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Seasonal Expression of OMT Gene in Relation to Lignin Biosynthesis in Two Poplar Species

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Abstract

We analyzed lignin content and expression of OMT gene during growth season in two hybrid poplar species. OMT gene expression was observed mainy in the developing secondary xylem where major quantity of lignin occurs. Lignin content in the xylem tissue increased as plant resumed growth in the spring and reached the highest in the late August. Change in lignin content was concurrent with that of OMT gene expression, indicating OMT is a key enzyme in lignin biosynthesis.

Key words : *wood quality, pulp, gene expression, developing secondary xylem*

Introduction

Wood is a leading industrial raw material and an important component of the world economy. This natural resource is diminishing rapidly by human demands and ecosystem destruction.

For a better and efficient use of trees, we need to improve traits that are directly involved in wood quality. One of the major goals in tree breeding is to improve the structural properties of wood and wood-derived fiber. However, it has been difficult to achieve the improvement using conventional breeding methods alone. The large size and long generation times of forest trees are significant barriers to rapid genetic improvement. Recombinant DNA techniques could accelerate the process of tree breeding by the use of the directed modification of wood properties. Most traits, however, related to wood quality are quqntitative ones that are under the multigene control. Thus, the application of genetic manipulation is restricted to single gene traits, so far.

Lignin in plant is biosynthesized from monomeric units which are made within cells of lignifying tissues. They are, then, secreted into intracellular spaced and between the plasma membrane and cell wall. Generally, lignin constitutes about 15 to 30% of the dry weight of woody tissue in plants[1]. From a quantitative point of view, lignins represent the second most abundant organic compound on earth after cellulose accounting for approximately 25% of plant biomass. Lignins also provide rigidity and structural support to plant tissues

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and water-proof the vascular elements allowing the conduction of water and solutes. Although lignins play important roles in plants, they are obstacle to the utilization of plant biomass, especially in pulp and paper industry where they have to be removed from wood. Removal of lignin from wood in paper pulping is the process causing environmental pollutions and energy waste. Pulping costs would be lowered by the use of less energy resulting in less pollution released into the environment.

The large variations of lignin content among plants indicate that the level of lignin could be genetically reduced without an adverse effect on the plant growth and development. Thus, reduction of lignin by genetic manipulation can offer an efective way for improving pulping properties of woods.

There are few genes that are involved in lignin biosynthesis. These are genes encoding phenylalanine ammonia-lyase (PAL)[2,3], O-methyltransferase (OMT)[4,5], and cinnamyl alcohol dehydrogenase (CAD)[3,6]. Among them OMT has been known for playing a key role in the pathway[4,7]. It catalyzes a meta-specific methylation of dihydroxy cinnamic acids. In the phenyl propanoid pathway from which monolignols are derived, OMT catalyses methylation of caffeic acid to ferulic acid and 5-hydroxyferulic acid to sinapic acid. Modulation of the lignin content and structure by antisense suppression of gene expression has been attempted for several enzymes in the phenylpropanoid pathway.

In the present paper, we describe the relationship between the expression of OMT gene and lignin synthesis in two hybrid poplar species as the first step toward the creation of pulp wood with reduced lignin content. Currently, isolation and cloning of OMT gene from these two species and transformation of plants with antisense-OMT gene is being underway by our group.

Materials and Methods

Plant material

Cuttings were taken from hybrid poplar stock plants grown in the nursery of Kyungpook Forest Environment Institute in Kyungju in Feb. 1996. Cuttings were rooted in the sandbed in the greenhouse. Rooted cuttings were transplanted in 30cm-diameter plastic pots in April and then placed outside. They were grown outside until they were collected for experiments in 1997.

Collection of developing secondary xylem

Stems (1.5 to 2 cm in diameter) were cut and the bark was peeled from the stem to expose the developing secondary xylem (DSX). The layer of developing secondary xylem was scraped from the stem with a razor blade and immediately frozen in liquid nitrogen. The secondary xylem was collected to determine lignin content and to extract total RNA for OMT gene expression at four week intervals from March to September.

RNA extraction and northern blot analysis

Total RNAs of leaf, cambium and DSX from the two species were extracted by LiCl based method described by Parsons *et al*[8]. Liquid nitrogen-frozen tissues (1.0 g) were ground and mixed with 2 ml of extraction buffer (100 mM Tris, pH 8.0 ; 20 mM EDTA ; 0.5 M NaCl ; 0.5% SDS ; 0.1 M 2-mercaptoethanol). One-fifth volume of buffer-equilibrated phenol was added to the mixture and incubated at 65℃ for 10 min. One-fifth volume of chloroform was added and the mixture was placed on ice for 15 min. After centrifuged at 1,4000 g for 10 min at 4℃ the aqueous phase was transferred to new tube and one-fifth volume of 10 M LiCl was added to precipitate the RNA. After 20 min on ice, RNA was recovered by centrifugation at 10,000 g for 10 min at 4℃. Then, RNA was dissolved in TE, precipitated by 3 M sodium acetate (pH 5.2) and 2 volume of ethanol, and finally dissolved in DEPC treated water. Electrophoresis and northern blot analysis (using OMT cDNA cloned from aspen plant as a probe)[4] were carried out using general methods described in the manual [9].

Lignin content determination

DSX was collected by the same method described above. Lignin content of the DSX was determined by the Klason-lignin method[10].

Collected DSX was dried in drying oven at 50℃ for 24 hr and ground until it could pass through 100-mesh (150㎛ in diameter). The milled sample (500 ㎎) was treated with 72% sulfuric acid (10 ㎖) at 20℃ for 4h. After the incubation, the mixture was diluted with water to a sulfuric acid of 3% and boiled for 4 h. The mixture was, then, filtered through IG4 glass filter and the acid-insoluble residue was dried at 105℃ overnight. Before the filtration, glass filters were completely dried and their weight was measured.

Results and Discussion

One of the difficult problems in the production of cellulose fibers from pulpwood species is the high amount of lignin that incrust cellulose fibers in wood, making the lignin-removing processes energy and chemical-intensive. As demands for wood fibers increases, the amount and cost for removing lignin also increase. Typical energy consumption levels for removing one ton of lignin are 60 to 80×10^6 BTU and 4,000 kWh and about 800 kg of NaOH are needed to remove every ton of lignin. Consequently, we can expect that new tree strains with low lignin content will reduce energy and chemical consumption for the production of wood fibers. To achieve this long-term goal, we first determined the relationship between OMT, a key enzyme in lignin biosynthesis, and lignin content.

OMT gene expression in different tissues

OMT gene expression was distinctive and high in the developing secondary xylem as compared to those in cambium and leaf (Fig. 1). As shown in Fig. 1, expression of OMT gene in cambium and leaf is barely detectable. It indicates that secondary xylem is the principal tissue

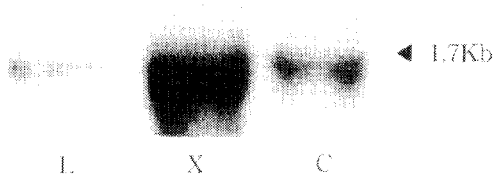

Fig. 1. OMT gene expression in different tissues of poplar species (L : Leaf, X : Developing secondary xylem, C : Cambial tissue).

in which OMT gene is expressing. Lignin biosynthesis occurs in greatest quantity in the secondary cell walls of fibers, xylem vessels and tracheids. Thus, high expression of lignin biosynthesis-related OMT gene in DSX, which includes all these cells, can be expected. The similar is expression pattern has also been demonstrated in other species[4,11,12].

Seasonal expression of OMT

Northern blot analysis of OMT DNA to RNA isolated from DSX at different times in the growing season revealed that OMT gene is actively expressed from late April to late September. Northern blot analysis demonstrated that OMT cDNA probe hybridized to a 1.7 kb transcript from DSX (Fig. 2). In both species, the gene expression showed high during the late July to August. The expression pattern observed in this study is different from the result reported by Bugos *et. al*[4]. in which two expression peaks were observed in early June and late July. They suggested that the biphasic pattern of gene expression may due to the characteristics of xylem tissue development which has two phases called earlywood and latewood. This biphasic pattern was not observed in our study. And expressions of OMT gene were detected from early June in their study with aspen. This different in OMT gene expression might be due to the climatic difference between the locations. Trees in Michigan's upper peninsula start growth in May while trees in our place resume growth in late March. Earlier

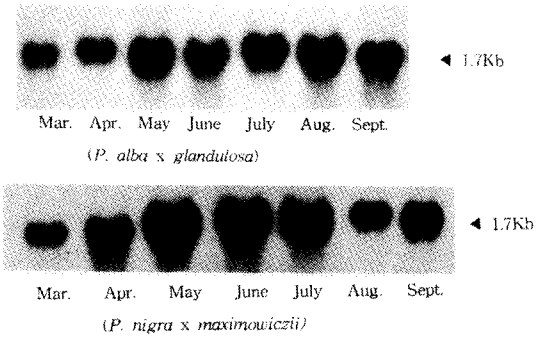

Fig. 2. Seasonal expression patterns of OMT gene observed in developing secondary xylem samples collected from March to September.

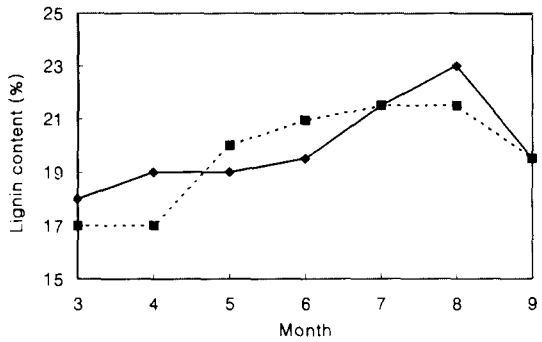

Fig. 3. Analysis of lignin content in developing secondary xylem of two hybrid poplar species from March to September
(◆*P. alba* x *glandulosa*, ■ : *P. nigra* x *maximowiczii*).

and longer growing season is believed to cause the expression pattern observed in our study. Many internal factors are known to be involved in the transition in xylem differentiation. Shoot-derived auxin, abscisic acid, ethylene and glutamine have been implicated in the differentiation. Thus, different expression patterns are expected due to internal and environmental factors in different places.

Lignin content analysis

To demonstrate that OMT is directly involved in lignin biosynthesis, the lignin content in developing secondary xylem was measured at three week intervals from March to September (Fig. 3). Contents of lignin in March were 17% in *P. nigra* x *maximowiczii* and 18% in *P. alba* x *glandulosa,* respectively. During active growing period, from April to August, they increased steadily and reached the peak in the late August. Then, the contents decreased as the growing season ends. The changing pattern of lignin content seemed to be in a good agreement with that of OMT gene expression. This result indicates that OMT plays an important role in lignin biosynthesis and the approach to reduce lignin content by antisense suppression using OMT gene is feasible. Previous studies have also proven the feasibility of this approach. Ni *et al*[7]., have transformed tobacco with a construct containing an alfalfa OMT cDNA fragment in antisense orientation under the control of the CaMV 35S promoter with a double enhancer. The constitutive expression of the transgene led to a significant reduction in the lignin content. Dwivedi *et al*[5]., also used an antisense OMT gene cloned from aspen to transform tobacco. They reported a reduction of lignin in transformed plants without causing any growth abnormality. In addition to the change in wood quality, reduction of lignin content can also affect the digestibility of forage crops.

The improvement of the pulping characteristics of transgenic plants will provide pulp industry with benefits. Minor changes in wood composition would have a significant economic impact in view of the huge consumption of pulp products. By using pulp wood with reduced lignin content, pulp industry can reduce energy needs and pollution.

Our present study, as a preliminary step toward the production of low lignin-containing pulpwood, clarified a close relationship between OMT gene expression and lignin biosynthesis and offers a firm base for further studies.

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초록 : 2 종의 포플라수종에서 리그닌생합성에 관계된 OMT유전자의 발현

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리그닌함량이 감소한 펄프재의 육성을 위해 OMT유전자와 리그닌생합성 간의 관계를 2종의 포플라 수종을 이용하여 실험하였다. OMT유전자의 발현은 리그닌생합성이 가장 많이 일어나는 developing secondary xylem에서 높았다. 이 사부조직에서의 리그닌함량의 증가는 봄에 성장을 개시할 때부터 증가하기 시작하여 8월 말 경에 가장 높았다. 이러한 리그닌의 증가는 OMT유전자의 발현 증가와 일치하였으며, 이는 OMT유전자가 리그닌생합성에서 매우 중요한 역할을 함을 나타내는 것이다.