CONTROL OF GIBBOSITY IN LEMNA GIBBA G3 BY ETHYLENEDIAMINE-DI-O-HYDROXYPHENYLACETIC ACID (EDDHA)

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SUMMARY

Flat forms of *Lemna gibba* became gibbous in the presence of EDDHA. The degree of the gibbosity varied with the concentration of the chelate. The maximum convexity was observed at 10 and 20 ppm. This clearly suggested that both flat and gibbous forms belong to the same taxon and merely represent the morphological modifications under different nutritional conditions.

1. INTRODUCTION

Lemna gibba has been described to occur in two forms. One is conspicuously gibbous and the other is essentially non-gibbous. Whether these are distinct genetic races or mere ecological modifications of the same race is disputed. The gibbous forms show obvious variation in the degree of gibbosity and are easily recognized by the swelling of their abaxial surface. Depending upon the degree of gibbosity, the intermediate forms are classified as *L. obscura* or *L. disperma* by DAUBS (1965). MASON (1957) observed that both gibbous and flat fronds flower profusely but fruiting specimens of gibbous forms are more frequently encountered, thereby suggesting that the non-gibbous form is a sterile hybrid. DE LANGE & SEGAL (1968) proposed, without any experimental support, that gibbous forms are produced under optimal growth conditions while only flat forms are found in sub-optimal conditions. However, DEN HARTOG (1968) considers the evidence for this conclusion to be insufficient and feels that it is yet to be demonstrated that these two forms do not belong to separate taxa. We have recently observed that flat fronds become gibbous *in vitro* in the presence of a chelate, ethylenediamine-di-o-hydroxyphenylacetic acid (EDDHA). The degree of gibbosity depends on the concentration of the chelate used, thereby indicating that flat and gibbous forms belong to the same taxon.

2. MATERIAL AND METHODS

Specimens of *Lemna gibba* G3 were aseptically cultured in 125 ml Erlenmeyer flasks. Each flask contained about 60 ml of the nutrient medium. M-medium (HILLMAN 1961) supplemented with 1% sucrose served as control. Each inoculum consisted of 3–5 fronds which were obtained from 6–12 day old stock cultures. Five replicates were subjected to each treatment. The cultures were maintained at 25° ± 2°C under continuous illumination from cool-white

fluorescent tubes of 225–250 foot candles intensity. Multiplication rates were calculated according to the method of CLARK (1925) taking into account the smallest visible daughter frond. The gibbosity and multiplication rate were determined 14 days after inoculation.

3. RESULTS AND DISCUSSION

The most obvious effects of EDDHA on vegetative growth were the appearance of gibbous character of the fronds and the decrease in the multiplication rate. In EDDHA medium the gibbosity was observed 3–4 days after inoculation. After 14 days of growth, 99% of the fronds were in the gibbous form. *Fig. 1* shows the flat a. and gibbous b. fronds of *L. gibba* raised in media without and with EDDHA.

Fig. 2 summarizes our finding on gibbosity and growth rate in M-medium at different concentrations of EDDHA. For each concentration of EDDHA, 100 fronds were measured for gibbosity. The degree of gibbosity varied with the concentration of the chelate (*fig. 2*). The maximum convexity was observed at 10 and 20 ppm while higher concentrations proved less effective. At the very low dose (0.1 ppm) of EDDHA, the multiplication rate (MR) was 140, the same as the control. The MR decreased in media containing increasing concentrations of EDDHA. At the highest concentration used (100 ppm), the MR was 100. Although the rate of multiplication declined in higher concentrations of

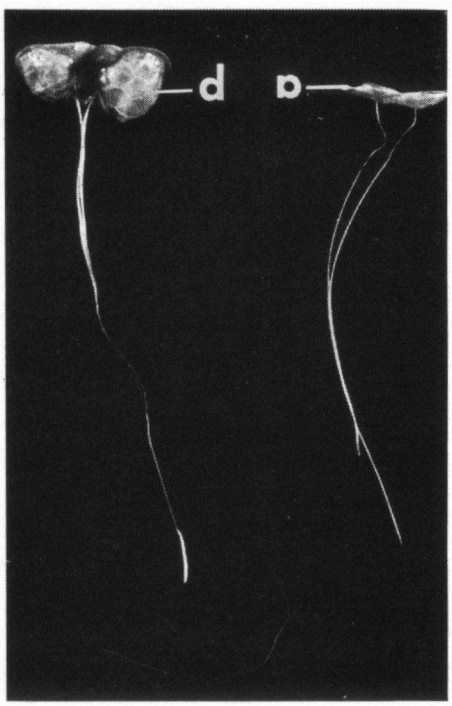

Fig. 1.
L. gibba: Note the flat fronds (a) raised in M-medium without EDDHA and gibbous fronds (b) produced in EDDHA medium 8 days after inoculation. (X4.)

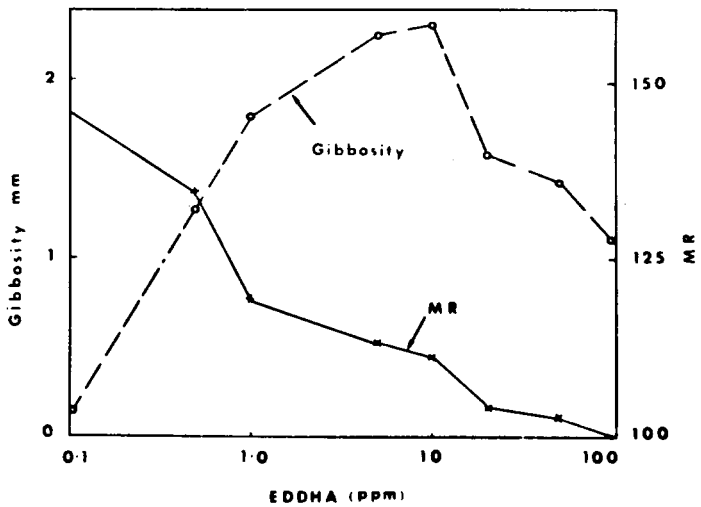

Fig. 2. Effect of EDDHA on *L. gibba*. The gibbosity and multiplication rate (MR) are shown under varying concentrations of EDDHA.

EDDHA, the size of the individual fronds increased. An apparent enlargement in the frond size was observed as early as 4 days after inoculation in the media containing 20–100 ppm of the chelate.

When gibbous fronds were transferred to the nutrient medium without EDDHA, the production of flat daughter fronds was observed 8–10 days after inoculation. This demonstrates that both gibbous and flat forms belong to the same taxon and merely represent the morphological modifications under different nutritional conditions. Intermediate gibbous forms described as *L. obscura* or *L. disperma* by DAUBS (1965) probably represent forms of *L. gibba* produced under different ecological conditions. Since EDDHA is known to be the most effective chelating agent (see WALLACE 1966), we believe that some metal ion is specifically involved in controlling gibbosity.

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