Studies on Photoperiodic Responses of Salvinia natans (V) *Effects of Some Inhibitory Treatments under the Influence of Certain Organic Acids*

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(Received Nov. 30, 1961)

Since it was recently reported that CO_2-fixation was essential to accomplish the dark process of photoperiod in some short day plants, a number of papers on this subject have been published by GREGORY et al. (1954)[2], LANGSTON et al. (1954)[3] and many other workers. According to their results, it has been found that an ability of CO_2-fixation in dark process is influenced by light quantity in light period preceding the dark period, and is gradually changed in course of time.

The author reported in the previous paper[4] that CO_2-fixation in dark period was indispensable also to photoperiodic process in a fern, *Salvinia natans*. The present experiment was designed to study the variation of CO_2-requirement for sporocarp initiation with the lapse of time in dark period, and to determine the possible influence of some organic acids on sporocarp initiation when it is suppressed by CO_2-free condition or light-interruption.

Experimental Material and Methods

As to the material, culture methods and general procedure of experiment, readers are referred to the papers[4, 5] previously published by the author.

To determine the time when CO_2 becomes more essential in dark period, plants were exposed to CO_2-free air for only a limitted time in 16 hour dark period. For the purpose of supplying CO_2-free air, an apparatus was devised which was illustrated in the previous report[4].

A 10 minute light-interruption was interposed using a Mazuda incandescent lamp (100 W), which was set 40 cm distant from the leaf surface.

Experimental Results

Experiment 1

(a). 8 hour CO_2-free condition during photoperiodic dark period.

The partly CO_2-free conditions were produced in the first-half or the second-

half of dark period, each untreated half period being exposed to the normal air.

Inhibition grade of sporocarp initiation was 38.4 per cent with the CO_2-free air at the first-half of dark period, while it was far less with the same condition of air at the second-half period.

(b). 4 hour CO_2-free condition and light-interruption at various stages of dark period.

Two experimental series were desired to investigate a relation between inhibitions caused by CO_2-free condition and that by light-interruption in the dark period.

Table 1. A schedule of inhibitions at different times in dark period.

	exposed to the CO_2-free condition for 4 hrs. from			light-interrupted for 10 mins. from	
phase 1	0 hr.	after the beginning of dark period.	treatment 1	3 hr. 50 min.	after the beginning of dark period.
phase 2	4 hr.	〃	treatment 2	7 hr.	〃
phase 3	8 hr.	〃	treatment 3	11 hr.	〃
phase 4	12 hr.	〃	treatment 4	15 hr.	〃

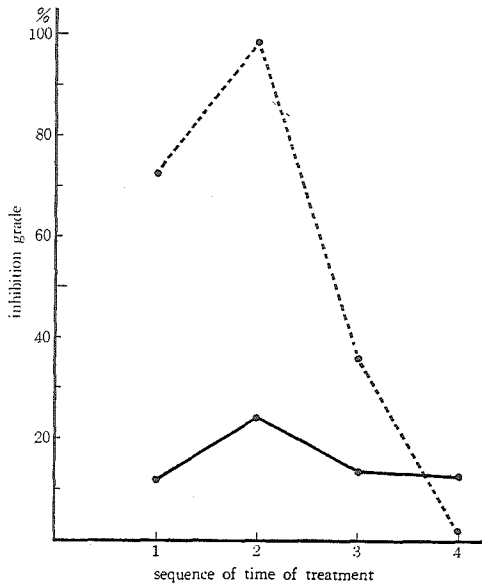

Fig. 1. A relation between the order of time of inhibition in the dark period and inhibition grade (I). Solid line: CO_2-free condition; broken line: light-interruption.

The inhibitory procedures were denoted in table 1.

The results were shown in figure 1. From these data, we can see that sporocarp initiation in the CO_2-free air was considerably inhibited in the earlier phase of dark period, and that the maximum inhibition was exhibited in the period 4-8 hours (phase 2) after the beginning of the condition.

The maximum inhibition of sporocarp initiation by light-interruption was observed at about the middle of dark period. The transition of inhibition by light-interruption with time was similar to that by the CO_2-free condition

(c) 2 hour CO_2-free condition and light-interruption at various stages of dark period.

To obtain more precise data on the inhibition of sporocarp initiation caused by CO_2-free condition or light-interruption in dark period, the same procedure as elucidated above was applied with an interval of 2 hours. Therefore, these experimental series included eight phases or treatments.

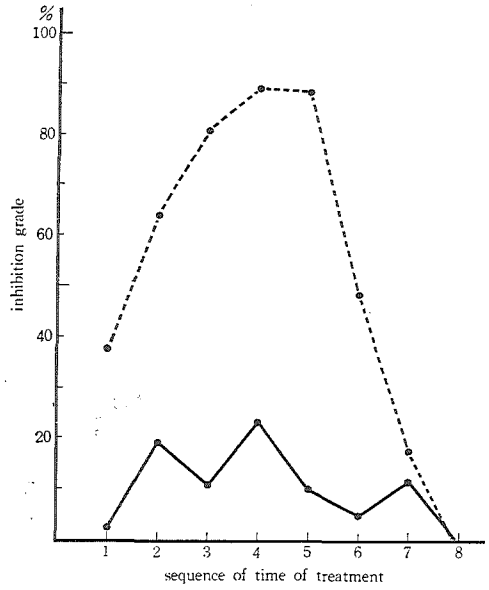

Fig. 2. A relation between the order of time of inhibition in the dark period and inhibition grade (II). Solid line: CO_2-free condition; broken line: light-interruption.

These results were shown in figure 2. In the case of CO_2-free condition, the inhibition of sporocarp initiation was most marked at the phase 4, and was decreased in later phases with the lapse of time in dark period. The inhibition curve had a peak in phases 2 and 7, which could not be found with the former experiment ((b) exp.). When the curve of inhibition grade was compared with that obtained in (b) experiment, however, there may be shown a similar tendency in both cases.

In the light-interruption, it was proved that the greatest inhibitions occurred in treatments 4 and 5, that is, about 9 hours after the beginning of dark period.

When these inhibition curves are compared with each other, it may be found that a peak of inhibition by light-interruption appeared about 2 hours later than that by the CO_2-free condition.

Experiment 2

According to the results obtained by BONNER et al. (1948)[1] in *Bryophyllum* leaves and our experiment described above, it was presumed that the inhibition by the CO_2-free condition might be directly caused by a disturbance of organic acid metabolism in the leaves. Therefore, the author conducted an experiment to see the influences of supplying some organic acids of citric acid cycle in two inhibitory treatments.

The condition of CO_2-free air was kept through the whole dark period, and light-interruption treatment was achieved by the 10 minute incandescent light at

the middle of 16 hour dark period. Each organic acid in citric acid cycle, i. e., citric, succinic, fumaric or malic acid, was supplied at 10^{-4} M concentration to culture solution in the dark period alone.

These results obtained were shown in table 2. It is proved that the effect of CO_2-free condition was affected by certain organic acids. The supply of citric acid enhanced this inhibition remarkably while the other acids reduced it, succinic acid being most effective, and fumaric acid being most inactive.

Table 2. Effects of some organic acids supplied during the inhibiting dark period of photoperiodic induction.

organic acid supplied	in CO_2-free air		in light-interruption	
	inhibition grade	induced plant	inhibition grade	induced plant
	%	%	%	%
none	15	100	92.2	33.3
citric acid	40.7	100	95.1	16.7
succinic acid	−16.8	100	93.1	33.3
fumaric acid	11.5	100	85.3	50
malic acid	0	100	80.4	83.3

In light-interruption, the effect of the organic acids on percentage of induced plant was more manifest than that on inhibition grade. In these results compared with inhibition in the absence of CO_2, some features were revealed that the citric acid had an effect similar to that of CO_2-free condition, and that a reducing effect on the inhibition as was found in the case of CO_2-absence was not obtained by succinic acid.

All sporocarps had initiated in spite of light-interruption treatment were remained as sporocarp primordia. When succinic, fumaric or malic acid was supplied, however, their development was more or less accelerated, and sporocarps in mature form were also seldom observed.

Discussion

As the cause of inhibition of sporocarp formation, some similarities were found between the CO_2-free condition and light-interruption in photoperiodic dark period in that these inhibitions were stronger in earlier stage of dark period, and attained to a maximum level at about middle of dark period. Strictly speaking, however, these two inhibitions were different from each other in the time duration required to attain to each maximum, and the maximum inhibition caused by light-interruption appeared more or less later than the case of air lacking CO_2. But a slight difference in this point seems to suggest the unique

character of each inhibition, though these inhibitions had a similar tendency in other ways.

As is shown in experiment 2, the inhibition caused by the absence of CO_2 was considerably reduced by supplying succinic, fumaric or malic acid, but not the case with citric acid. With the treatment of light-interruption, however, the inhibition was only slightly decreased by each organic acid. Such reduction in the inhibition by CO_2-free condition, which was observed with all other acids except for citric acid, seems to indicate a close relation between photoperiodic dark process and CO_2-fixation in the dark for the metabolism of organic acid as discussed in the previous reports[4, 5].

In the case of inhibition by light-interruption, a slight reduction in inhibition and a late appearance of its maximum may suggest that such inhibition occurred in a process which are probably subject to the CO_2-fixation. This process can be expected to play an important role to produce a physiological condition in the plants for sporocarp initiation. Further studies on this matter are in progress at present.

Summary

1. Experiments were conducted to investigate a feature of inhibition of sporocarp initiation is *Salvinia natans* by CO_2-free condition or light-interruption in the photoperiodic dark period.

2. The inhibition by CO_2-free condition during the dark period was more obvious in the first-half than the second-half phase of the dark period, and the inhibition was stronger when the condition was applied for 2 hours from 6th to 8th hour after the beginning of the 16 hour dark period.

3. The inhibition by light-interruption with the elapse of time was increased in the first-half stage, and then was decreased thereafter. The greatest inhibition occurred about 9 hours after the beginning of dark period, that is, soon after the appearance of the greatest inhibition in CO_2-free condition.

4. Succinic, fumaric or malic acid when supplied to the culture solution reduced rather effectively the CO_2-free inhibition, but less significant in the case of light-interruption. Citric acid, however, was found to enhance the inhibitions in either inhibitory procedure.

The author wishes to express his hearty gratitude to Prof. K. NAKAYAMA for his kind help and constant encouragement in the course of this study.

References

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