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슬래쉬 소나무의 花芽原基形成의 生理的 硏究
Ⅳ. 雌花形成期間동안의 頂芽의 PEROXIDASE의 変化*[1]

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Physiology of Strobilus Initiation in Slash Pine
IV. Peroxidase Activity during the Period of Female Strobilus Initiation*[1]

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Polyacrylamide gel 電気泳動法을 利用하여, 슬래쉬 소나무(接木苗, 20年生)의 花芽原基形成期間 동안에 雌花多量 開花個体와 少量 開花個体間에 頂芽의 peroxidase 同位酵素의 変化를 比較하였다. 頂芽에서 모두 9개의 band를 観察할 수 있었으며, 두 個体間에 同位酵素의 量的인 差異는 微細하였으나, 第五 band는 多量 開花個体에서 大部分 発見되었다. 이 第五 band의 同位酵素는 雌花原基形成에 直接 関聯되는 것 같이 생각된다.

With polyacrylamide gel electrophoresis, peroxidase isoenzyme patterns in the terminal buds of grafted, 20-year-old *Pinus elliottii* trees were compared between abundant-flowering and poor-flowering trees during the period of female flower bud initiation. A total of nine peroxidase bands were observed in the terminal buds. The total amount of peroxidase enzymes in slash pine buds showed no significant difference between the abundant-flowering and poor-flowering trees. However, the fifth band from the gel front was observed in all the samples of abundant-flowering trees throughout the sampling period (July 8 to September 17), while this band was absent in most of the poor-flowering trees.

INTRODUCTION

Peroxidases are enzymes which require hydrogen peroxide (H_2O_2) as an electron acceptor (oxidizing agent) to oxidize a wide range of substances (electron donors). They are widely distributed among higher plants and have been suggested to play important roles in many physiological processes, such as detoxification of H_2O_2, polymerization of lignin and rubber (Hahlbrock and Grisebach, 1979), oxidation of indoleacetic acid, and production of ethylene (Lieberman, 1979). They are also known to undergo both temporal and spatial shifts in the course of development and differentiation of higher plants.

The involvement of peroxidases in auxin metabolism, ethylene production and other physiological functions suggests the possibility that the expression of particular peroxidase isozymes in a developing plant may be related to a specific condition of the plant at the time of specific physiological activity.

The objective of present study was to determine whether peroxidase isozymes in abundant-flowering trees of *Pinus elliottii* can be related to the activity of the terminal buds at the time of floral bud initiation. Peroxidase isoenzyme patterns in the abundant-flowering trees were compared with those in the poor-flowering trees during the period of female flower bud initiation.

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MATERIALS AND METHODS

The slash pine (*Pinus elliottii* var. *elliottii* Engelm.) seed orchard used in this study was established in 1956 with grafted seedlings, and located in Gainesville, Florida in U. S. A.

Five clones with a history of abundant female cone production (abundant-flowering group) and five clones of poor female production (poor-flowering group) were used in this experiment. In the abundant-flowering group (AFG), branches with large terminal buds (above average bud diameter) and with one or more current-year female strobili were selected from the upper half of the crown, and the terminal buds were harvested. In the poor-flowering group (PFG) branches with small terminal buds (below average bud diameter) and with no current- or previous-year female strobili were selected from the lower half of the crown, and the terminal buds were harvested. The collection of samples started on July 8 and repeated every week in July, every other week in August, and on mid September. The buds were clipped and frozen in liquid nitrogen in the field. The frozen buds were brought to the laboratory and ground with a mortar and pestle to fine powder. Then the ground bud tissue was freeze-dried and stored in a freezer.

From freeze-dried bud tissue acetone powder was prepared by homogenizing with cold 70% acetone, sifting through a No. 60 screen, centrifuging, and drying the precipitate with 100% acetone (Fig. 1). All procedures should be done with chilled equipments and cold (−20℃) acetone. Three milligrams of acetone powder was placed in a test tube with 0.1 ml of extracting solution (Solution VI in Table 1) and left at 4℃ for 1 hour. The solution was centrifuged at 27,000g for 10 min. in a cold room (4℃). The supernatant was carefully decanted into a tapered tube, and 10 ul of the extract was used for electrophoresis.

Acrylamide gel electrophoresis: A method by Clarke (1964) and modified for reducing agents and detergents by Hare (1970) was used here. Acrylamide gels were prepared by mixing 1 : 1 : 2 ratio (by volume) of Solution I, II, and III (Table 1), and polymerizing the mixture overnight in glass gel-tubes under fluorescent light. Before polymerization the meniscus on the top of the gel solution was flattened by carefully adding water on the top. Before samples were loaded on the top of each gel in an upper chamber, the gels had been pre-electrophoresed with bromphenol blue. After 10 ul of extract was loaded, 2 milliamperes per gel was applied to the apparatus. Electrophoresis was performed in a cold room. Buffers for an upper and a lower chamber are described in Table 1.

After the electrophoresis the gels were stained for 6 min. in a mixture of 50 ml satuated benzidine dihydrochloride solution (500 mg/50 ml water), 2 ml 0.2% H_2O_2, and 10 ml 30% NH_4Cl. After staining, the gels were placed in a 7% acetic acid solution for 10 min. Then the gels were scanned at 550 nm with a recording spectrophotometer. Procedures for peroxidase essay were summarized in Fig. 1.

RESULTS AND DISCUSSION

A preliminary study of enzyme extraction methods showed that when the enzymes were extracted directly from freeze-dried samples of bud tissue, only a few bands were visible with dark background interference. Adding polyvinyl pyrrolidone to the extracting solution did not improve extraction of the enzymes. Acetone powder made from freeze-dried samples, as Hare (1970) used, gave 6 major and 3 minor bands (Figure 2). The minor bands (Nos. 1, 8, and 9) were observed occasionally, while 5 major bands Nos. 2, 3, 4, 6, and 7 were found in almost all samples. The No. 5 band was observed as a major band in all samples of AFG, whereas it was absent in most of the samples of PFG. This band seemed to be a qualitative difference between the two flowering groups.

Absolute amounts of the isozymes were not determined in this study, but values in areas (cm^2) under each peak in the photogram are illustrated in Figure 3. Peaks of six major bands were quantified and are shown in this Figure 3, while three minor bands produced small peaks which were difficult to be quantified, and are not shown here. The peak

areas of Nos. 2(Fig. B), 4(Fig. D), and 5(Fig. E) bands in AFG were larger than those in PFG, whereas the peak area of No. 7 band(Fig. G) was larger in PFG. Total peak areas(sum of individu al peak areas) of AFG(Fig. A) on 4 of 7 collection dates(July 23, July 30, August 13, and September 17) were larger than those of PFG. This indicated that the total activity of peroxidase enzymes in slash pine buds showed no significant difference between the two flowering groups. However, the presence of No. 5 band in all the samples of AFG and absence of it in PFG suggested a possible involvement of this isoenzyme in female strobilus initiation in slash pine.

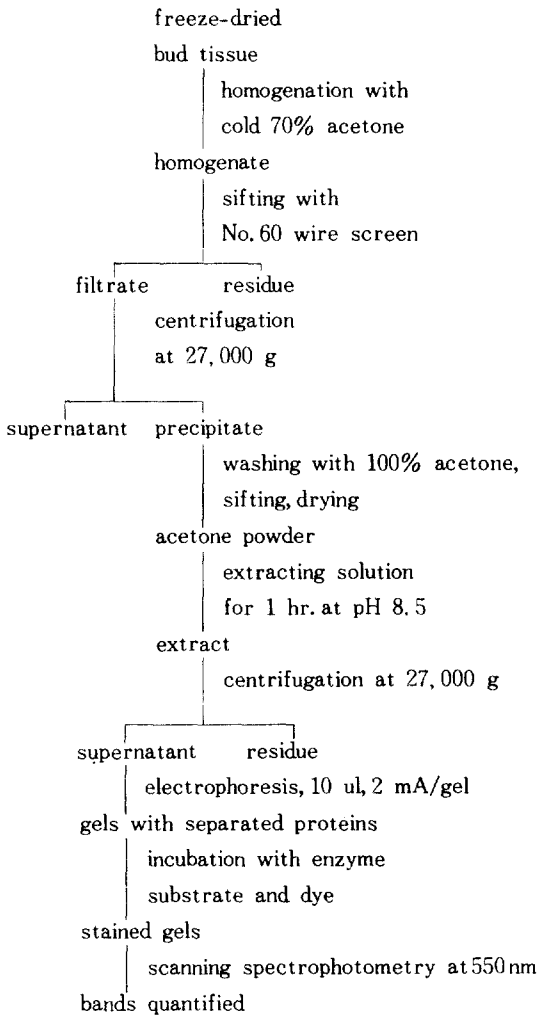


Figure 1. Summary of procedures for peroxidase essay with acrylamide gel elctrophoresis.

One of the interesting features in this study was unique characteristics of No.6 and 7 bands whose width was much wider than other bands. These two bands were found in almost all the samples regardless of flowering group. However, the presence and density of the bands(the degree of darkness) varied with different individual trees. The presence and absence of these two bands and a couple of other minor bands appeared to be an inherent characteristic of individual trees and can be used for the family analysis or for testing closeness of neighboring trees in a stand. This peroxidase analysis technique has already been used widely for this purpose(Park, 1977).

Table 1. Reagents for acrylamide gel, buffers for an upper and a lower chamber, and extracting solution.

Solution Ⅰ. Acrylamide 24.0g
Bisacrylamide 0.64g
Water to 100 ml.

Solution Ⅱ. Tris 18.15 g
HCl 1N 24.0 ml (to pH 9.1)
Temed 0.3 ml
Water to 100 ml

Solution Ⅲ. Riboflavin 5 mg
$K_2S_2O_8$ 150 mg
Water to 250 ml

Solution Ⅳ. (Buffer for upper chamber)
Tris 5.16 g(to pH 8.9)
Glycine 3.48 g
Water to 1,000 ml
A few drops of 0.05% bromphenol blue(12.5 mg/25 ml) to be added just prior to use.

Solution Ⅴ. (Buffer for lower chamber)
Tris 14.5 g
HCℓ 1N 60 ml (to pH 8.1)
Water to 1,000 ml.

Solution Ⅵ. (Extracting solution) pH 8.5

Urea 15.0 g
$K_2S_2O_5$ 0.5 g
Ascorbic acid 1.0 g
Cleland's reagent 0.1 g
Tween-20 4.0 ml 10%
Tris 1.5–2.0 g (to pH 8.5)
Water to 100 ml.

Temed : N, N, N', N' – tetramethylethylenediamine
Tris : 2–amino-2-hydroxymethyl 1, 3-propanediol
Bisacrylamide : N, N' – methylene bisacrylamide
Cleland's reagent : dithiothreitol
Tween 20 : polyoxyethylene (20) sorbitan monolaurate

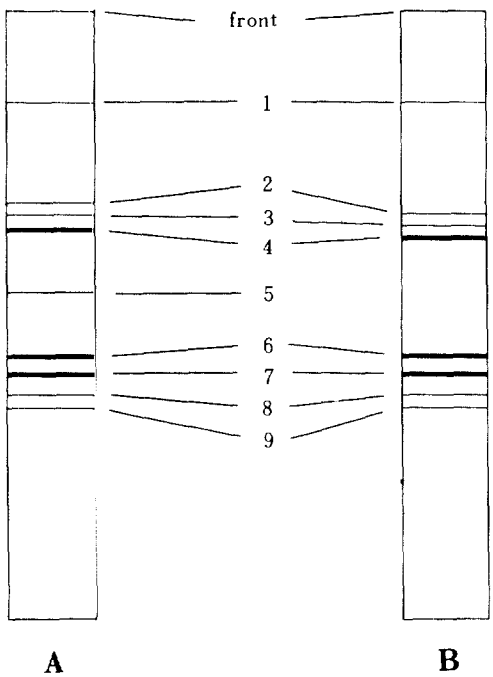
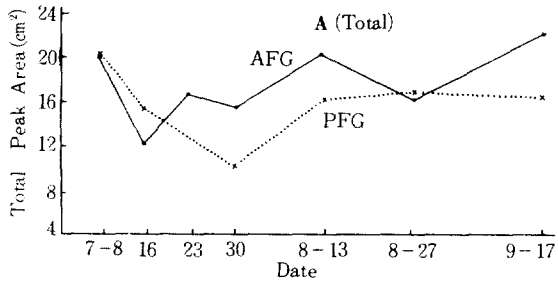
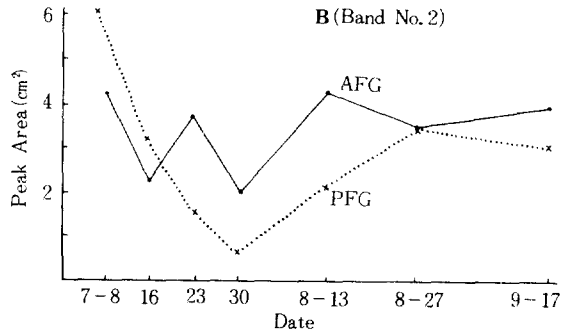
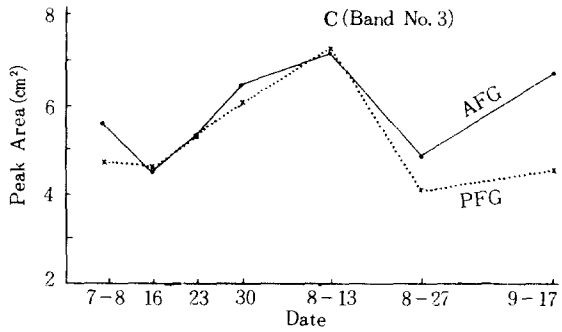
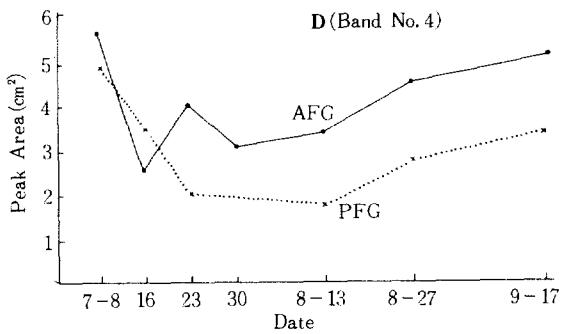


Figure 2 : Diagrams of gels after electrophoresis of bud tissue from abundant-flowering (A) and poor-flowering (B) groups of slash pine. Six major bands (Nos. 2, 3, 4, 5, 6, 7) and three minor bands (Nos. 1, 8, 9) were observed. The number 5 band was observed in all the samples of abundant-flowering group, while this band was absent in most of the samples of poor-flowering group.









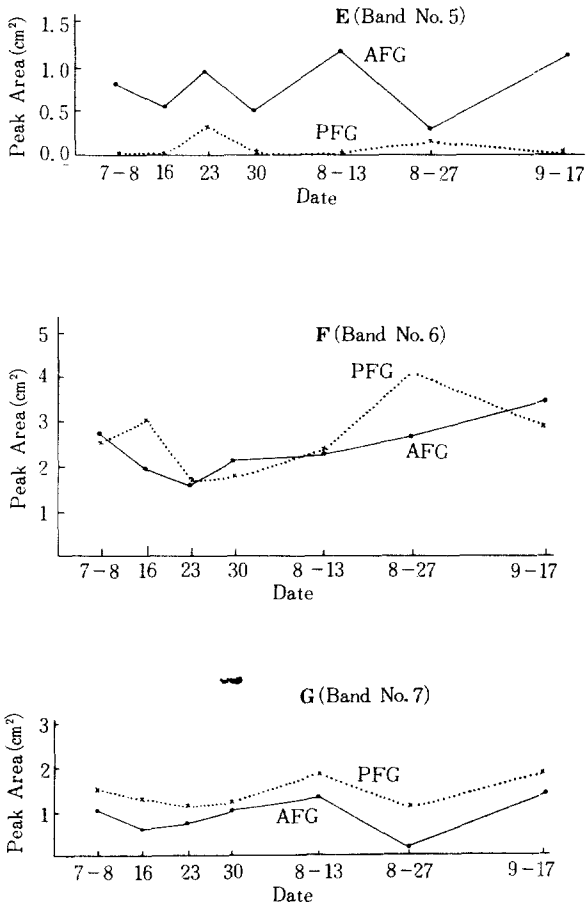


Fig. 3: Peroxidase isozymes in slash pine buds collected from abundant-flowering (AFG) and poor-flowering groups (PFG). Isozymes were determined by acrylamide gel electrophoresis as described in the text.

A. total peak area (sum of individual peak areas): B. No. 2 band; C. No. 3 band; D. No. 4 band; E. No. 5 band; F. No. 6 band; G. No. 7 band. The number 5 band in PFG (Figure E) was absent in most of the samples of PFG. A number given to each band and its relative position from gel front were illustrated in Figure 2.

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