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Floral Induction in a Photoperiodically Insensitive Duckweed, *Lemna paucicostata* LP6[1]

ROLE OF GLUTAMATE, ASPARTATE, AND OTHER AMINO ACIDS AND AMIDES

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ABSTRACT

The effects of 20 amino acids and two amides were studied on the flowering of a photoperiodically insensitive duckweed, *Lemna paucicostata* LP6. Alanine, asparagine, aspartate, cystine, glutamate, glutamine, glycine, lysine, methionine, proline, serine, and threonine induced flowering under a photoperiodic regime of 16 hours light and 8 hours darkness. Among these, glutamate and aspartate were found to be the most effective for flower induction. These acids could initiate flowering even at 5×10^{-7} molar level, though maximal flowering (about 80%) was obtained at 10^{-5} molar. Change in the photoperiodic schedule or the pH of the nutrient medium did not influence glutamate- or aspartate-induced flowering. The low concentrations at which glutamate and aspartate are effective suggests that they may have a regulatory role rather than simply acting as metabolites.

It is well established that both qualitative and quantitative changes in amino acid and protein content occur upon photoinduction of the flowering process. There are several studies of protein synthesis, some using inhibitors, where changes have been correlated with flowering (1). Although the role of amino acids in flowering of higher plants has not been investigated very extensively, there is some evidence for a regulatory role in the family Lemnaceae (6).

Nakashima (14) reported that high concentrations of several amino acids inhibit flowering in long-day *Lemna gibba* G3, but the inhibitory effect of arginine could be partially relieved by lysine (which was itself inhibitory). A high endogenous ratio of arginine to lysine was correlated with inhibition of flowering (15). Maeng and Khudairi (13) found an increase in the endogenous level of serine in *L. gibba* G3 during early phase of the photoinductive cycle, followed by a later decline in the total amino acid pool, occurring before the floral primordia were visible. Amino acids were also shown to influence flowering of the short-day duckweed, *Lemna paucicostata* 6746. Posner (16, 17) reported that the inhibitory effect of sugars on flowering of strain 6746 in a low-strength medium was reversed by several amino acids including aspartate and glutamate. Subsequently, reports of a more direct effect of amino acids on flowering in *L. paucicostata* 6746 have come from several different laboratories (7–9, 13, 19, 20).

L. paucicostata, strain LP6, is a photoperiodically insensitive duckweed which does not flower under any of the photoperiods tried when grown in the basal Bonner-Devirian (2) medium supplemented with 10^{-4} M EDTA (9, 12). Whether this strain lacks the photoperiodic sensitivity due to aberration at the genetic level or if there is some block in its metabolic pathway leading to flowering response remains to be investigated. However, it has been possible to induce flowering in this strain by chemicals such as ethylenediamine-di-*o*-hydroxyphenylacetic acid, 8-hydroxyquinoline, salicylic acid, and acetylsalicylic acid (10–12). In the present investigation, we demonstrate the effect of certain amino acids and amides on induction of flowering in *L. paucicostata* LP6. To our knowledge, this is the first report where such a profuse flowering could be obtained with amino acids.

MATERIALS AND METHODS

Aseptic cultures of *Lemna paucicostata* LP6 were regularly maintained in a medium containing major salts of Bonner-Devirian (2) and minor salts of Heller (5), supplemented with 1% (w/v) sucrose and 10^{-4} M EDTA. The pH was adjusted to 5.5 before autoclaving the nutrient medium at 1.08 kg/cm^2 for 15 min. The stock and experimental cultures were kept under a photoperiodic schedule of 16 h light and 8 h darkness. Light was provided from a mixed bank of fluorescent tubes (Philips TL 65-80 W/54 RS, 6800°K) and incandescent bulbs (Philips Argenta, 100 W). Fluence rate in the spectral range between 400 to 750 nm, measured with the help of the LiCor model 1800 portable spectroradiometer, varied from 10.0 to 10.5 W m^{-2}. The temperature was maintained at 26 ± 2°C during the light period and at 22 ± 1°C in darkness.

For experimental cultures, single 3-frond colony was inoculated in each 250 ml Erlenmeyer flask containing 100 ml nutrient medium. All amino acids and amides used for experiments were L-form (obtained from Sigma) and were filter-sterilized and supplied to 2-d-old cultures under aseptic conditions. Our earlier work on the kinetics of flowering in strain LP6, with respect to the effects of chemicals such as ethylenediamine-di-*o*-hydroxyphenylacetic acid, has shown that maximal flowering is usually obtained between 7 to 9 d after chemical-treatment (9, 12). Therefore, in the present investigation also multiplication rate and flowering were estimated 7 d after addition of the respective adjuvants. MR[4] was calculated according to Clark (3) as MR = ($\log_{10} Fd - \log_{10} Fo$) 1000/d, where F_o is the original frond number and Fd is the frond number on day d. Flowering per-

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[4] Abbreviation: MR, multiplication rate.

centage was calculated by dividing the number of flowering fronds by total number of fronds and multiplying by 100. All flowering stages were taken into consideration. For each replicate culture, percentage flowering was calculated from about 60 to 100 plants. The values so derived from three replicate cultures were averaged. Each experiment was repeated at least once and usually several times, but data of only single representative experiment are presented here.

RESULTS

Effect of Glutamate and Aspartate. The plants were precultured for 2 d before the addition of glutamate or aspartate. Both these amino acids caused the fronds to appear healthier and greener. Very high concentrations of both glutamate and aspartate (10^{-4} M) inhibited growth slightly and a decrease in MR was observed (Figs. 1 and 2). However, both glutamate and aspartate had a strong effect on induction of flowering: these amino acids could initiate flowering at a level as low as 5×10^{-7} M. Flowering increased remarkably (about 75–80%) as the concentration of glutamate and aspartate increased (up to 10^{-5} M), although thereafter, it declined moderately (Figs. 1 and 2).

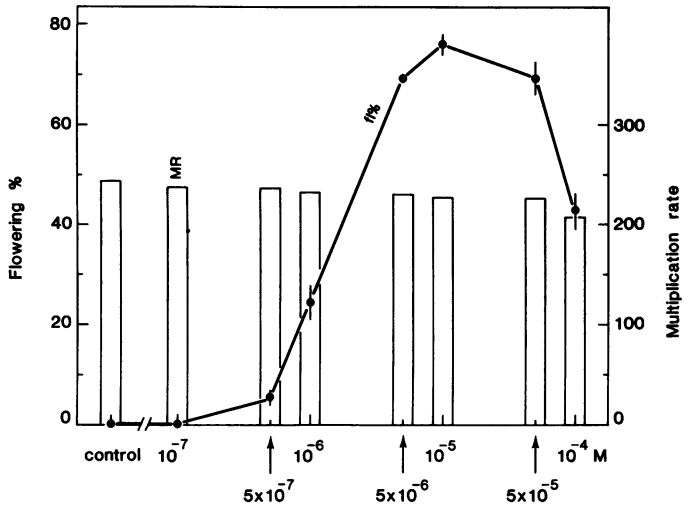

FIG. 1. Effect of glutamate on MR and flowering in *L. paucicostata* LP6, under a photoperiodic regime of 16 h light and 8 h darkness. Glutamate was filter-sterilized and supplied to 2-d-old cultures in Bonner-Devirian medium, and plants analyzed for flowering 7 d later. Vertical columns, MR; curve, flowering percentage (fl %). Each value plotted is the mean of three replicates, and vertical lines represent ± SE.

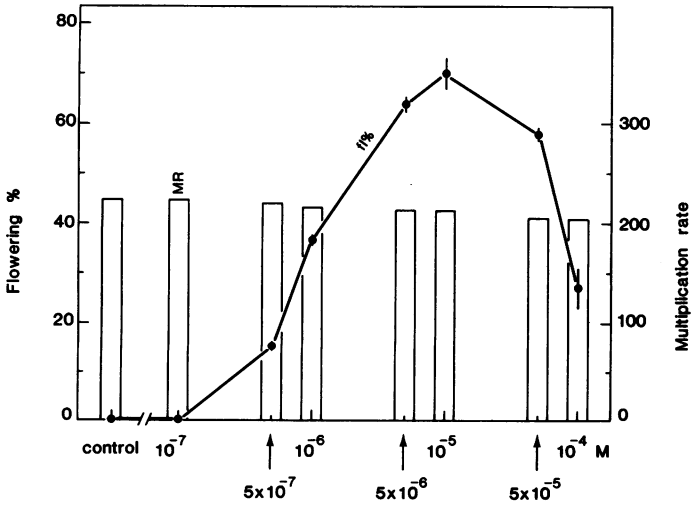

FIG. 2. Effect of aspartate on MR and flowering in *L. paucicostata* LP6, under a photoperiodic regime of 16 h light and 8 h darkness. Experiment was conducted under the conditions identical to those described in legend to Figure 1.

Effect of Other Amino Acids and Amides. Besides glutamate and aspartate, several other amino acids and two amides could also induce flowering in *L. paucicostata* LP6 under identical culture conditions. For floral initiation, alanine, methionine, and serine were effective at 10^{-6} M, whereas cystine, like glutamate and aspartate, elicited appreciable flowering even at 5×10^{-7} M level. With these amino acids maximum flowering (35–50%) was obtained only at 10^{-5} M (Table I). Though these amino acids did not show any significant effect on MR, however, a reduction in frond size was noticed at higher concentrations (*e.g.*, 10^{-4} M).

The results of the experiments conducted with rest of the amino acids have been summarized in Table II. Asparagine, glutamine, glycine, lysine, proline, and threonine were effective but higher concentrations (*e.g.*, 5×10^{-5} M) were required not only to obtain maximal flowering but even to elicit a response. Asparagine and glutamine were required at 10^{-4} M level for maximal response, whereas for glycine, lysine, proline, and threonine, such response was obtained at 5×10^{-5} M level (Table II).

Other amino acids do not appear to have any role in flower initiation (Table II). However, arginine, glutamine, glycine, phenylalanine, tryptophan, and tyrosine did not significantly affect the MR but fronds appeared greener as compared to the controls. On the other hand, cysteine, histidine, hydroxyproline, isoleucine, leucine, lysine, and valine inhibited growth (Table II).

The data presented in this section of results are from the experiments conducted under a photoperiodic schedule of 16 h light and 8 h darkness. Similar results were obtained, with regard to

Table I. *Effects of Alanine, Cystine, Methionine, and Serine on MR and Flowering in L. paucicostata LP6, under a Photoperiodic Regime of 16 h Light and 8 h Darkness*

For experimental details, see legend to Figure 1.

	Molarity	% Flowering ± SE	MR ± SE
Alanine	0	0	227.6 ± 1.1
	10^{-6}	2.6 ± 0.3	227.1 ± 1.9
	10^{-5}	46.8 ± 3.1	227.4 ± 3.3
	5×10^{-5}	54.5 ± 3.7	221.9 ± 2.6
	10^{-4}	54.9 ± 0.6	215.2 ± 1.8
Cystine	0	0	227.5 ± 0.8
	5×10^{-7}	8.9 ± 1.3	227.3 ± 1.5
	10^{-6}	23.9 ± 1.5	224.2 ± 2.0
	5×10^{-6}	26.6 ± 1.2	224.4 ± 0.9
	10^{-5}	35.0 ± 1.4	224.6 ± 1.6
	5×10^{-5}	16.2 ± 3.0	223.2 ± 1.2
	10^{-4}	5.5 ± 0.4	202.7 ± 0.9
Methionine	0	0	220.9 ± 1.4
	10^{-6}	8.6 ± 0.9	222.8 ± 0.5
	10^{-5}	31.2 ± 3.8	203.0 ± 1.3
	5×10^{-5}	17.9 ± 1.0	182.2 ± 2.5
	10^{-4}	7.6 ± 0.4	171.4 ± 2.7
Serine	0	0	216.0 ± 1.5
	10^{-7}	0	218.0 ± 1.2
	5×10^{-7}	0	211.1 ± 2.7
	10^{-6}	12.0 ± 2.0	210.3 ± 1.0
	10^{-5}	40.6 ± 2.1	204.9 ± 4.2
	5×10^{-5}	16.1 ± 2.7	202.3 ± 1.2
	10^{-4}	6.9 ± 0.8	190.1 ± 1.4

Table II. *Effects of Some Amino Acids and Amides on MR and Flowering in L. paucicostata LP6, under a Photoperiodic Schedule of 16 h Light and 8 h Darkness*

For experimental details, see legend to Figure 1. Figures in parentheses represent the MR.

	Control	10^{-6} M	10^{-5} M	5×10^{-5} M	10^{-4} M
Arginine	0 (231)	0 (233)	0 (230)	0 (231)	0 (210)
Asparagine	0 (211)	0 (206)	21.2 ± 2.1 (205)	32.4 ± 0.8 (205)	53.2 ± 2.3 (194)
Cysteine	0 (203)	0 (204)	0 (205)	0 (173)	0 (133)
Glutamine	0 (242)	0 (249)	0 (246)	11.1 ± 3.7 (247)	18.7 ± 1.0 (246)
Glycine	0 (237)	0 (246)	2.5 ± 0.7 (248)	37.4 ± 4.5 (238)	12.6 ± 1.2 (237)
Histidine	0 (226)	0 (234)	0 (213)	0 (163)	0 (135)
Hydroxy-proline	0 (220)	0 (218)	0 (187)	0 (85)	0 (78)
Isoleucine	0 (219)	0 (220)	0 (223)	0 (197)	0 (183)
Leucine	0 (232)	0 (234)	0 (196)	0 (102)	0 (93)
Lysine	0 (218)	0 (214)	10.7 ± 1.0 (210)	18.6 ± 1.8 (216)	9.3 ± 1.3 (156)
Phenyl-alanine	0 (220)	0 (215)	0 (230)	0 (230)	0 (224)
Proline	0 (220)	0 (226)	0 (223)	62.3 ± 0.8 (222)	24.4 ± 0.9 (219)
Threonine	0 (221)	0 (228)	0 (224)	40.8 ± 2.3 (206)	13.2 ± 1.2 (196)
Tryptophan	0 (233)	0 (228)	0 (224)		(209)
Tyrosine	0 (220)	0 (219)	0 (217)	0 (213)	0 (183)
Valine	0 (222)	0 (232)	0 (226)	0 (85)	0 (84)

both MR and flowering, when plants were exposed to a daily schedule of 8 h light and 16 h darkness.

Influence of Photoperiod on Glutamate- and Aspartate-Induced Flowering. To confirm whether glutamate- and aspartate-induced flowering is independent of photoperiod, three different photoperiodic schedules were used. There was no significant effect of any of the photoperiodic schedules tried on flowering induced either by aspartate or glutamate (Table III). However, in the set of plants treated with glutamate at 10^{-6} M (a suboptimal level) kept in continuous light, no flowering was obtained whereas under short days, some flowering occurred. This may imply that some degree of photoperiodic sensitivity exists, but it is difficult to draw conclusion from such limited evidence.

Influence of pH on Glutamate- and Aspartate-Induced Flowering. Earlier work on the Lemnaceae has shown that pH of the nutrient medium has an effect on flowering. Therefore, it was thought worthwhile to study the effect of pH on flowering induced by the most effective amino acids, glutamate and aspartate. The pH of the nutrient medium was kept at 4.0, 5.5, or 6.5 (before autoclaving) and both glutamate and aspartate tested at 10^{-6} and 10^{-5} M levels. The results of this experiment are summarized in Table IV. At pH 4.0, both flowering and MR were slightly suppressed in glutamate- and aspartate-treated plants, whereas at higher pH, *i.e.* 5.5 and 6.5, flowering percentage and MR were not affected significantly. It appears that glutamate- and aspartate-induced flowering is thus not affected by pH. The MR of the plants kept as controls at pH 4.0 was not affected significantly but frond size was reduced considerably. Therefore, it is likely that both glutamate and aspartate, which are acidic amino acids may further retard the growth (at higher concentrations) and hence are detrimental for MR at low pH of 4.0.

DISCUSSION

The results show a strong inductive effect of certain amino acids on flowering in *L. paucicostata* LP6. On the basis of concentrations required for initiation of flowering and the maximal response, aspartate and glutamate and cystine form one group (group I). Within the group, the first two are the most effective amino acids—both could induce flowering at 5×10^{-7} M, and at the optimal level, nearly 75 to 80% flowering was obtained (Figs. 1 and 2). Although the threshold value for cystine to initiate flowering was also 5×10^{-7} M, maximum flowering could go up to only 40% (at 10^{-5} M level, Table I).

The remaining amino acids can be subdivided into two further groups (groups II and III). Alanine, methionine, and serine constitute group II—the threshold was at 10^{-6} M and the maximal flowering was obtained between 10^{-5} and 5×10^{-5} M (Table I). On the other hand, asparagine, glutamine, glycine, lysine, proline, and threonine (group III) were clearly less effective. For this group, significantly higher concentrations (10^{-5} and 5×10^{-5} M) were required even for initiation of flowering (Table II)

Table III. *Effect of Daylength on Glutamate- and Aspartate-Induced Flowering (fl) in L. paucicostata LP6*

Experimental cultures were initiated under a photoperiodic schedule of 16 h light and 8 h darkness. Two-d-old cultures were supplied with the desired adjuvant, and 24 h later, separate sets of plants were subjected to respective photoperiods. The MR was calculated and plants analyzed for flowering 7 d later. Each figure is mean value of 3 replicates ± SE.

Photoperiod	Molarity	Glutamate		Aspartate	
		% fl	*MR*	*%fl*	*MR*
CL	0	0	240.6 ± 0.8	0	238.1 ± 2.2
	10^{-6}	0	239.2 ± 1.5	35.6 ± 1.8	239.3 ± 1.8
	10^{-5}	71.4 ± 2.7	232.1 ± 1.3	69.0 ± 1.4	234.8 ± 0.6
16L:8D	0	0	227.6 ± 1.1	0	229.5 ± 0.9
	10^{-6}	17.5 ± 1.2	225.2 ± 1.1	40.7 ± 1.0	222.7 ± 1.0
	10^{-5}	72.6 ± 1.5	219.2 ± 2.0	72.6 ± 0.6	216.4 ± 1.1
8L:16D	0	0	220.3 ± 2.2	0	219.9 ± 1.9
	10^{-6}	21.2 ± 5.3	218.8 ± 1.4	34.5 ± 0.4	217.9 ± 1.8
	10^{-5}	64.5 ± 0.9	206.1 ± 2.6	66.5 ± 3.2	204.7 ± 1.3

Table IV. *Influence of pH on Glutamate- and Aspartate-Induced Flowering (fl) in L. paucicostata LP6*
For details, see legend to Figure 1.

pH	Molarity	Glutamate		Aspartate	
		% fl	*MR*	*% fl*	*MR*
4.0	0	0	230.5 ± 1.0	0	230.5 ± 1.0
	10^{-6}	26.0 ± 1.8	223.4 ± 0.3	39.1 ± 0.8	229.9 ± 1.1
	10^{-5}	53.4 ± 2.6	220.5 ± 1.2	19.6 ± 5.1	197.2 ± 3.4
5.5	0	0	239.2 ± 0.8	0	234.2 ± 0.8
	10^{-6}	22.5 ± 0.6	232.2 ± 0.3	54.5 ± 1.6	232.8 ± 0.6
	10^{-5}	79.3 ± 0.8	220.8 ± 2.1	78.1 ± 5.8	223.5 ± 1.3
6.5	0	0	232.5 ± 1.0	0	232.5 ± 1.0
	10^{-6}	18.6 ± 1.3	230.3 ± 2.1	51.0 ± 1.2	232.1 ± 1.0
	10^{-5}	73.0 ± 0.9	223.5 ± 1.7	77.9 ± 3.7	222.1 ± 1.2

and, to obtain maximal effects, in certain cases one required as high a concentration as 10^{-4} M (*e.g.* asparagine).

So far as we are aware, this is the first evidence where such a strong induction of flowering by amino acids, especially those in group I, has been achieved in any higher plant. Appreciable flowering was obtained even under continuous light as is evident from the experiments conducted with glutamate and aspartate (Table III). By and large, these amino acids did not show any significant effect on MR, at least at concentrations around the physiological range ($<10^{-5}$ M), though in some cases growth was retarded at higher concentrations, *e.g.* 5×10^{-5} and 10^{-4} M (see Table I).

The earlier work on the role of amino acids in flowering has been mainly confined to the SD *L. paucicostata,* strain 6746. Maeng and Khudairi (13) found a flower-promoting action of serine, threonine, and tryptophan and a flower-inhibitory effect of cysteine, for flowering of this strain. On the basis of changes in endogenous levels of amino acids during light and dark phase of photoinductive cycles, Khudairi and Hemberg (7) proposed that serine may in fact act as a precursor of flower initiating substance. Tanaka and Takimoto (19) demonstrated that certain other amino acids including aspartate, glutamate, alanine, and glycine also enhanced flowering apart from serine. These amino acids not only promoted flowering under inductive SD but also shortened the critical dark period requirement for flowering by 1 to 2 h. The fact that certain amino acids may have a role in the flower inducing dark reaction(s) was further confirmed when a red light exposure during the inductive dark period failed to inhibit flowering in the presence of the "active" amino acids.

The situation with strain LP6 of *L. paucicostata,* employed in the present investigation, is different from the SD strain 6746. Since *L. paucicostata* LP6 does not flower under any photoperiodic schedule when grown in the Bonner-Devirian medium, it is likely that either its photoperiodic sensitivity (or a day neutral behavior) is arrested due to a metabolic block or some component of the nutrient medium is inhibitory for flowering. Though the latter possibility cannot be ruled out completely, earlier attempts in our laboratory to initiate flowering in this strain, when grown in various standard media (of different ionic strengths) like Bonner-Devirian, Hoagland, Hutner, and Pirson-Seidel, along with various combinations of EDTA and sucrose, and under different photoperiods, have not been successful (9). It is therefore possible that amino acids have a direct effect on the flowering process rather than merely removing the inhibition caused due to one or more of the media constituents. The low concentrations (5×10^{-7} and 10^{-6} M) at which glutamate and aspartate cause floral induction in strain LP6 also support this contention. Whether the effect of amino acids (like glutamate and aspartate) is on the primary events of floral induction or whether they influence the sequence of events later in the transduction pathway cannot be concluded from the data presently available.

It is worthwhile to mention here that glutamate has been shown to play an important role in the sexual morphogenesis in the alga *Volvox* (18). Also, both glutamate and aspartate act as excitatory transmitters in the mammalian central nervous system (4). Whether a system analogous to that in animals exists in plants too, and glutamate and aspartate exert their effect on flowering in *Lemna* through their role as signal transducers, is an interesting possibility and thus deserves further investigation.

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