucicostata* flowers only in the presence of either EDDHA or ethylenediaminetetraacetic acid (EDTA) in the culture medium. These observations raise the question whether initiation of flowering by EDDHA is common to other species of *Lemna* or restricted to *L. gibba* and *L. paucicostata*. In the present report it is shown that EDDHA can induce flowering in *L. minor* in M medium using long-day photoperiods. *L. minor* can be distinguished from *L. gibba* by the darker green surface of the fronds, more symmetrical apex, and smaller air spaces. The plants of *L. minor*, like other species of *Lemna*, are ideally suited for investigation on flowering since they are small and can easily be cultured under experimental conditions. Plants easily grow and multiply vigorously under axenic condition.

2. MATERIAL AND METHODS

The plants were grown in M medium (HILLMAN 1961). A litre of medium contained 1% sucrose in addition to the following in mg: KH_2PO_4 680, $MgSO_4 \cdot 7H_2O$ 492, $Ca(NO_3)_2 \cdot 4H_2O$ 1180, KNO_3 1515, $ZnSO_4 \cdot 7H_2O$ 0.22, H_3BO_3 2.86, $CuSO_4 \cdot 5H_2O$ 0.08, $Na_2MoO_4 \cdot 2H_2O$ 0.12 $MnCl_2 \cdot 4H_2O$ 3.62, $FeCl_3 \cdot 6H_2O$ 5.40, and tartaric acid 3.0. The pH of the medium was adjusted to 5.0. Plants obtained from a single clone were used for this investigation. Ten replicates were subjected to each treatment and the experiment was repeated 5 times. The plants were kept at 25 $\pm$ 2°C, under either long-day or short-day

conditions. The short-day photoperiod consisted of 8 hours of light and 16 hours of darkness, while the long-day was 16 hours of light and 8 hours of darkness. The illumination was obtained from cool white fluorescent tubes and gave 225–250 foot candle intensity upon the culture flasks. The multiplication rates were calculated according to the method of CLARK (1925):

$$\frac{\log_{10}(Fd) - \log_{10}(Fo) \times 1000}{d}$$

(where Fo is the original frond number, Fd is the frond number on day d). Observations for the appearance of flowering fronds were taken 14 days after inoculation. Every visible frond was taken into consideration. The endogenous copper levels of flowering and non-flowering plants were analysed by Ohio Plant Analysis Laboratory, Wooster, Ohio. The copper estimation was done using a direct emission spectrometer; lithium served as the internal standard.

3. RESULTS AND DISCUSSION

When plants were grown in M-medium, there was no flowering either under long-day or short-day photoperiodic conditions. The plants remained healthy and grew vegetatively. The multiplication rates approached 90. *Fig. 1* illustrates the effect of the incorporation of varying amounts of EDDHA in M-medium on flowering and the multiplication rate (MR) under long-day conditions. The addition of EDDHA influenced the MR; the MR slightly increased at 10 ppm, while increasing the concentrations above 10 ppm resulted in a progressively lowered MR.

The more interesting effect of EDDHA was, however, on flowering. In the medium devoid of EDDHA, plants did not flower under either long or short days. When EDDHA (5–100 ppm) was incorporated into the medium, flowering was discernible 7–8 days after inoculation (*fig. 1*). The maximum number of

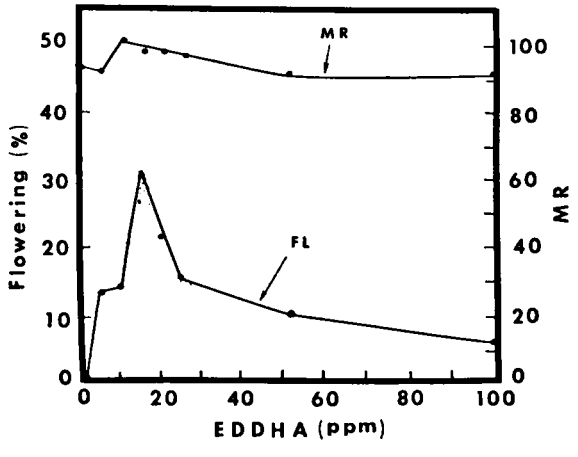

Fig. 1. Effect of various concentrations of EDDHA on flowering (FL) and multiplication rate (MR) of *Lemna minor*.

flowering plants were observed in the medium containing 15 ppm of EDDHA.

The effect of EDDHA on flowering in *L. gibba* G3 (PIETERSE *et al.* 1970c) and *L. paucicostata* (GUPTA & MAHESHWARI 1970) has been previously reported. In *L. gibba*, a long-day plant, EDDHA has profound influence on growth (PIETERSE *et al.* 1970a) and flowering (PIETERSE *et al.* 1970c). In 1/3 strength Hutner's medium, flowering occurred only in the presence of EDDHA. *L. paucicostata*, a short-day plant, flowers in the medium supplemented with EDTA and EDDHA. In the present investigation EDDHA induced flowering in *L. minor* in M-medium. There is some evidence that in *L. gibba* EDDHA exercises its role by chelating some metallic ion or ions, perhaps Cu^{++} (PIETERSE *et al.* 1970c). To investigate the possible role of copper in induction of flowering in *L. minor*, endogenous copper in flowering plants cultivated in the nutrient medium containing EDDHA and non-flowering plants grown on medium devoid of EDDHA was investigated. In all the experiments the levels of endogenous copper in vegetative and flowering plants showed a difference. The level of copper decreased by 45–60% in flowering plants, thereby clearly demonstrating the role of copper in the induction of flowering in *L. minor*. However, the mechanism by which copper regulates flowering remains to be investigated. This and other related questions are under study, and the results will be reported subsequently.

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