Korean Jour. Botany
Vol. 19, No. 1, 14-18 (1976)

Studies on Inhibition Factors and the Role of Phytochrome in the Floral Induction in Short-day Plants

Maeng, Jueson
(Department of Biology, Sogang University, Seoul)

短日植物의 開花誘導를 調節하는 開花抑制要素의 糾明과 Phytochrome의 役割에 關한 硏究

孟 柱 善
(西江大學校 理工大學 生物學科)

ABSTRACT

Inhibition of flowering in *Lemna perpusilla* 6746 by 30 mM sucrose was reversed by the addition of acetylcholine ($>10^{-4}$M) supplemented with 10^{-4}M ascorbic acid to 1/10-strength Hutner's growth medium. The reversible effect of acetylcholine was found to be greater at early stages of flowering than in the later period.

Promotive effects of both acetylcholine (10^{-3}M) and eserine (10^{-5}M) on flowering in the short-day plant under various photoperiodic conditions were studied. It was indicated that the application decreased length of the critical dark period for the floral induction, and it was also shown that the endogenous status of acetylcholine was involved in the floral response which had a correlation with phytochrome. Interruption of inductive dark periods by red irradiation (1 min) immediately followed by far-red light (1 min) completely inhibited flowering, while the addition of acetylcholine and eserine to the medium under the same condition slightly promoted flowering, indicating possible involvement of phytochrome system in acetylcholine activity for photoperiodic sensitivity of floral response in *Lemna perpusilla* 6746.

INTRODUCTION

Photoperiodically sensitive floral response of a short-day plant, *Lemna perpusilla* 6746 has been found to be regulated by environmental factors such as sugars, chelating agents, ammonium and nitrate ions present in the growth medium. Sucrose inhibits the floral response of *Lemna* while it promotes the vegetative propagation. The inhibitory effect of sucrose depends on the composition of growth medium (Esashi and Oda, 1964; Posner, 1967, 1969; Hillman and Posner, 1971).

Acetylcholine present in all organs of mung bean (*Phaseolus aureus*) seedlings was found to function in roots as it did in animal systems, and it also acted as a local hormone which regulated several phytochrome-mediated phenomena (Jaffe, 1970). In *Lemna* system, there were several reports showing acetylcholine seemed to regulate the flowering and diurnal temperature sensitivity (Kandeler, 1972;

Oota and Hoshino, 1974).

Since the inhibition of flowering in *Lemna perpusilla* 6746 by sucrose is extremely variable, it is assumed that unidentified factors in the inhibition remain uncontrolled in the previous experiments carried out in many laboratories.

The following report describes attempts to reveal the sucrose inhibition of flowering related to acetylcholine function, and also to relate the acetylcholine effect to phytochrome system in *Lemna perpusilla* 6746.

MATERIALS AND METHODS

Lemna perpusilla Torr. 6746, a short-day plant, was grown aseptically in 125 ml Erlenmeyer flasks containing 50 ml of Hutner's medium of various dilutions. The composition of the medium of full strength expressed in mg/*l* of final solution is: $Ca(NO_3)_2 \cdot 4H_2O$, 354; K_2HPO_4, 400; KOH, 200; EDTA, 500; NH_4NO_3, 200; $MgSO_4 \cdot 7H_2O$, 500; $FeSO_4 \cdot 7H_2O$, 24.9; $MnCl_2 \cdot 4H_2O$, 17.9; $ZnSO_4 \cdot 7H_2O$, 65.9; $CuSO_4 \cdot 5H_2O$, 3.95; $Na_2MoO_4 \cdot 2H_2O$, 25.2; and H_3BO_3, 14.2. The *p*H of the culture solution was adjusted to 6.3 before autoclaving.

Vegetative stock cultures were kept under continuous light in half-strength Hutner's medium supplemented with 30 mM sucrose. Experimental cultures were started with two 3-frond colonies per culture flask. All cultures were grown in growth chambers (Freas 815, Precision Scientific Co.) at 26±1°C during the light period and 24±1°C in the dark. The light source in the growth chambers consisted of two 15-watt cool-white fluorescent lamps(General Electric, Standard). The light intensity, as measured with a photometer (LI-COR, 185 with LI-210S photometric sensor, LAMBDA Inst. Corp.), was kept at 7000 lux at plant level.

The total frond number of a culture (#TF) was determined by counting all fronds which visibly projected beyond the margin of their mother fronds. Flowering was evaluated by the flowering percentage (FL%) as described in previous reports (Maeng and Khudairi, 1973; Khudairi and Maeng, 1973).

The red and far-red light treatments were given in a specially designed irradiation cabinet in a dark room. The red light source consisted of two 10-watt cool-white fluorescent lamps above a 3 mm thick piece of Rohm and Haas #2423 red plexiglass which transmitted less than 1% below 580 nm and 85~90% from 650~700 nm. As the far-red light source, the light from four 60-watt incandescent bulbs was passed through 3 mm thick Rohm and Haas FRF far-red plexiglass which transmitted less than 1% below 700 nm. To minimize possible temperature increases when the far-red light was on, a fan was turned on to blow the hot air out of the light source compartment above the far-red filter. The red and far-red light energy, measured with LI-190S Quantum Sensor (LAMBDA Inst. Corp.), were 2.3 and 0.75 microeinsteins·m^{-2}· sec^{-1} respectively.

Sucrose was added, if needed, to the culture medium before autoclaving, and acetylcholine chloride (Sigma), ascorbic acid (Sigma) and eserine sulfate (Sigma) were sterilized by membrane filtration before adding to the medium. Acetylcholine treatments were supplemented with 10^{-4}M ascorbic acid in all experiments. All experiments were repeated twice and a treatment had 3 or 4 replicas.

RESULTS AND DISCUSSION

The inhibitory effect of sucrose on flowering of *Lemna* is extremely variable depending on intensity of light, growth conditions of stock cultures, size of culture vessel, concentration of growth medium, agitation of vessels, and carbon dioxide content (Esashi and Oda, 1964; Posner, 1967; Hillman and Posner, 1971).

In dilute Hutner's medium (1/10-strength), there was complete inhibition of flowering by sucrose at concentration of 15 mM or higher, while the sucrose effect was diminished as the concentration of Hutner's medium increased toward its full strength (Posner, 1967). As shown in Figure 1, sucrose (30mM) in 1/10-strength Hutner's medium inhibited flowering by 51.3%–35.6% in 4–7 days. It was observed that the inhibitory effect during early culture period was greater than that at later period, which led to an assumption that unidenti-

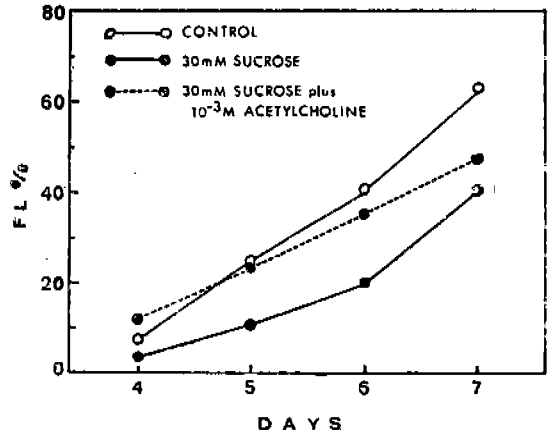

Fig. 1. Effect of acetylcholine supplemented with 10^{-4}M ascorbic acid reversing the sucrose inhibition of flowering in *Lemna perpusilla* 6746 grown on 1/10-strength Hutner's medium under 8-hour light, 16-hour dark cycles. FL% was evaluated at the end of each dark period. Treatments were at the beginning of the first light period.

fied factors preventing the sucrose effect became gradually activated in the later stage. It could also be possible that the concentration of sucrose decreased in later period, but a preliminary experiment showed the degree of decreasing was too low in 7 days of culture to even partially diminish the sucrose effect. This was consistent with results obtained by Posner in 1967 and 1969.

Among many diverse factors reversing the sucrose inhibition, ATP and intermediates of the tricarboxylic acid cycle were reported to be effective, and it was shown that there was a correlation between carbohydrate inhibition of flowering and enhancement of glucose 6-phosphate dehydrogenase (Posner, 1971). Sucrose inhibition of flowering in a long day plant, *Lemna gibba* G3 was reversed by 3′,5′-cyclic AMP and catecholamines (Oota, 1972, 1974), while in *Lemna perpusilla* 6746, several adenine derivatives including 5′-AMP, cyclic 3′, 5′-AMP, 5′-ADP, 5′-ATP and K_2HPO_4 were reported to reverse at least partially the inhibitory effect of sucrose (Posner, 1973). So far, however, there has been no satisfactory explanation on the mechanism of sucrose action. The present report made attempts to relate the sucrose action to phytochrome system in *Lemna perpusilla*.

Various concentrations of acetylcholine supplemented with 10^{-4}M ascorbic acid were added to a dilute Hutner's medium (1/10-strength) to reveal

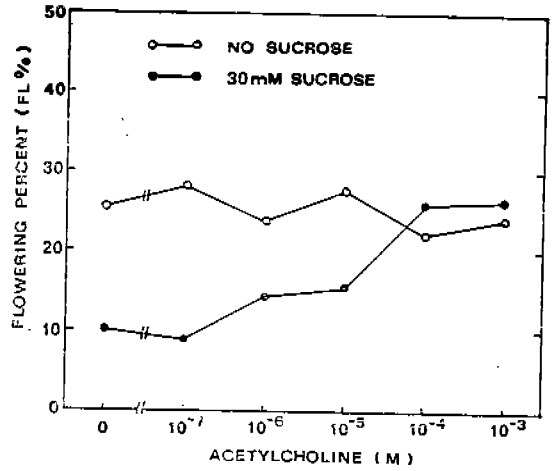

Fig. 2. Promotion of flowering in *Lemna perpusilla* 6746 by acetylcholine supplemented with 10^{-4}M ascorbic acid. The plants were grown on 1/10-strength Hutner's medium under 8-hour light, 16-hour dark cycles. FL% was evaluated on Day 5. Treatments were at the beginning of the first light period.

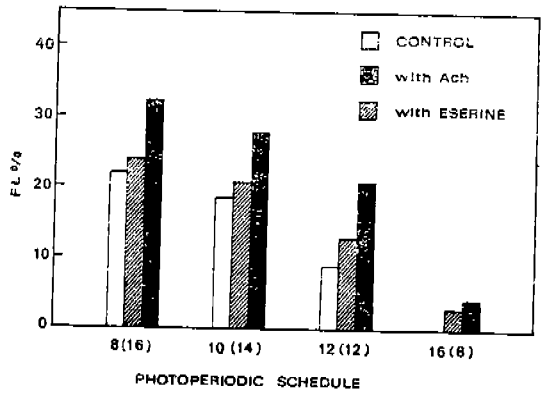

Fig. 3. Promotive effects of acetylcholine (Ach; 10^{-3}M) supplemented with 10^{-4}M ascorbic acid and of eserine (10^{-5}M) on flowering in *Lemna perpusilla* 6746 grown on 1/10-strength Hutner's medium with 30mM sucrose under various photoperiods for 6 days. Treatments were at the beginning of the first light period and FL% was evaluated at the end of the last dark period. Photoperiodic schedule : X(Y) represents X-hour light, Y-hour dark.

a relationship between sucrose and acetylcholine effects (Fig. 2). With no sucrose in the growth medium, acetylcholine (10^{-7} through 10^{-3}M) had little or no effect on flowering. In the presence of 30 mM sucrose, however, acetylcholine (>10^{-4}M) exerted its effect.

In Figure 1, acetylcholine at its concentration of 10^{-3}M could completely reverse the inhibitory effect of 30 mM sucrose up to Day 5, while its reversible action became effective only to a certain extent at later stage. It was shown that acetylcholine effect on the flowering in *Lemna perpusilla* was greatly enhanced as photoperiod approached its critical level which was 12-hour light, 12-hour dark in 24-hour cycle (Fig. 3 and Table 1). Furthermore, acetylcholine application to the medium could decrease length of the critical dark period for the plant. Thus, flowering could be induced even under 16-hour light, 8-hour dark cycles, which was a long-day condition for the plant.

To prepare an evidence that the endogenous acetylcholine level rather than the exogenously applied one was involved in this action, the effect of eserine, a specific inhibitor of acetylcholinesterase, was studied. Eserine was found to have a similar effect to that of acetylcholine.

Based on the results stated above, endogenous level of acetylcholne in the plant is assumed to be altered under different photoperiodic conditions. An alternative assumption for the results is that acetylcholine status in the plant has a correlation with factors controlling the photoperiodic sensitivity such as phytochrome system as discussed later.

Table 1. Percentages of increase in flowering in *Lemna perpusilla* 6746 by acetylcholine(Ach) or eserine under various photoperiods

Addition of:	Photoperiodic schedule			
	8(16)	10(14)	12(12)	16(8)
10^{-3}M Ach plus 10^{-4}M Ascorbic acid	147.7%	152.7%	237.5%	∞
10^{-5}M Eserine	109.6%	112.5%	145.5%	∞

See Fig. 3 for experimental conditions.

There have been a few reports showing a possible correlation between acetylcholine action and phytochrome system. Acetylcholine, a neurohumor in animal system, was found to be present in organs of mung bean seedlings and it was able to substitute for red light in reducing the formation of secondary roots by mediating changes in ion flux across cell membrane, showing an evidence that it acted as a local hormone regulating several phytochrome-mediated phenomena (Jaffe, 1970). Another finding should be mentioned: when *Lemna gibba* G3 was in contact with acetylcholine or eserine, floral response to chilling changed diurnally under continuous light (Oota and Hoshino, 1974). They surmised that exogenous acetylcholine modified the relative rates of chemical and physical component reactions involved in the floral evocation processes, resulting in the rhythmical floral response to chilling. Membrane permeability of the plant showed fluctuation similar to the one revealing endodiurnal rhythm in *Albizzia pulvinule*, which was related to phytochrome in some ways (Satter and Galston, 1971).

The data presented in Table 2 gave an evidence in favor of the idea that phytochrome system was involved in acetylcholine action. Interruption of 12-hour dark period by 1 min red light or red light immediately followed by far-red light completely diminished the inductive dark period effect for flowering. However, in the presence of acetylcholine (10^{-4}, 10^{-3}M) or eserine (10^{-6}, 10^{-5}M) in the growth medium, the flowering was slightly restored in group Ⅵ showing that the compounds overcame the inhibitory effect of red followed by far-red irradiations in the middle of the dark period. Flowering did not occurred in group Ⅴ where only red light interrupted the dark.

Since reversible effect of far-red irradiation following red light treatment was not observed, the result in group Ⅵ in Table 2 could make postulation that P_r form of phytochrome with adequate levels of acetylcholine might have initi-

Table 2. Effects of acetylcholine and eserine on the floral induction in *Lemna perpusilla* 6746 related to phytochrome system.

Group*	Treatments	FL% ±s.e.	#TF ±s.e
I	none	12.4±0.70	71.3±0.52
II	none	0	98.1±2.53
III	none	0	86.5±4.31
IV	Ach: 10^{-4}M	28.5±1.22	56.6±2.02
	10^{-3}M	26.8±1.00	60.9±5.35
	Eser: 10^{-6}M	20.5±1.83	57.3±6.51
	10^{-5}M	20.6±0.46	59.3±2.55
V	Ach: 10^{-4}M	0	88.1±2.56
	10^{-3}M	0	80.3±1.40
	Eser: 10^{-6}M	0	92.6±3.98
	10^{-5}M	0	90.8±1.22
VI	Ach: 10^{-4}M	2.5±0.53	70.7±4.12
	10^{-3}M	6.7±0.49	70.5±6.22
	Eser: 10^{-6}M	0	85.5±4.84
	10^{-5}M	2.0±0.50	84.1±5.13

Plants were grown on 1/10-strength Hutner's medium with 30mM sucrose. Photoperiodic condition: 3 cycles of 12(12) followed by 2 cycles of 24 (0). Addition of Ach and Eser (eserine) at the beginning of the first light period. FL% and #TF were measured at the end of the last cycle.
*Group I & IV : control. II & V : red light (1 min) in the middle of dark periods. III & VI : red light (1 min) followed by far-red light (1 min) in the middle of darkperiods.

ated chemical reactions fo rthef loral induction in *Lemna perpusilla* 6746.

Further studies are required to detect changes of endogenous levels of acetylcholine in *Lemna* in the process of floral induction. Investigations on effects of other related compounds such as norepinephrine, 5-hydroxytryptamine, atropin and acetylcholinesterase are also to be suggested.

This work was supported by Korean Traders Scholarship Foundation Grant in 1975.

REFERENCES

Esashi, Y. and Y. Oda. 1964. Effects of light intensity and sucrose on the flowering of *Lemna perpusilla*. *Plant & Cell Physiol.* 5: 513—516.

Hillman, W. S. and H. B. Posner. 1971. Ammonium ion and the flowering of *Lemna perpusilla*. *Plant Physiol.* 47: 586—587.

Jaffe, M. J. 1970. Evidence for the regulation of phytochrom-mediated processes in bean roots by the neurohumor, acetylcholine. *Ibid.* 46: 768—777.

Kandeler, R. 1972. Die Wirkung von Acetylcholin auf die photoperiodishe Steuerung der Blütenbildung bei Lemnaceen. *2. Pflanzenphysiol.* Bd. 67. S, 86—92.

Khudairi, A. K. and J. Maeng. 1973. Studies on the flowering mechanism in *Lemna*. II. The dark reaction of the short-day plant *Lemna perpusilla*. *Physiol. Plant.* 28: 271—277.

Maeng, J. and A. K. Khudairi. 1973. Studies on the flowering mechanism in Lemna. I. Amino acid changes during flower induction. *Ibid.* 28: 264—270.

Oota, Y. 1972. A possible mechanism for sugar inhibition of duckweed flowering. *Plant & Cell Physiol.* 13: 195—199.

_____.1974. Removal of the sugar inhibition of flowering in *Lemna gibba* G3 by catecholamines. *Ibid.* 15 : 63—68.

_____, and T. Hoshino. 1974. Diurnal changes in temperature sensitivity of *Lemna gibba* G3 induced by acetylcholine in continuous light. *Ibid.* 15: 1063—1072.

Posner, H. B. 1967. Inhibitory effect of sucrose on flowering in *Lemna perpusilla* 6746 and mutant strain 1073. *Ibid.* 8: 535—539.

_____.1969. Inhibitory effect of carbohydrate on flowering in *Lemna perpusilla*. I. Interaction of sucrose with calcium and phosphate ions. *Plant Physiol.* 44: 569—566.

_____.1971. Inhibitory effect of carbohydrate on flowering in *Lemna perpusilla*. III. Effects of respiratory intermediates, amino acids, and CO_2. glucose 6-phosphate dehydrogenase activity. *Ibid.* 48: 361—365.

_____.1973. Reversal of sucrose inhibition of *Lemna* flowering by adenine derivatives. *Plant & Cell Physiol.* 14: 1199—1200.

Satter, R. L. and A. W. Galston. 1971. Potassium flux: a common feature of *Albizzia* leaflet movement controlled by phytochrome or endogenous rhythm. *Science* 174: 518—520.

(Received February 17. 1976)