

Communication

Role of Catecholamines in Promotion of Flowering in a Short-Day Duckweed, *Lemna paucicostata* 6746¹

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ABSTRACT

L-Epinephrine, L-norepinephrine, and L-isoproterenol substantially promote flowering under a photoperiodic regime of 8 hours light and 16 hours darkness in *Lemna paucicostata* 6746 when grown on the modified Bonner-Devirian medium devoid of ethylenediaminetetraacetic acid. If catecholamines are provided to plants at 10⁻⁴ molar level prior to transferring them to the short-day regime, they not only induce more floral primordia but also significantly improve flower development and sustain the flowers for a longer period. Propranolol (10⁻⁴ molar), a β -adrenergic blocking agent, partially suppresses flowering and the inhibition of flowering is relieved by catecholamines.

There has been a great deal of interest in identifying chemicals or hormones involved in flowering. *Lemna paucicostata* 6746, a short-day duckweed, has been widely employed for the study of physiology and biochemistry of flowering. This plant is sensitive to a single long night and flowers even without chelating agents in the modified Bonner-Devirian medium (8).

Catecholamines are well-known for their strong regulatory effect in animal systems, though little is known of their role in plants. Nevertheless, catecholamines have been shown to relieve inhibition of flowering in *Lemna gibba* caused by the presence of sugar in the M medium (10) or by the simultaneous presence of sucrose and ammonium ions in *Lemna paucicostata* 6746 when grown in half-strength Hutner medium (4). In our culture conditions—in the modified Bonner-Devirian medium—which is free of ammonium ions, sucrose is not inhibitory and indeed it is required for healthy growth and flowering in *L. paucicostata* 6746 (7). Nonetheless, in view of the effects of catecholamines and their role in plants reported earlier, we investigated the effect of catecholamines under our culture conditions. Here we report a significant promotion of flowering in *L. paucicostata* 6746 by these compounds under inductive photoperiods.

MATERIALS AND METHODS

The aseptic cultures of *Lemna paucicostata* 6746 were maintained in the modified Bonner-Devirian medium containing

major salts of Bonner-Devirian (2) and minor salts of Heller (3). The medium was supplemented with 1% sucrose (w/v) and 10⁻⁴M EDTA. The pH of the medium was adjusted to 5.5 with 0.1 N HCl or 0.1 M NaOH, before autoclaving at 1.08 kg/cm² for 15 min. The stock cultures were kept under long days of 16 h light and 8 h darkness and light provided by a bank of mixed cool daylight fluorescent tubes (Philips TL 65-80W/54 RS, 6,800 K) and incandescent bulbs (Philips Argenta, 100 W). The irradiance level in the spectral range between 400 and 750 nm varied from 10.0 to 10.5 W m⁻². The temperature was 26 ± 2°C during light conditions and 22 ± 1°C in darkness.

All experimental cultures were started with a single 3-frond colony in 250 ml Erlenmeyer flask containing 100 ml modified Bonner-Devirian medium supplemented with 1% sucrose but without EDTA. Catecholamines (purchased from Sigma) were filter-sterilized and added to the nutrient medium 2 d after inoculation. Multiplication rate and flowering percentage were determined as per details described in an earlier paper (8). All experiments have been repeated at least once.

RESULTS

Lemna paucicostata 6746 is a short-day duckweed which, in modified Bonner-Devirian medium, flowers irrespective of presence or absence of a chelating agent. However, it has not been found possible to substitute EDTA for the photoperiodic requirement of this plant, although inclusion of this chelating agent in the nutrient solution does cause profuse flowering (about 70%). These basic observations were reported earlier from our laboratory (8).

We studied the effects of different concentrations of epinephrine, norepinephrine and isoproterenol (ranging from 10⁻⁶ to 2 × 10⁻⁴M) on flowering and vegetative growth in *L. paucicostata* 6746 in the modified Bonner-Devirian medium under a regime of 8 h light and 16 h darkness separately in three sets of experiments. As can be seen in the left column of Figure 1 (A, B, and C), epinephrine, norepinephrine, and isoproterenol all enhanced the multiplication rate and flowering percentage. A small but clear effect could be seen even at the 10⁻⁶M level on flowering. However, an increase in concentration of epinephrine, norepinephrine, and isoproterenol from 10⁻⁶ to 10⁻⁴M elicited excellent flowering response. At 10⁻⁴M the fronds were also more green and healthy than fronds grown on control medium. Nonetheless, a further increase in its level (e.g. 2 × 10⁻⁴M) caused a decrease in multiplication rate as well as size of fronds, although flowering percentage remained essentially unaltered.

The data shown in Figure 1, in the right column, show the effects of added catecholamines on flowering under different photoperiods. Flowering was observed only if the photoperiod was 14 h or less. But the noteworthy aspect is that, under identical

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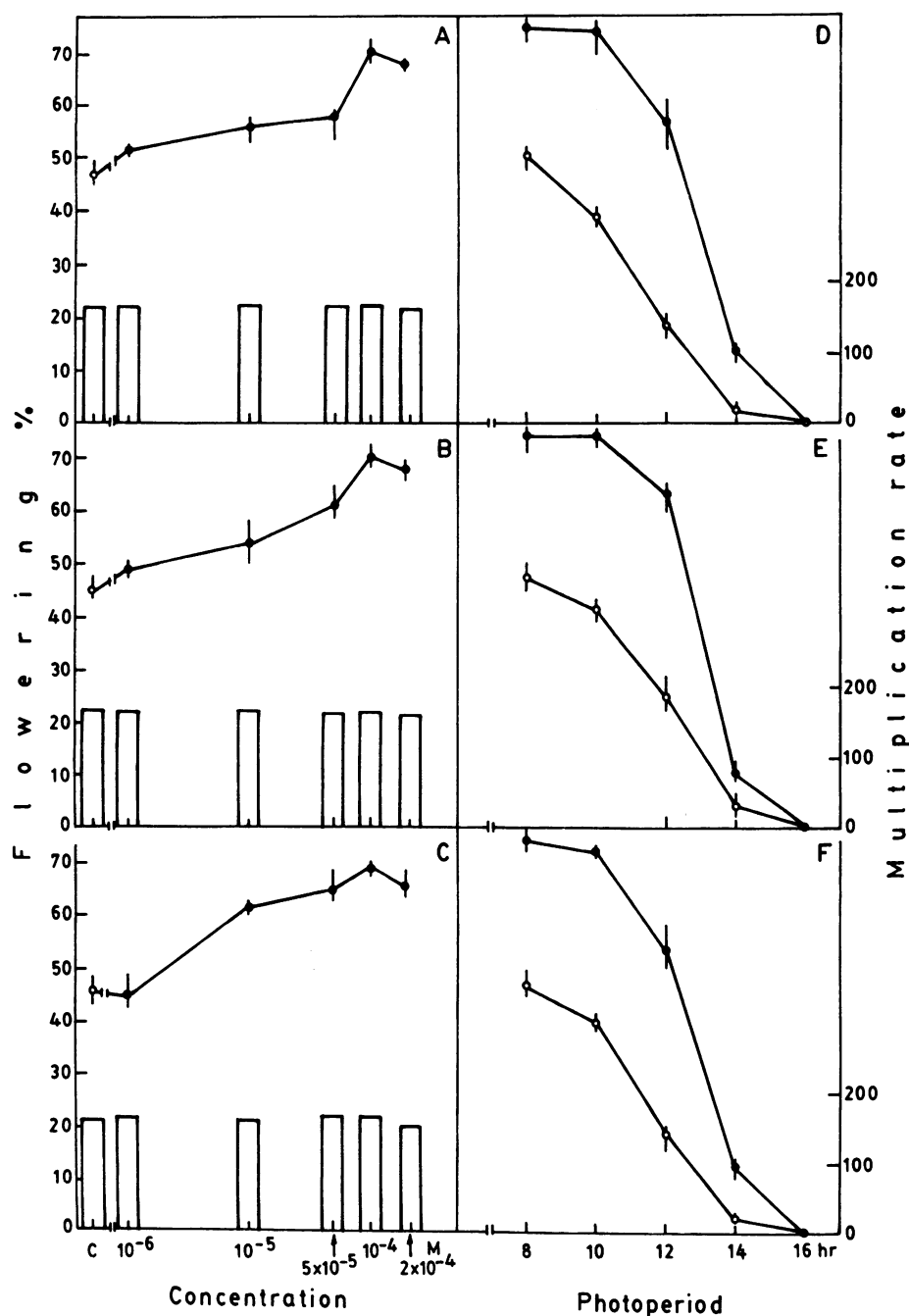


FIG. 1. Effect of catecholamines on multiplication rate and flowering in *L. paucicostata* 6746. Left column: effect of different concentrations of epinephrine (A), norepinephrine (B), and isoproterenol (C) on multiplication rate and flowering under short days. Experimental cultures were initially grown under long days of 16 h light and 8 h darkness. These compounds were filter-sterilized and provided to plants 48 h after inoculation, and after 24 h cultures were subjected to 3 short-day cycles of 8 h light and 16 h darkness, followed by 3 long days. Flowering percentage was calculated 7 d after addition of these chemicals in the medium. The vertical bars and curve represent the multiplication rate and flowering percentage, respectively. The results shown here are the average of three replicates with maximal and minimal variation. Right column: effect of epinephrine (D), norepinephrine (E) and isoproterenol (F) on flowering under various photoperiods. (○—○) Control; (●—●) 10^{-4} M of respective adjuvant.

conditions, the flowering percentages were significantly higher under all photoperiods in 10^{-4} M catecholamine-treated plants. Under daily photoperiodic schedules of 10 to 12 h light, flowering was 2 to 3 times higher than in control media. None of these chemicals could, however, induce flowering (e.g. under a photoperiodic regime of 16 h light and 8 h darkness).

The effect of 10^{-4} M epinephrine on flowering as a function of number of days elapsed after three short-day photoinductive cycles is shown in Figure 2. It is clear that the maximal effect is seen on the 3rd day after completion of the 3 short days. In parallel experiments it was noticed that catecholamines sustained flowers for a longer period and the flowering percentage was higher even on the 5th and 6th day after photoinductive treatment than in the controls. A further significant observation is that the flowers remained well developed for as long as 10 days in the nutrient medium containing catecholamines.

To determine the specificity of action of catecholamines on

flowering, the effect of DL-propranolol, a β -adrenergic blocking agent, was also studied. Propranolol (10^{-4} M) did not have much effect on multiplication rate; but it inhibited flowering by about 20% in comparison to the control. Further, the inhibitory effect of 10^{-4} M propranolol was overcome by 10^{-4} M epinephrine, norepinephrine, or isoproterenol (Fig. 3).

Finally, although the effects of amino acids on flowering in *L. paucicostata* 6746 have already been reported by Tanaka and Takimoto (15), a few experiments were performed to determine whether the effects on flowering were specific to catecholamines or could be elicited by tyrosine, an amino acid and a precursor of catecholamines. Tyrosine, however, had no promotive effect on flowering—rather it inhibited flowering at higher concentrations (data not communicated).

DISCUSSION

The results clearly indicate a strong promotive role of catecholamines on flowering in *L. paucicostata* 6746 under inductive

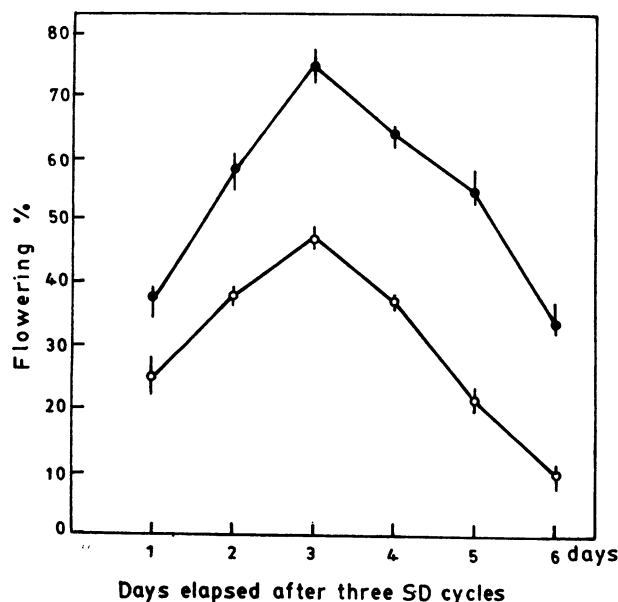


FIG. 2. The effect of epinephrine on flowering as a function of number of days elapsed after three short-day treatments. The plants were fixed for calculating the flowering percentage each day (1-6) after 3 short-day treatments. For further details see Figure 1. (○—○) Control; (●—●) 10⁻⁴M epinephrine.

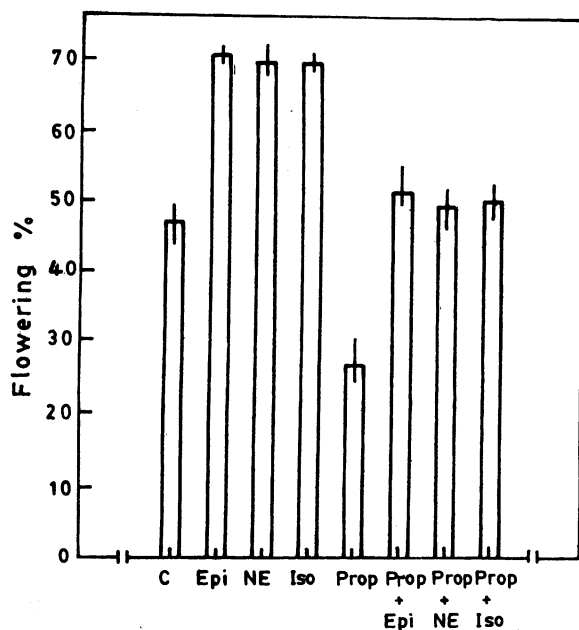


FIG. 3. Interaction of propranolol with epinephrine, norepinephrine and isoproterenol in relation to their effect on flowering in *L. paucicostata* 6746 under short days. At 10⁻⁴M, epinephrine, norepinephrine, and isoproterenol were added separately to nutrient medium after addition of 10⁻⁴M propranolol as described in earlier experiments. For further details, see Figure 1. C, control; Epi, epinephrine; NE, norepinephrine; Iso, isoproterenol; Prop, propranolol.

short-day photoperiods. Epinephrine, norepinephrine, and isoproterenol, all are equally potent. To our knowledge, this is the first report in the plant kingdom where *direct promotion* of flowering by these chemicals was obtained. It is true that for maximal response rather high concentrations (10⁻⁴M) are required; however, this could be due either to poor absorption by the plants or rapid degradation of these compounds once taken

in by cells. This is a matter that would need to be looked into by use of radiolabeled catecholamines. It is also a matter of great interest that propranolol, an inhibitor of β -adrenergic receptor, inhibits catecholamine-stimulated flowering, further supporting a role of catecholamines in plants.

The presence of norepinephrine in tissues of flowering plants has been reported by several investigators (1, 14, 16, 17), although its role is largely unknown. In the Lemnaceae, no catecholamines have so far been isolated. Nonetheless, some indirect evidence for a role of these compounds has existed in duckweeds for some time. Thus, an inhibition of flowering in *L. gibba* imposed by presence of sugar in the medium was overcome by catecholamines as reported by Oota (10). More recently, Ives and Posner (4) have shown a remarkable effect of epinephrine in relieving the inhibition of flowering in *L. paucicostata* 6746 due to the presence of sucrose and ammonium ions. In this connection, it is of interest to review also reports indicating a role of cyclic AMP and adenylate cyclase in Lemnaceae, since in animals adenylate cyclase is affected by many hormones including of course epinephrine. Oota (9, 11) and Posner (13) reported that cyclic AMP can overcome the inhibitory effect of sugar and sucrose on flowering in duckweeds. The inhibitory effect of ammonium ions and water-treatment on flowering of *L. gibba* could also be negated by cyclic AMP (12). Although no one has yet demonstrated the existence of cyclic AMP and adenylate cyclase in the Lemnaceae unequivocally, Kato *et al.* (5, 6) have reported the presence of cyclic AMP-dependent protein kinases in *L. paucicostata* which makes it likely that there is a system analogous to that in animals. We have unpublished observations indicating a strong inductive effect of cyclic AMP on flowering in *L. paucicostata* 6746 (details to be published elsewhere). Thus, if the existence of cyclic AMP and adenylate cyclase in plants is taken as real, a role of catecholamines in metabolism of plant cells will become not only understandable, but also very likely.

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