

*Effects of CO on the Change in Dehydrogenase Activity with Floral Induction in *Pharbitis nil**

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The change in respiratory capacity in the plants by photoperiodic treatment has been reported by some workers (2, 3). This fact has been described to suggest a change in some metabolic processes by the photoperiodic treatment. In short-day plants, CHARLAKHYAN (2) has suggested the increased content of respiration oxidase except for metal-containing oxidase in these plants. Though some workers (4, 7) have reported that floral formation in long-day or short-day plants is not influenced or is inhibited by anaerobic condition, SHIBATA (8) reported in *Salvinia natans* that the inhibitors for metal-containing enzymes which were given during photoperiodic dark periods accelerated the sporocarp initiation when glucose was supplied during its light periods though the inhibitors suppressed the sporocarp initiation without glucose. In order to investigate enzymologically these facts, dehydrogenase activity in cotyledone of *Pharbitis nil* was measured on the plants with or without CO treatment.

Pharbitis nil was grown under a continuous illumination by fluorescent lamps, and the cotyledons of 5 day old seedlings were used as experimental materials. Six plants were subjected to one experiment, and they were given from 1 to 7 short-day cycles, and one of them consists of 8 hr light and 16 hr dark periods. Experiment was undertaken 3 times. In order to supply CO to the cotyledons during the dark period, the cotyledons alone were enclosed at the beginning of the dark period in an air-tight box made in two parts, upper and lower. The uppers sat above the lowers, the two parts were clamped together by a clip, and then were sealed with vaseline. Petioles were passed between these edges. CO (70%) which was admitted to the box through a tube on one of the parts was substituted for air in the box which was exhausted through a tube on another part. This apparatus was set in a light-proof box. After being exposed to given number of short-day, most of the plants were used for measurement of enzyme activity at 4th hr after the end of dark period. Some of the plants given the short-day treatment were returned to the continuous illumination till the observation of the inflorescence at 20th day after the treatment.

Table 1. Floral initiation as affected by CO-application in *Pharbitis nil* cotyledon during photoperiodic dark periods.

condition of dark period	average node-number bearing the first flower bud	average number of flowers per plant	plants initiated with flowers (%)
air (1 cycle)	3.6 ± 0.54	2 ± 0.3	100
air (2 cycles)	3.3 ± 0.58	3.3 ± 0.58	100
CO (2 cycles)	3.4 ± 0.45	4 ± 0.23	100

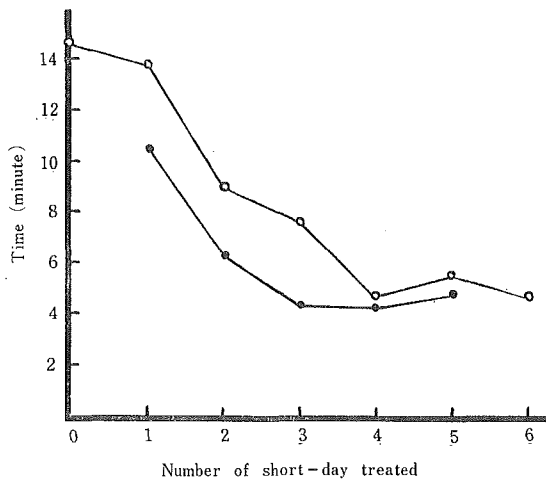


Fig. 1. Change of time required for TTC reduction in *Pharbitis* cotyledonary tissues in presence or absence of CO during photoperiodic dark period. —○—, CO absent; —●—, CO present.

Dehydrogenase activity in the cotyledonary tissue was histochemically evaluated by the ability to reduce triphenyl tetrazolium chloride (TTC). The reduction ability was measured as follows; the cotyledonary tissue handsectioned were set on a slideglass, and were added with each one drop of 1/15 M phosphate buffer, pH 7.2, and 1% TTC solution, and were crushed by pressing the coverglass to facilitate TTC penetration into them. The slideglass was kept at about 25°C, and the time required from the TTC addition to the microscopical formation of the formazan granules was measured.

More floral primordia were produced on the plants in CO than that in air, but no difference was found on the node number bearing the first flower primordia (Table 1). The change of the dehydrogenase activity in the plants given from 1 to 7 cycles of the short-day treatment as well as that in the plants treated by CO is shown in Fig. 1. The time required for TTC reduction in the plants given short-day in air was decreased with increasing number of short-day cycles, but,

as to the number of the cycles more than 4, the required time was constant essentially. In the plants treated with CO in the dark period, the time required for TTC reduction was more shortened than that in air.

The fact that the time required for TTC reduction was shortened with increasing short-day cycles indicates that some dehydrogenase systems were activated with increase in the number of the cycle. The increased dehydrogenase activity by short-day treatment in air has previously been histochemically studied by THEIN (8) with *Xanthium*, and the present data were consistent with his results.

It appears that shortening of the time required for TTC reduction under CO application was resulted from more activation of some dehydrogenase in CO than in the normal air. Together with the results obtained in the floral response, this fact may suggest that the activity of the dehydrogenase system enhanced under the anaerobic condition in the dark period was similar to that activated under the aerobic condition, and that the increased activity of dehydrogenase had a relation with floral-initiation ability. It is not clear, however, whether the metabolic system activated under the anaerobic condition has a relation with a KCN-resistance system as was reported by CHANCE and HACKETL (1) with skunk cabbage. Previously, KINOSHITA and SHIBATA (6) have observed the acceleration of the floral induction in *Pharbitis purpurea* under a moderate Fe-deficient condition, and also have reported that such condition promotes light absorption at 260nm. Moreover, HIGASE (5) have reported that ethyl alcohol supplied to culture media accelerates the floral formation in tobacco plants. As many workers has suggested, the results obtained here may suggest the existence of fermentative process in the reaction of floral stimulus formation.

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