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Changes of Catalase and Peroxidase Activities with Indole Acetic Acid in the Dormant Bark of *Populus euramericana* cv. *gelrica* *1

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休眠期間中의 *Populus euramericana* cv. *gelrica* 樹皮 組織內에서 일어나는 過酸化物除去酸素 活性및 植物 호르몬의 變化에 관한 硏究*1

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摘 要

포플라 細胞의 Sucrose gradient 遠心分離에 나타난 細胞분획은 6개의 band 및 침전으로 分離되었다.

Peroxidase의 活性變化는 休眠期에 높아졌으며, 自發的 休眠이 타파되면서 낮아지기 시작하였다. 休眠期에 급격히 높아진 Peroxidase 活性은 Peroxidative 活性을 지닌 細胞內 Microbody에 存在하는 것으로 사료된다.

Catalase의 活性은 11月, 12月, 1月의 休眠期에 活性이 낮았으며, IAA의 농도는 休眠初期에 감소하기 시작하여, 12月에 最低가 되었다. 自發休眠이 타파된 1月부터는 IAA 농도가 증가하기 시작하여 4月의 發芽期까지 계속되었다. 이와같은 Peroxidase, Catalase를 비롯한 IAA의 變化는 봄철의 發芽 및 再生長과 가을철 休眠調節에 영향을 미치는 것으로 사료된다.

I. Introduction

The deciduous trees resume the growth in the spring after dormancy. The dormancy and regrowth cycle acts important role in the growth regulation and wood formation (1, 7). As the dormancy has developed, the plant cells show the resistance to dehydration and freezing(2, 13). Sagisaka(11) reported that the water content in dormant bark was reduced from 70%(growth period) to 50% of the total fresh weight not to be freezed. The accumulation of protein and starch relating with antifreezing was shown in the dormant shoot of popular(9, 11, 13). This accumulated starch slowly degraded into sugars during winter.

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Asada(2) found the microbody which has the peroxidase activity in the dormant cells of popular, cherry, and pear trees. In the treatment of Glucose-1-$C^{14}$and Glucose 6-C^{14} to doramnt shoot, most $^{14}CO_2$ was obtained from the former indicating the pentose phosphate cycle is more active than the glycolysis cycle in trees(10). It was reported that the NADPH supplying system can supply enough electron not only to Glucose-6-Phosphate(G-6-P) dehydrogenase and Sphogluconate-6-P (S-6-P) dehydrogenase and but to glutathione and ascorbic acid metabolism in the tissue of high activities of G-6-P dehydrogenase and S-6-P dehydrogenase(9, 10, 11, 13, 14). The concentration of the oxidized products in trees is stable as micro mole through the year. On the other hand, the dormant shoot cell treated long-term cold contains increased oxidized products and decreased esterized sugar-phosphate, reduced glutathione and inactive G-6-P dehydrogenase(12).

The increase of oxidized products resulted from accumulation of oxidized products due to the low activity of removing system rather than the *de novo* synthesis of oxidized molecules(4, 6). Thus accumulated oxidized product resulted in plant death by subsequent biochemical reaction (9, 14). Alvin (3) found that the concentration of ascorbic acid in the willow had increased as dormancy developed. Others reported that each tissue contains the appropriate indole acetic acid regulating the cell division, growth, and formation of organ(1, 8).

Ⅱ. Material and Methods

1. Plant Material

The 1-year-old shoots from 30-year-old *Populus euramericana* cv. *gelrica* were collected for sample. The bark was removed with sharp razor and analyzed immediately.

2. Sucrose Gradient Centrifugation

The bark was extracted in 50mM Tris-HCl, pH 7.5, containing 0.4M sucrose, 1mM EDTA, and 0.4% BSA with polytron(Kinematica CH-6010) at 0-4℃. The crude extract was centrifuged at 2,000×g for 5min. After discard pellet, the supernatant was ultracentrifuged with Centrikon T 2080 at 40,000×g for 25min using TFT 65.3g angle rotor.

The pellet was suspended in extraction buffer. The sucrose density gradient ultracentrifugation with pellet was performed with Beckman sw 27-1 swing rotor at 100,000×g for 60 min in 2ml of 1.80M, 2.5ml of 1.30M, 3ml of 0.88M, 3ml of 0.66M, and 3ml of 0.55M sucrose in Polyallomer test tube. Six bands and yellowish brown preciptation were collected with Pasteur pipet and used for enzyme and hormone source.

3. Enzyme Assay

Peroxidase activity was assayed in 0.1ml of 50mM phosphate buffer, pH 6.4, 2ml of 0.3 mM guaiacol, and 0.01ml of 0.14mM H_2O_2(5). The enzyme source(0.1ml) was then added with distilled water to make 3ml mixture and incubated the mixture at 25℃. The activity was measured at 470nm with spectrophotometer Catalase activity was assayed according to Luck(7) using 0.15ml of 50mM phosphate buffer, 3μl of 11.6mM H_2O_2, 0.05ml of enzyme source and

1.297ml of distilled water at 25℃. The activity was checked at 240nm.

4. ELISA(Emzyme Linked Immuno Sorbent Assay) for IAA

IAA measurement by ELISA was conducted by Weiler's method with monoclonal antibody of mouse(16). Freezed alkaline phosphate tracer of IAA was dissolved in the 50mM Tris-HCl buffer containing 0.1M NaCl, and 1.0 mM $MgCl_2 6H_2O$.

The dissolved 100μl of tracer and 100μl of sample were mixed and incubated in polyacrylamide well at 4℃ for 3 hrs. After incubation, 200 μl of p-nitrophenyl phosphate was added after discard the solution and incubated at 37℃ for 60min. The incubation was stopped with 100μl of 5mM KOH and measured at 405nm using IAA(MW-175.2) as standard.

Ⅲ. Results and Discussion

The popular bark tissue was separated into two fractions, supernatant and pellet at 40,000 ×g for 25 min. This supernatant containing soluble cell constitues was used for enzyme and IAA assay. The pellet was again fractioned into 6 bands and yellowish-brown precipitation in sucrose density ultracentrifugation at 100,000×g for 60 min.

Fragments of cell wall and plasmamembrane were found in band 1 which was in top. Band 2, 3, and 4 had the liposome. Chloroplasts were present in band 4 and 5. Mitochondrias were present in band 5 and 6. The yellowish-brown pellet which was in bottem contained nucleus. The microbody showing peroxidase activity in dormancy was found in band 6. The density of microbody was similiar to that of mitochondria. These six bands and a pellet were analyzed for peroxidase and catalase activity and IAA concentration from early stage(September) of spontaneous dormancy to late stage(February) of compulsory dormancy.

Peroxidase activity in supernatant began to increase from September to October, then it was decreased continuously until the breakage of spontaneous dormancy(Fig.1).

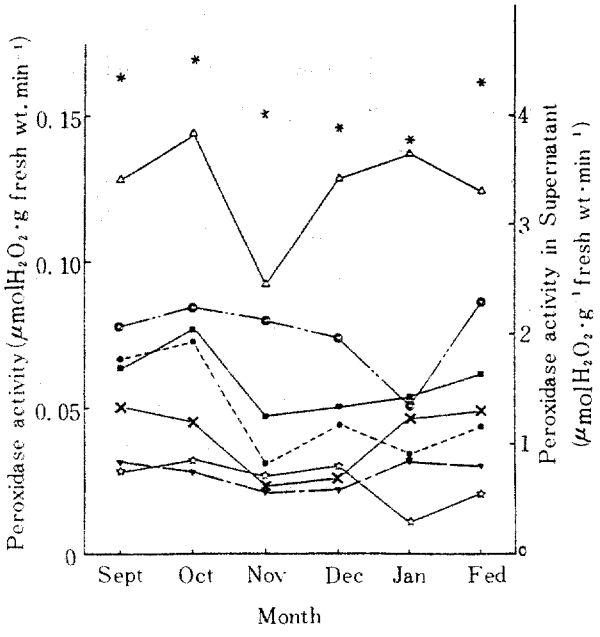

Fig. 1. Changes in the peroxidase activity in the soluble and particulate fractions in the poplar bark. Soluble peroxidase activity(✱) was measured in the supernatant after centrifugation at 40,000xg for 25min. The symbols of the bands were band 1(×), band 2(▼), band 3(☆), band 4(●), band 5(■), band 6(△), and pellet(◎).

After a flush in the compulsory dormancy, it was decreased prior to bud germination (data not shown). In the 7 fractions of sucrose density, these activities showed similiar pattern to supernatant although the activity was quite low comparing with soluble fraction. The cha-

nge of activity in the band 6 obtained from microbody was apparent and showed higher activity in the 7 fractions. During the spontaneous dormancy, band 6 showed high activity. And from the begining of compulsory dormancy, the activity was again increased prior to regrowth. Upon germination, the activity was sharply decreased showing lowest value in the year.

Sagisaka(13) and Asada(2) found that the peroxidase activity in poular was low during growth period. This low activity began to increase as dormancy progressed. They also reported that the high activity was found in the microbody which is apparent in the bark from the early dormancy.

The activity of catalase in the soluble fraction was extremely high showing 120 μM H_2O_2 per gram fresh weight per minute at October which is middle of spontaneous dormancy.(Fig 2). From October, it was gradually decreased down to 20 μM until breakage of dormancy, then increased with spring growth. The general pattern of activity change in the 7 fractions was similiar to each other although band 6 showed higher activities.

In the cells, the peroxide is hydrolyzed to water and oxygen by peroxidase and catalase. The peroxide produced through photosynthesis is degraded by catalse which is present in chloroplast. It is generally accepted that the high activity of catalase during growing season is to remove the peroxide produced in the photosynthesis. But the high activity in early dormancy in this experiment indicates another function of this enzyme to protect plant from the high concentration of oxidized products obtained in the process of dormancy. Peroxidase seems to have more sensitive protect

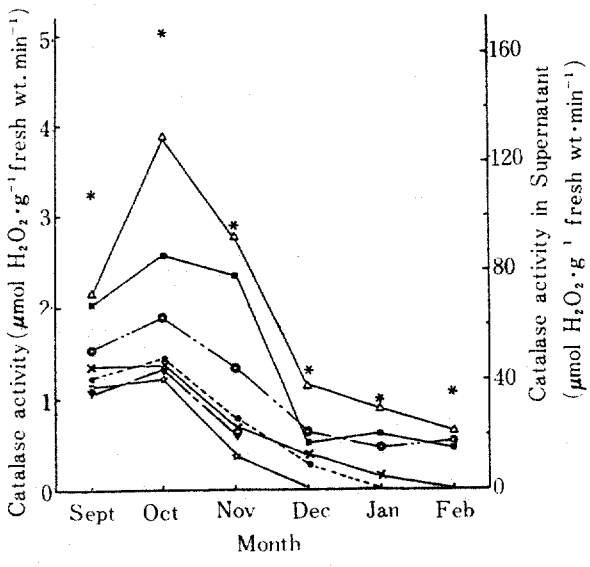

Fig. 2. Changes in the catalase activity in the soluble and particulate fractions in the poplar bark. Soluble catalase activity (✱) was measured in the supernatant after centrifugation at 40,000xg for 25min. The symbols of the bands were band 1 (×), band 2 (▼), band 3 (☆), band 4 (●), band 5 (■), band 6 (△), and pellet (◎).

function because it was present in special microbody and its activity was proportional to the depth of dormancy. Several worker also found that peroxidase activity was low in growing season and high in dormancy (2, 13). The change of IAA content in soluble and 7 fractions showed typical pattern in perennial plant(Fig. 3). In the supernatant and 7 fractions, decline of IAA as dormancy developed was observed and increase was accompanied with regrowth Especially the band 6 which was consisted of microbody appeared high IAA content comparing with other pellet fraction. This high concentration and changes against with the peroxidase activity is likely to be a complexed regulation system for plant growth and development. Peroxide present in popular bark affects the synthesis of lignin, flavonoid, and ethylene and regulation of IAA(12). But the

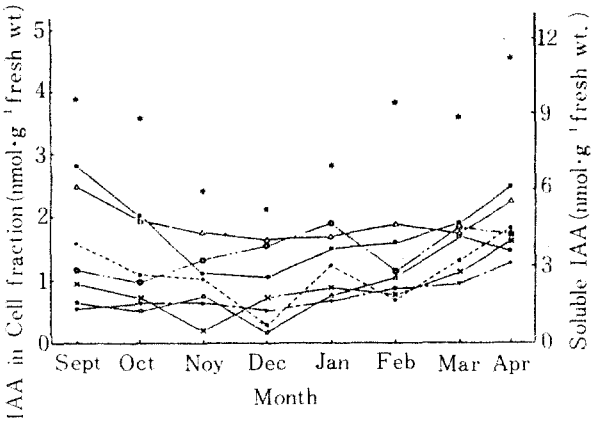

Fig. 3. Seasonal changes in the IAA concentrations in the soluble and particulate fractions in the poplar bark. The symbols of the soluble IAA (*), band 1 (×), band 2 (▼), band 3 (☆), band 4 (●), band 5 (■), band 6 (△), and pellet (◎).

high concentration than normal can be critical to living organism. In the popular, peroxidase and catalase relating with IAA seem to have function of removal of overproduced peroxide to protect itself in the dormancy.

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