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Short-day flowering of *Lemna gibba* G3 induced by salicylic acid

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Salicylic acid, probably as a chelating agent of the EDTA-salicylaldoxime type, can eliminate the light requirement during the inductive phase of *Lemna gibba* G3, and thus is able to induce short-day flowering of this long-day plant.

Cleland and Ajami (*1*) reported that salicylic acid is capable of inducing short-day flowering of the long-day plant *Lemna gibba* G3. The primary effect of the acid seems to be on flower initiation rather than flower development, since in the presence of salicylic acid few flowers were produced when the duckweed was exposed to 11 cycles of a short-day schedule ($[9(15)]_{11}$) and many with 8 cycles of the same short-day schedule followed by 3 cycles of a long-day schedule ($[9(15)]_8\,[24(0)]_3$). If the medium contained no salicylic acid, neither schedule induced any flower primordia (*2*).

We recently found that irradiation of both initial and terminal 1-hr portions of the subjective day fraction of a day, designated respectively the L1- and L2-phases, must be the necessary and sufficient condition to serve as a long day for *Lemna gibba* G3 (*9*). Thus, a day with its L1- and/or L2-phase darkened functions as a short day even if its remaining portions are all irradiated. Note that the time interval of these two light-sensitive phases is equal to the critical daylength for the duckweed, i.e., 11.5 hr (cf. *9*). Although the L1-phase is involved in the main photoperiod of either natural long- or natural short-day regime, the L2-phase is illuminated only under the long-day conditions. Therefore, the L2-phase should closely be identified with the inductive phase, the illumination of which is determinative for floral induction (*9*). If so, salicylic acid must remove the light requirement during the inductive phase, in order for the acid to render a short day physiologically equivalent to a long day, as was demonstrated by Cleland and Ajami (*1*). This assumption was proved valid as reported below.

Materials and methods

All experiments were carried out with aseptic cultures of *L. gibba* G3 in M-medium supplemented with 1% sucrose at 26±1°C. White fluorescent light of

Abbreviations: $[p(q)]_n$, n cycles of a photoperiodic schedule consisting of p hr light followed by q hr dark; min-[p(q)], minimum number of [p(q)]-cycles needed for flower induction. In this paper, min-[p(q)] is expressed in hr instead of cycle number: n cycles = n × 24 hr.

2600 lux (at plant level) was given during the photoperiods. Culture schedules are detailed in figure legends. Solutions of reagents to be tested were sterilized by membrane filtration before use. At the end of the experimental culture period, numbers of fronds and flowers (see Ref. *11* for definition of frond and flower) were counted under a dissecting microscope (×20). Mean values of 4 to 8 flasks are shown in the text. See Oota (*4*, *9*) for further explanation of experimental procedures.

Results and discussion

Fig. 1 shows the effects of salicylic acid at various concentrations on frond and flower production of *L. gibba* G3 in continuous light. Although the data are not detailed here, the duckweed responded essentially similarly to salicylic acid under the [16(8)]-regime or even short-day ([9(15)]) condition. That is, regardless of the photoperiodic schedules applied, optimum concentrations for frond and flower production were always at about 10^{-8} and 10^{-6} M, respectively.

Fig. 2 shows that the min-[24(0)] extension due to dark treatment of the L2-phase of the first day of the experimental culture period was completely

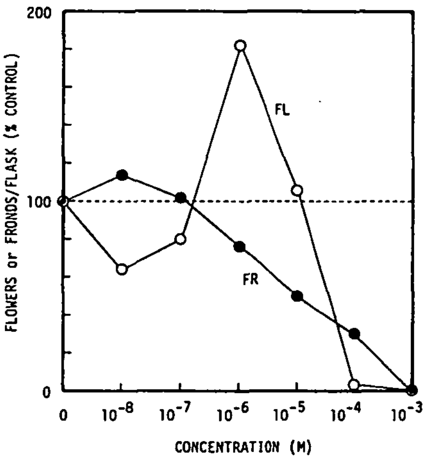

Fig. 1.

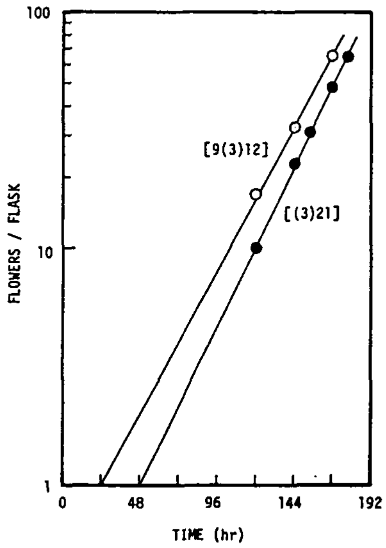

Fig. 2.

Fig. 1. *Effects of salicylic acid at various concentrations on frond and flower production.* Culture schedule: $[9(\mathit{15})]_m$*[9(15)]†$[24(0)]_7$, m=ca. 25. Schedules in italic, roman and gothic types are respectively for stock, preliminary and experimental cultures. *: Time of transfer of a 4-frond colony to fresh medium. †: Time of application of salicylic acid at designated concentrations. FR: Fronds. FL: Flowers.

Fig. 2. *Effects of salicylic acid on darkened L1- and L2-phases.* Culture schedules: $[9(\mathit{15})]_m$*[9(15)][9(3)†12]$[24(0)]_{n-1}$ for ○ and $[9(\mathit{15})]_m$*[9(15)][(3)†21]$[24(0)]_{n-1}$ for ●. †: Time of application of 10^{-6} M salicylic acid. Ordinate: Number of flowers per flask. Abscissa: Experimental culture period (n×24 hr). See legend to Fig. 1 for further explanation.

eliminated by 10^{-6} M of salicylic acid given just after the darkened L2-phase, and that the increased min-[24(0)] due to the darkened L1-phase of the same day was quite resistant to salicylic acid. Note that the min-[24(0)] is extended from 48 to 72 hr when the L1- and/or L2-phase of the first day is darkened (cf. *9*), and is decreased from 48 to 24 hr by salicylic acid (Fig. 3). That the min-[24(0)] was 48 hr in the presence of salicylic acid together with the darkened L1-phase of the first day (Fig. 2) means that here the induction reaction began to proceed at the end of the first day and reached completion in 24 hr.

These results clearly support our anticipation that the light during the inductive phase or L2-phase, but not the L1-phase, can be entirely replaced by salicylic acid. In the same experiment as in Fig. 2, however, salicylic acid was ineffective when administered at hr 24 of the experimental culture period or later (data not shown).

Salicylic acid is known to chelate a variety of metals (e.g., *10*). In previous papers, we proposed to divide chelating agents into two groups with respect to their actions on frond and flower production of *L. gibba* G3 (*3*, *7*). The dose-response pattern for salicylic acid (Fig. 1) resembles the one disclosed for the EDTA-salicylaldoxime group of chelators, in that the optimum concentration for vegetative growth is lower than that for reproductive growth (*5*, *6*). Inversely, chelators of the oxine-phenanthroline group are characterized by their higher optimum doses for vegetative growth (*3*, *5*).

It is also known that, at 26°C, either the min-[24(0)] or min-[16(8)] has a

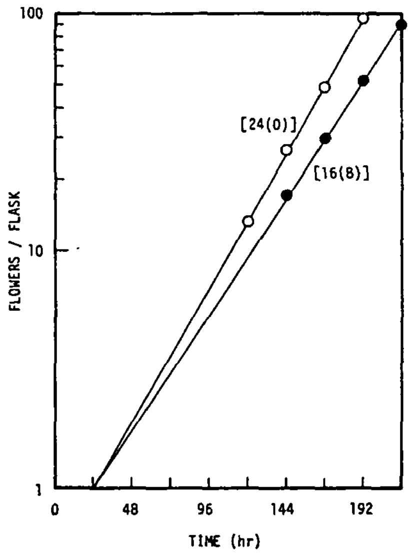

Fig. 3.

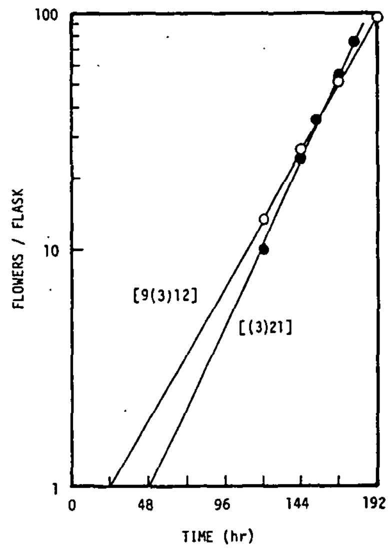

Fig. 4.

Fig. 3. *Effects of salicylic acid on min-[24(0)] and min-[16(8)].* Culture schedules: [9(*15*)]$_m$*[9(15)]†[24(0)]$_n$ for ○ and [9(*15*)]$_m$*[9(15)]†[16(8)]$_n$ for ●. See legend to Fig. 2 for further explanation.

Fig. 4. *Effects of EDTA on darkened L1- and L2-phases.* Culture schedules were the same as in Fig. 2, except that 10^{-5} M EDTA (tetrasodium salt used) was administered instead of 10^{-6} M salicylic acid. See legend to Fig. 2 for further explanation.

value of 48 and 24 hr, in the absence and presence, respectively, of the first group of chelators at flower-promoting concentrations (*6*, *7*). However, oxine and *o*-phenanthroline at flower-promoting concentrations can decrease the min-[24(0)] only by a few hours, although they decrease the min-[16(8)] by 24 hr (*7*). As shown in Fig. 3, 10^{-6} M of salicylic acid curtailed by half both min-[24(0)] and min-[16(8)] at 26°C.

That 10^{-5} M of EDTA would similarly remove the light requirement of the duckweed during the L2-phase has been suggested by an observation that in the presence of the chelator, [8(8)1(7)] represents a long-day schedule, despite the fact that the 8-hr dark period included in the schedule completely covers the L2-phase (*9*). This suggestion was verified by the experiment shown in Fig. 4. The first day of the experimental culture period with the darkened L2-phase functioned as a long day in the presence of EDTA at 10^{-5} M, so that the min-[24(0)] value was 24 hr as in the EDTA culture without inserted darkness (*6*). Fig. 4 also indicates that EDTA was unable to reverse the min-[24(0)]-extending action of the darkened L1-phase, as was the case for salicylic acid. Although salicylaldoxime has not yet been examined, action similar to EDTA or salicylic acid is expected. Oxine at 10^{-7} M was unable to reverse the extended min-[24(0)], i.e., 72 hr, due to the darkened L1- or L2-phase of the first day of the experimental culture period (Fig. 5). The concentrations used of EDTA and oxine in these experiments were those optimal for floral promotion in continuous light (*3*, *6*).

It is as yet obscure how salicylic acid or EDTA can substitute for the illuminated L2- or inductive phase. Some metal-sensitive membrane process(es) could be involved in the inductive phase, and the metal inhibition could be overcome by light as well as the chelators in question. Analyses of the photoreceptor system involved are urgently needed. Our preliminary experiments deny the participation of phytochrome.

Another question remains to be answered. Although a short day is considered

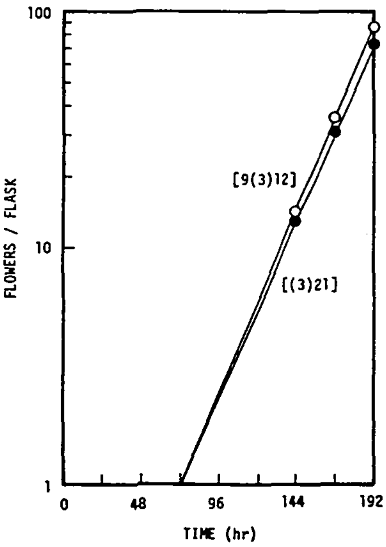

Fig. 5. *Effects of oxine on darkened L1- and L2-phases.* Culture schedules were the same as in Fig. 2, except that 10^{-7} M oxine was administered instead of 10^{-6} M salicylic acid. See legend to Fig. 2 for further explanation.

to function as a long day in the presence of EDTA, actual flower production of duckweed grown in an EDTA-containing M medium under short-day conditions has been reported to occur only when the plant is subjected to a marginal daylength (Fig. 3 in Ref. *1*). In a salicylic acid-containing M medium, *L. gibba* G3 is able to produce flowers even under strictly short-day conditions. What then is the difference in action between EDTA and salicylic acid? Is it only of quantitative or of qualitative nature?

That cyclic AMP at 10^{-5} M can eliminate the light requirement during the L1-phase, but not that during the L2-phase, has briefly been reported (*8*). Therefore, a combination of cyclic AMP with salicylic acid or EDTA provides the means of inducing flowering of *L. gibba* G3 in complete darkness. This will be detailed elsewhere.

Our tentative conclusion is that salicylic acid as a member of the EDTA-salicylaldoxime group of chelating agents provokes the short-day flowering of *L. gibba* G3 by replacing the light required during the inductive phase.

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