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Immunological Analysis of Proteins in the Leaf of *Pharbitis nil* during Photoinduction of Flowering

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Pharbitis nil 開花 誘導時 葉蛋白質 變化에 對한 免疫學的 分析

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ABSTRACT

Using double immunodiffusion and immunoelectrophoretic techniques, attempts were made to detect any protein changes in leaf tissues of a short-day plant, *Pharbitis nil* Chois. variety Violet during floral induction under 8 hr light, 16 hr dark cycles. Immunoprecipitin systems showed at least four proteins newly appeared in the induced leaf tissues. Accumulation of the proteins were observed as the induction proceeded.

INTRODUCTION

Among the enormous amount of information on the physiological and biochemical aspects of flower initiation in photoperiodic plants, understanding the basic internal mechanisms, especially the molecular events of the floral transition is still in its infancy.

Analyses of nucleic acids and proteins in various plant tissues in transition to flowering have been attempted. Using electrophoretic technique, proteins specific to flowers have been demonstrated in *Pharbitis* (Murashige and Murashige, 1962) and in *Xanthium* apices (Nitsan, 1962). Transforming apical meristsms of *Cannabis* (Heslop-Harrison and Heslop-Harrison, 1970) and of *Sinapis* (Jacqmard *et al.*, 1972) have shown increase in the amount of nucleic acids and proteins by quantitative microchemical techniques. Isoelectric focusing detected the distinct loss of a single protein band in cotyledons of *Pharbitis* on floral induction (Stiles, Jr. and Davies, 1976). More recently, immunological tests found qualitative changes in the protein complement of evoked *Sinapis* meristems (Pierard *et al.*, 1977; Pierard *et al.*, 1980). The search for new proteins associated with floral evocation has been often unsatisfactory due to limitations of techniques to detect minute changes in total protein complement of such small plant tissues. More convin-

cing analytical methods are needed.

In this paper, double immunodiffusion(ID) and immunoelectrophoretic (IE) techniques were adopted to detect any antigenic protein changes in the leaves of *Pharbitis nil* during floral induction by short-day treatments.

MATERIALS AND METHODS

Growth of *Pharbitis nil*. Seeds of *Pharbitis nil* Chois. variety Violet were soaked in concentrated sulfuric acid for 30 minutes, rinsed and imbibed in running water for 24 hours. The seeds were placed on wet filter paper in Petri dishes and kept in the dark for germination. The germinating seeds were transplanted in flats of vermiculite in an environmental growth chamber at 25±1°C under continuous illumination of 3,000 lux provided by a mixture of fluorescent and incandescent lamps. The plants grown vegetatively under constant illumination were induced to flower by 8 hr light, 16 hr dark cycles.

Preparation of antigen. Antigenic material was obtained from newly expanded leaves. Ten gram portion of the tissues was homogenized in 40 ml of cold 0.05 M sodium borate buffer, 0.2% NaCl solution at pH 8.0. Centrifugation was performed at 1,200 *g* for 20 minutes at 0°C. Presence of soluble proteins in the supernatant was tested in 10% (w/v) trichloroacetic acid. The soluble protein fraction was concentrated 10 times in Amicon Pressure Cell(Model 12 with PM 10 filters) under nitrogen gas at 1,500 $g \cdot cm^{-2}$ as described by Lee and Dickinson(1979). The concentrated protein fraction thus obtained was used as antigenic materials.

Four different antigenic fractions were prepared; antigens S_4, S_8 and S_{12} were extracted from leaf tissues collected at Day 4, 8 or 12, respectively after start of the short-day treatments.

Immunological Techniques. Antisera were produced in rabbits, New Zealand White, against the protein extracts obtained from leaves grown under continuous light, a non-inductive photoperiod. The first intramuscular injection was done with Freund's complete adjuvent(Crowle, 1973), followed by 4 consecutive weekly injections of the antigenic fractions and booster series. Antisera were isolated by the standard method(Clausen, 1981) from the blood taken 7 days after the start of the last booster series. Temperature was set at -25°C for the storage of the antisera.

Double diffusion method(Ouchterlony and Nilsson, 1978) was adopted for analysis of immunoprecipitin system. Ouchterlony plates were prepared in Petri dishes (6 cm in diameter) with agarose gel layer containing 1.5%(w/v) agar, 0.05 N sodium 5,5-diethylbariturate and 0.015 N HCl. The pH of the medium was 8.2. Seven wells 5 mm in diameter were made. Six wells on periphery were filled with the protein extracts and a center one received antiserum. Plates were kept at room temperature with 100% RH.

Garber-William methods(Weir, 1978) were used for immunoprecipitin(IP) tests. The same agarose solution prepared in ID was used. The solution (14ml) was layered on an immunoplate (8.4×9.4cm) and the well received the protein extracts. After electrophoresis(130V, 70mA) had been carried out at 4°C for 1.5 hours, the troughs were filled with the antisera. The plates were incubated at room temperature with 100% RH. Photographs of precipitin bands on Ouchterlony plates and arcs on immunoplates were made using dark field illumination.

RESULTS AND DISCUSSION

Two types of immunological analyses, double ID and IE, showed results consistent to each other. Qualitative analyses by double ID (Ouchterlony test) were attempted by identifying precipitin bands resulting from reactions between antibodies in rabbit serum and antigenic protein extracts from leaf tissues previously treated with various photoperiodic cycles. The precipitin bands developed on Ouchterlony plates showed 3 types of typical reaction patterns: Type Ⅰ, identity; Type Ⅲ, partial identity; Type Ⅳ, reaction-inhibition (Ouchterlony and Nilsson, 1978).

At the end of 24 hour incubation period (Fig. 1), the multivalent antisera showed multiple immunoprecipitin bands representing multiple immunoprecipitin systems with

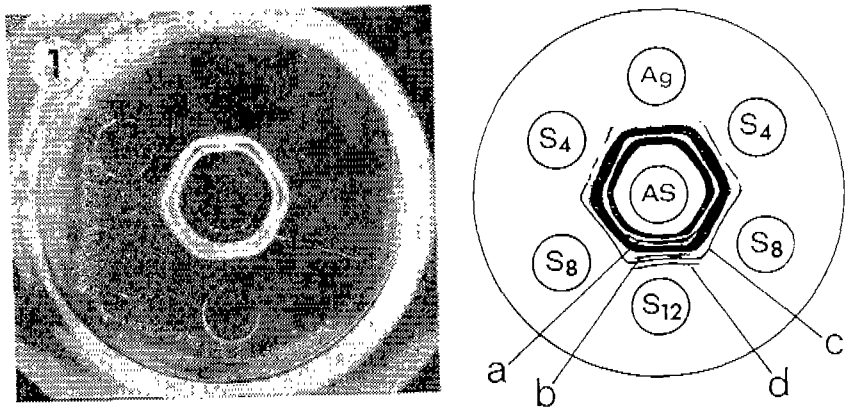
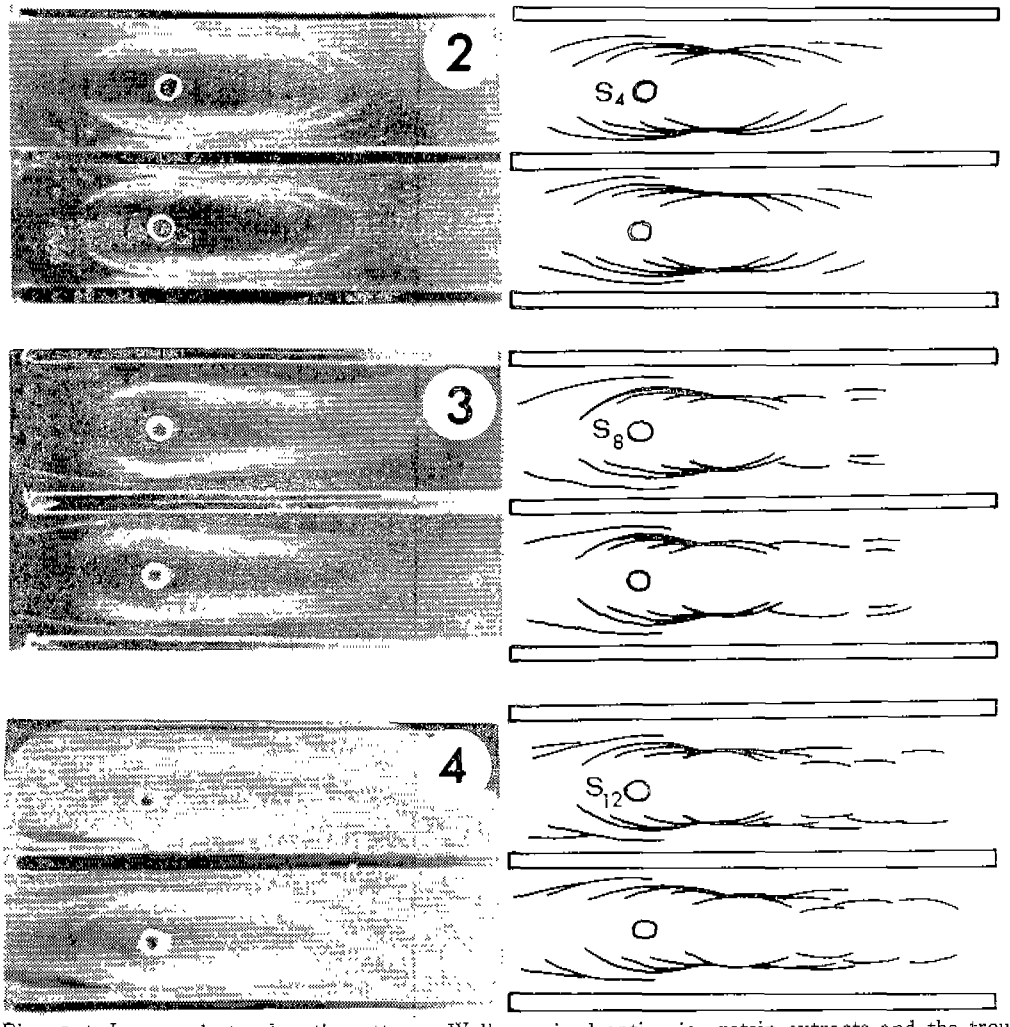


Fig. 1. Immunoprecipitin bands developed for 24hr incubation were photographed (left) and sketched(right). Center well received rabbit antiserum (AS) against protein extracts from leaves under non-inductive photoperiods. Each of the peripheral well was filled with various antigenic materials; reference antigen or the protein extract used for the antibody production(Ag), leaf protein extracts at the end of 4 (S_4), 8 (S_8), or 12 (S_{12}) short-day cycles.

antigenic materials from the plant tissues having exposed to various photo-treatments. The bands developed in all cases were shown to be composed mainly of 2 groups of distinct immunoprecipitin systems, which were equivalent to Type Ⅰ, indicating there

were common antigenic determinants in all antigenic materials tested. The immunoprecipitin systems thus detected are thought to be from reactions of structural proteins in cells of the leaf tissues.

Four specific precipitin bands, a-d, could be detected for Group S_{12}, and a single band, c, for Group S_8, while Group S_4 showed much fainter one. No specific reaction



Figs. 2-4. Immunoelectrophoretic patterns. Wells received antigenic protein extracts and the troughs were filled with the rabbit antiserum. Incubation period was 5 days. Duplicates shown in each figure. Abbreviations are the same as in Fig. 1.

was shown for the reference antigen, Ag. To trace the behavior of immunoprecipitin bands, further observation was performed. As incubation was extended, Groups S_4 and S_8 also began to show the 4 bands. But they later on started to diffuse and fade out gradually first from S_4 and then S_8. The concentration of the protein fractions was

assumed to be highest in S_{12} which retained the bands for longer than any other groups. The order of concentration was; Groups $S_{12}>S_8>S_4$.

In IP tests(Figs. 2~4), serological characteristics of antigenic materials could also be revealed by the number of arcs and their loci. Two groups of arcs were detected in IP. One group of arcs, with no electric charge, was developed around the center well, while the other, with negative electric charge, was formed at a distance from the well. The antigenic materials used were the same as the ones for ID tests. Group S_4(Fig. 2) developed 7~8 arcs which showed distinguishable differences from Group S_8(Fig. 3) or Group S_{12} (Fig. 4). Especially, arcs corresponding to those weak bands and developed in later period in double ID tests were found to move with negative electric charges.

Although there was limitation of identifying arcs on the plates, approximately 14 arcs for S_{12} could well be observed. Increasing number of arcs in IP analysis as the induction cycles for flowering was prolonged confirmed the results of double ID.

The apparance and longevity of the immunoprecipitin bands observed in the present study leads to postulation that at least 4 different proteins somewhat related to floral induction processes may continuously be synthesized and accumulated in the leaf tissues of *Pharbitis nil*. It is not known at present whether the proteins thus detected actually particitipate in the induction or the changes occur as a consequence of the processes. More precise techniques are to be developed to trace quantitative protein changes in leaf tissues at the threshold of floral induction. Although most of the relevant investigations have been focused on protein complement changes in shoot apical meristems, attempts to trace any molecular differentiation in leaf cells, where floral induction processes actually take place, are more essential and will provide more fundamental approach to the problem.

摘 要

短日植物인 *Pharbitis nil* Chois. variety Violet의 開花(花芽發生)를 8時間 光, 16時間 暗期로 誘導한 후, 이에 따른 葉組織內 蛋白質의 變化를 double immunodiffusion과 immunoelectrophoresis로 分析하였다. 營養生長 光週期下의 葉組織에서는 나타나지 않았던 4種의 새로운 蛋白質이 開花誘導過程이 進行되는 葉組織에서 出現하였으며 誘導處理가 연장됨에 따라 이들의 濃度는 增加되었다.

REFERENCES

Clausen. J. 1981. Immunochemical Techniques for the Identification and Estimation of Macromolecules. Elsevier, New York. 388 pp.

Crowle. A. J. 1973. Immunodiffusion. Academic Press. New York. 545 pp.

Heslop-Harrison, J. and Y. Heslop-Harrison. 1970. The state of the apex and the response to induction in *Cannabis sativa*. *In* Cellular and molecular aspects of floral induction, G. Bernier (ed.), pp. 3~26. Longman, London.

Jacqmard, A., J. P. Miksche and G. Bernier. 1972. Quantitative study of nucleic acids and proteins in the shoot apex of *Shinapis alba* during transition from the vegetative to the reproductive condition. *Am. J. Bot.* **59** : 714~721.

Lee, Y. S. and D. B. Dickinson. 1979. Characterization of pollen antigen from *Ambrosia* L. (Compositae) and related taxa by immunoelectrophoresis and radial immunodiffusion. *Am. J. Bot.* **66** : 245~252.

Murashige, K. and Y. Murashige. 1962. An electrophoretic study of tissue extracts from leaf and flower in *Pharbitis nil* Chois. *Plant & Cell Physiol.* **3** : 319~322.

Nitsan, J. 1962. Electrophoretic patterns of *Xanthium* leaf extracts as affected by physiological age of leaf, photoperiod, and age of plant. *Plant Physiol.* **37** : 291~295.

Ouchterlony, O. and L. A. Nilsson. 1978. Immunodiffusion and immunoelectrophoresis. *In* Handbook of experimental immunology, Vol. 1. Immunochemistry, D. M. Weir(ed.), Blackwell Sci. Pub., London. 543 pp.

Pierard, D., A. Jacqmard and G. Bernier. 1977. Changes in the protein composition of the shoot-apical bud of *Sinapis alba* in transition to flowering. *Physiol. Plant.* **41** : 254~258.

—— ——, ——, —— and J. Salmon. 1980. Appearance and disappearance of proteins in the shoot apical meristem of *Sinapis alba* in transition to flowering. *Planta* **150** : 397~405.

Stiles, J. I., Jr. and P. J. Davies. 1976. Qualitative analysis by isoelectric focusing of the protein content of *Pharbitis nil* apices and cotyledons during floral induction. *Plant & Cell Physiol.* **17** : 855~857.

Weir, D. M. 1978. Handbook of experimental immunology, 3rd ed., Blackwell Sci. Pub., London. 348 pp

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