On Ultraviolet Absorption of a Component from Leaves of the Photoperiodically Induced Plant

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Many studies have been made to determine the chemical compositions of photoperiodically induced or non-induced plants, indicating that there were significant differences in some metabolic products between two series of the plants. These differences may presumably be caused by some divergences in metabolic activities. Concerning to the matter, recently THEIN[7], using the stem apex of *Xanthium*, investigated the reduction of 2, 3, 5-triphenyltetrazolium chloride, and he found that dehydrogenase activity in the apex was considerably increased by short day treatment.

The authors investigated spectrophotometrically a change in some metabolic states of plant caused by photoperiodic treatment. The data in this paper concern a change in optical density of aceton-insoluble fraction in leaves following a short day treatment at a distinct wave length of light.

Material and Methods

The experimental material *Pharbitis purpurea* VOIGT, a short day plant, was cultured on Knop's solution from the seed germination under 24 hour photoperiod supplemented by illumination with a fluorescent light. For the floral induction, a short day consisting of 8 hour light and 16 hour dark periods was given to the plants which have had only a mature first-leaf and cotyledon cut. Control plants were cultured under a continuous illumination.

For the preparation of leaf-protein, following methods were applied: Immediately after the photoinduced mature leaves were collected, they were weighed and homogenized in cold aceton by using a homogenizer. By this procedure, the leaf-protein was precipitated cell-freely, and pigments including chlorophyll were removed through a filtration. For the purpose of complete removal of the pigments, the sediments were washed out with ethyl ether, and were dried in air to evaporate the ether. The dried sediments were then dissolved in 40ml. of pure water per gm. of fresh leaves. After the solution was maintained in an ice-box for 24 hours, it was centrifuged with 1000 r.p.m. for two minutes, and its supernatants were used as a water-soluble protein solution. It can be considered,

however, that, in practice, so-called protein solution obtained here may contain also the other components like flavin compounds.

For the extraction of leaf-protein, the first leaves taken from more than nine plants were used as one extraction. A determination of the optical density was made by using a photoelectric spectrophotometer of Beckman type.

Experimental Results

1. *The change of absorption of ultraviolet and visible lights.*

The optical density of the protein solution was determined in two zones of ultraviolet and visible lights to know whether it was changed by short day treatment. One cycle of short day was given in this experiment.

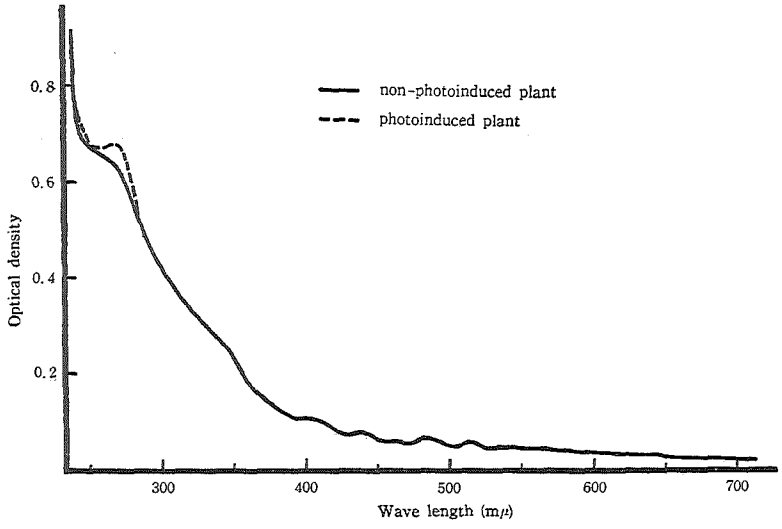

Fig. 1. Optical densities of an aceton-insoluble fraction isolated from photoinduced and control plants in ultraviolet and visible lights.

The result was represented in fig. 1. The extracts of control as well as photoinduced plants revealed a considerable strong absorption of ultraviolet light. With a transition from ultraviolet to visible light zone the optical densities in both plant groups decreased sharply, and were kept on a low level with little fluctuation. Here, a difference in this respect between these two groups was observed only in a wave length ranging 250—270 mμ, in that the photoinduced plants showed higher optical density than the controls. In the other zones of light, however, no significant difference was found between them. Accordingly, the optical densities in subsequent experiments were determined only in the zone of ultraviolet light.

2. *The effect of the length of time from the end of dark period to the collection of photoinduced leaves.*

The protein solutions prepared just after, 4 or 8 hours after the dark period were compared with each other as to the optical density. No difference was found among these values determined, so the ultraviolet absorption was thought never to be affected by the time duration from the end of the dark period. Then, the protein solutions in subsequent examinations were prepared immediately after the dark period.

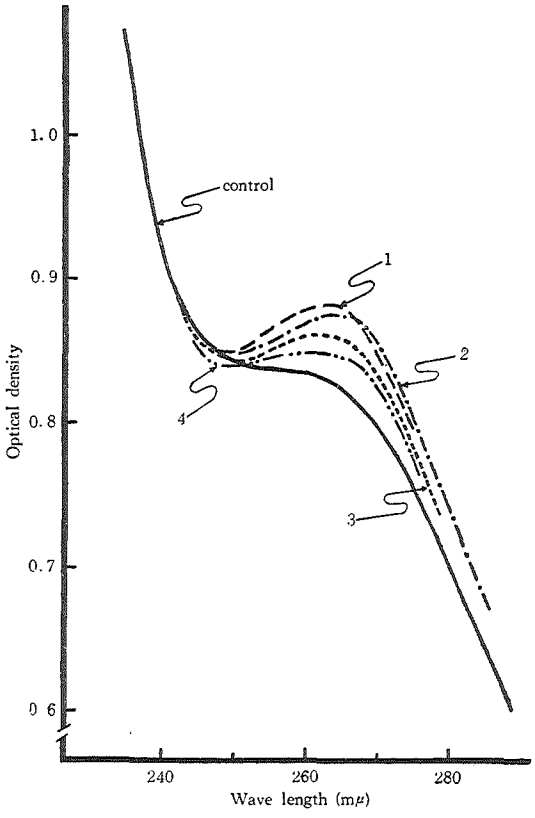

Fig. 2. A change in the optical density by the number of photoperiodic induction. Number denoted by the arrow means the number of short day cycle.

3. *The change of ultraviolet absorption with the number of photoinductive cycles.*

A series of plants were photoinduced with from one to four short day cycles.

The change in optical density affected by the number of cycle was represented in fig. 2. In these results, the density of the control plants was represented by only one case, because there was no difference in optical densities among controls taken for each short day cycle.

In contrast with the case in controls, optical density of the treated plants was increased suddenly by the first cycle of short day, then decreased gradually by the following cycles, and finally attained an almost constant value. But it never fell to the level of controls so far the number of the photoinduction applied here was concerned.

4. *The effect of a light-interruption on the ultraviolet absorption.*

As a fact that the floral formation is inhibited by a light-interruption of the dark period has been well established, it was investigated whether this interruption had an influence on the change in optical density.

A series of plants consisted of four plant groups which were given one to

four short day cycles, and each group was light-interrupted for 10 minutes at the middle of every 16-hour dark period. This treatment failed to disturb the proper change in the optical density in every plant group.

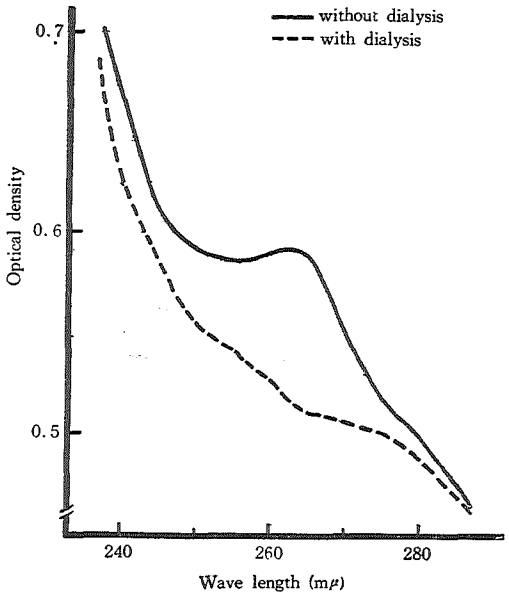

Fig. 3. An effect of the dialysis on the optical density.

5. *The effect of dialysis of the protein solution on ultraviolet absorption.*

A qualitative experiment was made to determine a chemical charactor of the substances which were considered to change the ultraviolet absorbing capacity by short day treatment. The protein solution extracted from the photo-induced leaves was subjected to a dialysis to pure water through a collodion membrane in an ice-box for 24 hours, and then its optical density was determined.

As shown in fig. 3, the substances effective for ultraviolet absorption were diminished by such procedure, so that all or some parts of the substances were found able to be dialyzed.

6. *Ultraviolet absorption affected by culturing the plants with different iron contents.*

The authors[2] have observed that floral formation of *Pharbitis purpurea* cultured with a moderate deficient iron (5mg/l of $FeCl_3 . 6H_2O$), which did not affect on the chlorophyll content, was as good as that with a sufficient iron (9mg/l of $FeCl_3 . 6H_2O$). Accordingly, the optical densities were compared between two plant groups cultured with different iron contents.

Fig. 4 indicates the cases of non-induced plants cultured with the different iron contents. The optical density was higher in plants cultured with the

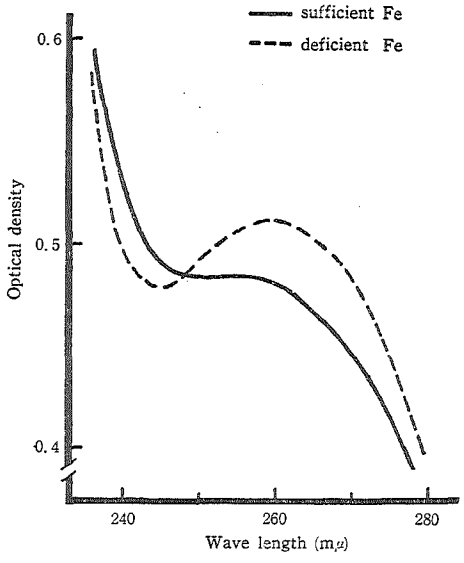

Fig. 4. The optical density affected by culturing with different iron contents in non-photoinduced plants.

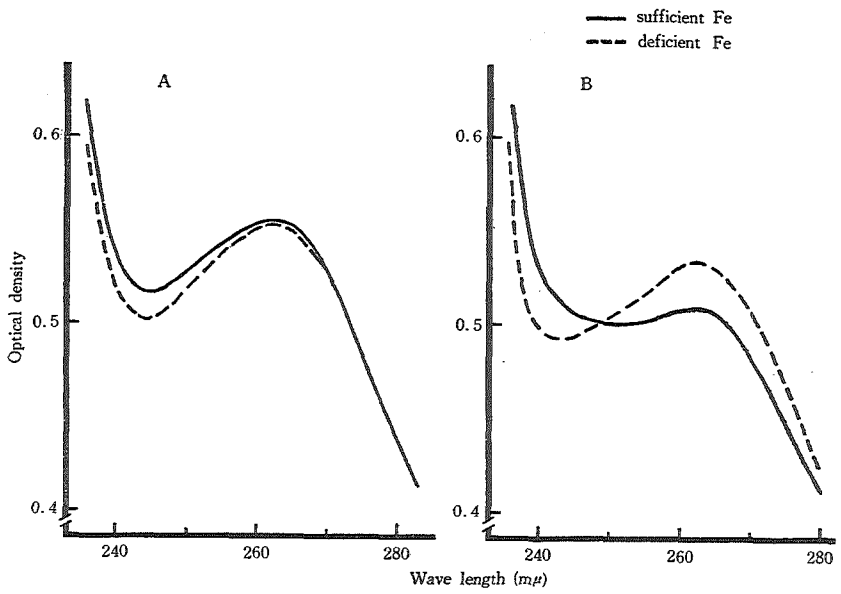

Fig. 5. A——B. A change in the optical density of photoinduced plants by different culture conditions in iron content. A: one photoperiodic cycle, B: two photoperiodic cycles.

moderate deficient iron than in those on sufficient iron. A relation of the optical density to a given number of short day cycles was shown in fig. 5-A and -B with the results obtained by the different contents of iron. The optical densities were always higher in iron deficient plants than that of sufficient iron.

Discussion

As an usual method for preparation of crude enzymes was applied to obtain the leaf-protein, the extracts may be regarded as those for the most part consisting in certain protein components.

It can be considered that these extracts may contain the flowering substances of *Pharbitis* plant. Such possibility, however, appears to be denied by the fact that the optical density was not affected by the time durations from the end of dark period, nor was affected by a light-interruption in the dark period.

The transition curve in ultraviolet absorption may arouse our interest in comparing it to some data reported by other workers. ELLIOT and LEOPOLD[1] reported that, in some short day plants, the respiratory activity was increased remarkably up to the third cycle of short day treatment, decreasing thereafter through the following cycles. They also have suggested a relation between the extent of changes in respiratory activity and the degree of flowering response to photoperiodic induction. NAKAYAMA[4] also has reported a similar change in

respiratory activity in *Pharbitis Nil.* One of the authors[6] suggested, from the results of an analytical experiment on photoperiodic induction of *Salvinia*, that a certain metabolic system might perhaps be activated by the beginning of photoperiodic treatment, and that its activity was reduced with the more inductive cycles after its arrival to the maximum. The change in the ultraviolet absorption found here had an analogy to that in the respiratory activity.

SALISBURY[5] has reported that the floral development in *Xanthium* plant became suppressed when the plant was debudded after the inductive treatment, and LAM and LEOPOLD[3] have observed that photoinduced plants, when they were debudded or decapitated, reversed to show vegetative growth. They, as an interpretation of this reversibility, presumed that a specific agency of flowering stimulus, that is, self-perpetuating or immobilization, might be caused by above operations. The results obtained here were considered to be caused by the change of metabolic activity in synthesis of flowering substances, and such our consideration seems more reasonable than supposing the specific agency in interpreting the reversibility of reproductive to vegetative condition.

Another interesting fact was resulted by dialysis of the protein solution. Since this procedure reduced degree of the ultraviolet absorption of the solution, it is suggested that the ultraviolet absorbing substances responsible to the absorption can be expected to be of low molecule. At present, however, further details on this substances are unknown.

Summary

1. *Pharbitis purpurea* VOIGT was exposed to a short day consisting of 8-hour light and 16-hour dark periods, and then an acetone-insoluble fraction obtained from leaf extracts was prepared for the determination of optical density.

2. An absorption of ultraviolet light was increased suddenly by the first cycle of the short day treatment, and then was reduced gradually in spite of number of the inductive cycles.

3. This ultraviolet absorption was not disturbed by a 10 minute light-interruption of the dark period, and was kept on a constant level regardless of time duration from the end of the dark period to the leaf-protein extraction. But, it was reduced by a 24 hour dialysis.

4. Such absorption was increased by growing the plants with deficient iron even in the case of non-photoinduced plant, and much more in the photoinduced one.

5. From the results obtained here, it was discussed that the ultraviolet absorption may probably be caused by some enzyme-like components relating the formation of flowering substances.

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