Studies on Photoperiodic Responses of Salvinia natans (I)

A Role of Carbon Dioxide in Photoperiodic Responses

By Osamu SHIBATA

Department of Biology, Faculty of Liberal Arts and Science, Shinshu University

(Received Nov. 27, 1958)

It has been found that under the limited supply of CO_2 in the light or in the dark period, the plants are less induced to flowering indicating that the CO_2-fixation is essential to the completion of photoinductive processes in green plants.

Applying CO_2-free air in the light period, Parker and Borthwick[10] reported a close relation between these two factors, CO_2-fixation in the light and photoinductive effects. Recently Bonner and Liverman[1] described that sugar added to the solution accelerated the flower formation of Xanthium plants.

It was also confirmed that CO_2-fixation was necessary to the flower formation in the dark period. Langston and Leopold[4] and others[2] have dealt with the substances produced by the CO_2-fixation from the view point of organic acid metabolism.

The present paper represents a study on the significance CO_2-fixation has in the photoinduction in the light and in the dark periods.

Material and Methods

The experiments were undertaken on a fern *Salvinia natans*, a short day plant of floating habit, cultured in 0.1% Knop's nutrient solution[7].

All plants were grown under a vegetative condition in a continuous illumination by daylight supplemented with two 100 watt incandescent lamps. At every experiment when the sixth air-leaf was completely unfolded, from seven to nine plants were used for photoinduction with five exposures of 8 hr. daylength (9 A.M.-5 P.M).

All young air-leaves which were expected to develop during the period of photoinduction were cut off. The plants thus operated were replaced under the vegetative conditions described above after receiving a given number of

photoinductive cycles.

The effect of short-day treatment was determined on the 15th day from the beginning of the experiment, and was expressed in a percentage of induced plants or in the average number of initiated sporocarps. A term "inhibition-grade" was used for comparing the inductive effects expressed by the average number of sporocarps.

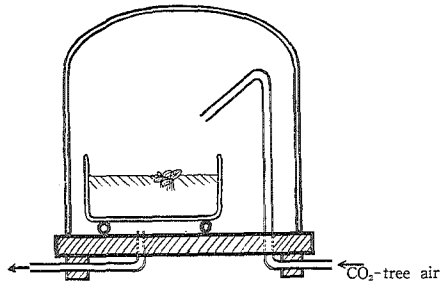

Fig 1. Apparatus used for photoinduction in CO_2-free air.

An apparatus shown in fig. 1 was applied for the examination of photoperiodic responses under the condition of CO_2-free air. The test plants were placed in a bell-glass sealed at the beginning of each testing period. The bell-glass was kept under the continuous operation of a suction pump, and the air exhausted was substituted with the same quantity of CO_2-free air obtained by passing through two soda-lime tubes. This apparatus was placed under natural daylight during the light period while in the dark period it was covered with a black paper bag during the required time. Control plants were photoinduced only in natural atmosphere.

Experimental results

1. *CO_2-free condition in the light period*

In the preliminary experiments, the CO_2-free condition was found to cause a remarkable reduction of the induction effects owing to the probable deficiency of photosynthesized carbohydrates.

For the purpose of understanding these facts further, the plants were cultured in the solution containing glucose only in the light period.

A relation between the glucose concentrations and the induction effects is shown in table 1. The inductions were enhanced with the increase of glucose

Table 1. Effects of glucose supplied to the culture solution under the CO_2-free condition in the light period of photoinduction.

glucose concentration (%)	inhibition-grade (%)	induced plants (%)
0	80.8	76.9
0.1	14.3	100
0.5	1.9	100
1.0	−2.9	100

concentration. The percentage of induced plants amounted to 100 even with 0.1% glucose and the inhibitions decreased against the increase of glucose concentration. In the case of the 1% concentration the inductions were a little more effective than in that of control. The plants cultured with supplementary 0.5% glucose in the light in natural air were induced to have the inhibition -grade of about 12%.

The growth of the plants was not influenced by the additional glucose in the solution.

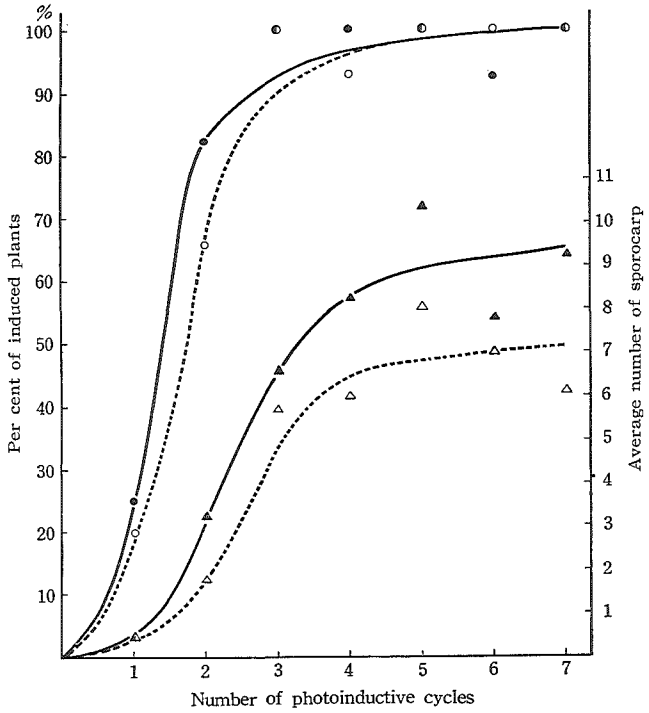

Fig 2. A relation between the induction effects and the number of photoinductive cycles.
The photoinduced plants in natural air : ▲, average number of sporocarps, ●, induced plants
The photoinduced plants in CO_2-free air : △, average number of sporocarps, ○, induced plants

2. *CO_2-free condition in the dark period*

A relation between the number of photoinductives cycles and induction effects in CO_2-free air as well as in natural condition is shown in fig. 2. In these results, excepting the first two cycles, there is no differences in the percentage of the induced plants, while some differences are found in the

average number of sporocarps but for the first cycle. Thus the CO_2-free condition was found to inhibit the sporocarp initiation or formation, and it was also indicated that the dark fixation was essential for the development of the reproductive phase of the plant.

Table 2. Effects of biotin supplied in the dark period of photoinduction.

condition	biotin	inhibition-grade (%)	induced plants (%)
natural air	−	0	100
	+	5.6	100
CO_2-free air	−	22.7	100
	+	16.3*——2.3**	100

* the maximum value. ** the minimum value.

In the next series of experiments the plants were supplied with biotin known to be an accelerator of CO_2-fixation[9]. The concentration of biotin used was adjusted to be 10 γ per ml. of the culture solution. The test plants were transferred to this solution only in the dark period, and in the light period they were furnished with the solution containing no biotin.

The inhibition in the plants supplied with biotin was small in contrast with those not supplied with it. When biotin was supplied in natural air a little larger inhibition was to be observed.

The effect of biotin as a growth factor was verified.

Discussion

The results reported here with CO_2-free air during the light period accord with those observed by Parker and Borthwick[10]. Furthermore, the data obtained by supplying glucose under this condition may give an immediate support to their suggestion that the carbohydrates produced by the CO_2-fixation in the light are responsible for the photoperiodic induction, as the induction effects were increased with the increase of glucose concentration. So that, the inhibition that should otherwise have been made under the CO_2-free condition in the light period was obscured by glucose added to the culture solution. It indicates that photosynthetic substances have some important roles in the light process in the photoperiodic reaction. This interpretation can be applied to all the other experimental results[8,11].

As for the results obtained in the dark period, CO_2-fixation, as has been found by other workers[2,4], was observed to be essential also for the development of the reproductive phase of *S. natans*.

Under CO_2-free condition during the dark period, some sporocarps have differentiated though more suppressed in further development. This can be

interpreted by supposing that, as it was almost impossible to remove all of the respiratory CO_2 from the leaf tissues, the CO_2 produced by respiration might have been fixed in the leaf. In view of the organic acid metabolism, the obscurity of the inhibition caused by the supply of biotin may be due to the promoted fixation of respiratory CO_2.

The postulation of the biotin effect seems to be contributive to the interpretation of photoperiodic reaction on the principle above, for if the metabolism activated by biotin has a relation to the induction it can be assumed that the supplied biotin must have changed the organic acids contents in the plants (especially, those in citric acid cycle) and that it may have affected the development of the phase. This imagination appears to be supported by the data that the acids in citric acid cycle supplied to plants promoted flowering[3,6] or sporocarp formation[12]. Furthermore, this may be admitted by the observation of Langston and Leopold[5] on biotin and pantothenic acid contents of leaves.

The role of carbohydrates may be interpreted on a relation with the dark fixation as a precursor of CO_2-acceptor suggested by Gregory, Spear and Thimann[2], for the reduction of the induction effects was caused to the lack of CO_2 in the light or in the dark, being particularly remarkable in the case of the light period.

These facts appear to indicate the primary functions of carbohydrate in the CO_2-acceptor or in a respiratory substance.

Summary

Some experiments were conducted to determine the role of CO_2 in both the light and the dark periods in photoperiodic treatment of *Salvinia natans*, an aquatic short day plant.

The results obtained may be summarized as follows.

1. In both the light and the dark periods, carbon dioxide fixations were found to be essential for the photoperiodic induction.

2. In the light period, the CO_2-free condition remarkably reduced the induction effects, which were recovered by supplying glucose to the culture solution. These facts may indicate that the carbohydrate level is closely associated with the sporocarp formation.

3. The reduction of induction effects, which occurred under the CO_2-free condition in the dark period, was found to be impeded by biotin supplied to the culture solution. From these facts it may be suggested that the organic acid metabolism in citric acid cycle is responsible for the photoinductive processes in the plant.

The writer wishes to express his hearty gratitude to Prof. K. Nakayama for his valuable guidances and criticisms.

References

(1) BONNER, J. and LIVERMAN, J. L. (1952) *Ann. Rev. Plant Physiol.*, 3, 265.
(2) GREGORY, F. G., SPEAR, I. and THIMANN, K. V. (1954) *Plant Physiol.* 29, 220.
(3) KLEIN, W. H. and LEOPOLD, A. C. (1952) *Ann. Rev. Plant Physiol.*, 3, 265.
(4) LANGSTON, R. G. and LEOPOLD, A. C. (1954) *Plant Physiol.*, 29, 436.
(5) LANGSTON, R. G. and LEOPOLD, A. C. (1954) *Physiol. Plantarum*, 7, 397.
(6) LIVERMAN, J. L. (1952) *Ann. Rev. Plant Physiol.*, 3, 265.
(7) NAKAYAMA, S. (1952) *Bot. Mag. Tokyo*, 65, 274.
(8) NAKAYAMA, S. (1955) *Bot. Mag. Tokyo*, 68, 61.
(9) OKUNUKI, K. (1954) *Shokubutsuseirikaqaku. 1st ed.* 728p.; *Tokyo : Asakura Co. LTD.* (in Japanese).
(10) PARKER, M. W. and BORTHWICK, H. A. (1940) *Bot. Gaz.*, 102, 256.
(11) SHIBATA, O. (1955) Summary of the lecture on the local meeting of Kwanto district of Botanical Society of Japan, 6.
(12) SHIBATA, O. (1955) Summary of the lecture on the 20th Annual Meeting of Botanical Society of Japan, 39.