Wood Characteristic of Superior Sengon Collection and Prospect of Wood Properties Improvement through Genetic Engineering

N. Sri Hartati, Enny Sudarmonowati, Widya Fatriasari, Euis Hermiati, Wahyu Dwianto, Rumi Kaida, Kei'ichi Baba, and Takahisa Hayashi

Abstract

Many tree breeding programs ranging from conventional to molecular genetics approach were applied to produce clone or tree genetic industrially desirable wood. This study was aimed to evaluate the wood properties of selected sengon (*Paraserianthes falcataria*) tree which has high score of growth parameter and stem form and to evaluate stem properties of transgenic sengon and mangium (*Acacia mangium*) overexpressing wall hydrolases. Physical and chemical wood properties including basic density, shrinkage and thickness swelling, estimated stand volume, cellulose, lignin and water content were examined for two selected plus tree sengon namely PI and PII, which were grown at Germ Plasm Collection Garden of Research Centre for Biotechnology - LIPI. Both of two sengon tree has high value of estimated stand volume and basic density (0.43 and 0.49 g/cm^3). The PII tree has lower lignin and water content than P I. Cellulase overexpression in sengon and xyloglucanase overexpression in mangium could alter stem cell walls composition. Transgenics mangium stem have higher cellulose content (37.70~53.64%) and lower hemicelluloses content (30~40%) than the wild type.

Key words: sengon (*Paraserianthes falcataria)*, mangium (*Acacia mangium*), wood properties, transgenics, cellulose, xyloglucanase.

Introduction

Wood is a natural and renewable resource used for a diversity of product such as building materials for house construction, furniture, energy, pulp and paper. Increasing population and improving standards of living in both developing and developed countries are expected to drive the growth of demand for wood for both solid wood and pulp products (Lutz 2003). With a growing human population the demand for wood and woody product will generally increase faster than the supply of wood in the future. Much recent research on forest trees is focused on improving not only wood biomass production, but also the properties of wood. To a large extent, future forestry is likely to rely on plantations with highly productive trees grown for specific purposes.

Wood is an extremely versatile material with a wide range of physical and mechanical properties among the many species of wood. It is also a renewable resource with an exceptional strength-to-weight ratio. Wood is a desirable construction material because the energy requirements of wood for producing a usable end-product are much lower than those of competitive materials, such as steel, concrete, or plastic. Wood quality concept can be generally defined as a measure of the characteristics of wood that influences properties of products made from. The suitability of wood for a variety of purposes is influenced by several factors including density, uniformity, proportion of heartwood, fiber length, occurrence of juvenile or reaction wood (or both), cellular composition, presence of knots, grain orientation, and chemical composition. The important characters in wood to be used for one product are often different from those for another product. In one case, their quality could be measured in terms of density, uniformity of growth rings, and percent of knot-free wood, whereas in another instance, properties such as proportion of latewood, cellulose yield, and the fiber-to-vessel ratio may be primary quality indices. In solid wood product, strength is important.

The emphasis of selective tree breeding program was placed on increasing volume and yield and changing the mechanical properties of wood. The field of genetics offers perhaps the greatest potential for improvement of wood yield and quality. The efforts in this area were conducted on identifying trees that exhibites superior growth or form. Seed were collected from these trees and planted in nurseries to raise new generations of trees having many of the same characteristics. Vegetative propagation was also employed in reproducing clones of certain species.

A more recently developed technology involves tissue cultures and biotechnologies, including DNA recombinant technology, to the tree-improvement field. New techniques in biology have provided us with research tools to accelerate tree breeding. Such techniques offer opportunities for very rapid gains in tree improvement by passing the long developmental periods of woody plants.

Sengon belongs to the subfamily Mimosoideae of Leguminosae is an exceptionally fast growing tree, native to the eastern island of the Indonesian archipelago and New Guinea. It is widely planted in Indonesia, in industrial plantations in Java, Sumatra, Kalimantan, Sulawesi, Nusa Tenggara and Molluccas, and in small holder plantations in Java. The tree is useful for timber material, packing case and furniture. Therefore, it is expected to be one of the most useful tree species for industrial forests. This species could have the ability to grow in poor or marginal land and make the land fertile as it is categorized as nitrogen fixing tree species (Binkley *et al*. 2003; Shively *et al*. 2004; Kurinobu *et al*. 2007; Siregar *et al*. 2007).

Acacia mangium is a tropical species that is capable of colonizing infertile sites. The tree grows well not only in cleared forest sites, but also in degraded sites, such as weedy *Imperata* grasslands and mining sites. Important attributes of *A. mangium* include rapid early growth, good wood quality (for pulp and timber), and tolerance of a range of soil types and pH. Since its introduction to Sabah, Malaysia, as an exotic in 1966, *A. mangium* has become one of the three or four most common plantation tree species in Asia. As a good characteristic of *A. mangium* growth, it is selected as one of the industrial Timber Estate (HTI = *Hutan Tanaman Industri*) commodities to promotes the production of raw material for wood-based industry.

Tree improvement program through identification of the tree that exhibited superior growth performance and wood quality and application of genetic engineering technique to obtained superior tree resources could support the wood raw material supply. This study was aimed to evaluate the wood properties of superior sengon tree and to evaluate stem properties of transgenic sengon and mangium.

Materials and Methods

Plant Materials

Two superior sengon trees grown at Germ Plasm Collection Garden of Research Centre for Biotechnology - LIPI were used as a material of physical and chemical wood properties. We also used 1 year old transgenic sengon overexpressing poplar cellulase and 8 months old mangium overexpressing xyloglucanase that were produced previously.

Wall Analysis

Polysaccharides of hemicelluloses were successively extracted three times with 24% KOH containing 0.1% NaBH4. The soluble fraction was used for total sugar determination. Total sugar was determined by the phenol-sulfuric acid method. The amount of cellulose was determined by measuring the acid-insoluble residue; the samples were extracted with acetic/nitric reagent (80% acetic acid/concentrated nitric acid, 10 : 1) in a boiling water bath for 30 min. Lignin content was determined by the Klason method.

Physical Property Analysis

Wood sengon sample of 2 x 2 x 2 cm strips were used for basic density, shrinkage, water content and thickness swelling. The basic density was calculated by dividing the air-dry weight or oven-dry weight (60°C) by the green volume measured from the water displacement.

Results and Discussion

Physical and Chemical Properties of Superior Sengon Trees

Physical and chemical wood properties including basic density, shrinkage and thickness swelling, estimated stand volume, cellulose, lignin and water content of 13 years old superior sengon tree were presented in Table 1. Both of selected sengon tree exhibited best growth performance.

Wood density is an important determinant of wood quality and dry weight yields per volume of woody material. Because of this, even large increases in volumetric growth might be viewed as undesirable if the result were a significant decrease in specific gravity. Thus breeding program generally aimed at controlling wood density as well as improving growth rate. The oven-dry density of P1 and P2 were 0.49 g/cm^3 and 0.43 g/cm^3, respectively (Table 1). The density value was similar with the result of the basic density of the five selected sengon trees grown at Serpong that indicated 0.46 g/cm^3 of maximum basic density (Ishiguri *et al*. 2007). The P1 tree that had higher density value indicated higher cellulose content. Based on the density value, cellulose content and stem volume estimation the result indicated that P1 has a great potential as a genetic resource tree breeding program. As a follow-up to superior tree identification, the seed were collected from these trees and planted in nurseries to raise new generations of trees having many of the same characteristics (Figure 1).

Lignin content analysis shows that the P2 tree had low lignin content (17.93%). During the manufacture of high-quality paper, lignin is chemically separated from the polysaccharide components of wood during pulping and bleaching reactions. Lignin extraction consumes large quantities of chemicals and energy leading to a poor environmental image for the industry (Baucher *et al*. 2003). Any woods, which have low lignin content would be expected to be useful as a raw material for pulp and paper. The benefits of removing as much lignin as possible in order to avoid residual lignin, which causes discoloration and reduces paper brightness have to be balanced against the loss of pulp quality and strength that result when cellulose is significantly degraded.

Wood Chemical Properties of Transgenic Sengon and Mangium

Xyloglucan is one of the major hemicellulosic polysaccharides in the primary cell walls of higher plants, making up about 20~25% of the dry weight of the primary cell wall in dicotyledons angiosperms, 10% in grasses and 2~5% in soft woods. The uniform assembly, structural similarity to cellulose and decreasing amount of xyloglucan as plant growth progresses could indicate the important role for this xyloglucan in the biosynthesis of the cell wall in the growing plant. Xyloglucan is intimately associated with cellulose microfibrils by hydrogen bonding, thus providing the load-bearing network of the cell wall which protects the

Figure 1. Growth performance of superior sengon P1 (left) and 2 years old sengon stand derived from P1 seed.

Table 1. Physical and chemical properties of 13 years old superior sengon.

Wood properties	Sample	
	P 1	P 2
Physical properties:		
Air-dry density (g/cm^3)	0.49	0.43
Oven-dry density (g/cm^3)	0.39	0.35
Thickness shrinkage (%)	2.04	2.37
Volume shrinkage (%)	4.19	4.93
Thickness swelling (%)	2.68	6.37
Volume swelling (%)	3.70	7.85
Stem volume estimation (m^3)	1.586	0.67
Chemical properties:		
Cellulose content (%)	52.23	51.54
Lignin content (%)	26.57	17.93
Water content (%)	16.57	12.64

Table 2. Chemical properties and stem volume estimation of transgenic sengon and mangium.

Wood properties	Plants			
	Sengon overexpressing cellulose (1 year old)		Mangium overexpressing xyloglucanase (8 months)	
	Wild type	Transgenic	Wild type	Transgenic
Cellulose (%)	45.42	46.13	59.98	62.62
Lignin (%)	26.50	25.30	19.14	22.18
Hemicelluloses (%)	21.00	20.30	22.45	27.05
Stem volume estimation (cm^3)	428.51	1225.10	63.69	101.41

cell wall from collapsing due to osmotic stress. In addition to this structural function, xyloglucan also has a role in the regulation of cell enlargement during plant growth (Benko *et al*. 2008). The interaction between cellulose microfibrils and the hemicellulose-xyloglucan network is believed to represent the major load-bearing structure in the primary cell walls (Shani 2004; Hartati *et al*. 2008) .

Overexpression of plant cellulase in plants does not lead to a lack of cellulose; rather, it modifies the cell walls by trimming off disordered Glc chains from the microfibrils. This action has been demonstrated in Arabidopsis (*Arabidopsis thaliana*) overexpressing poplar (*Populus alba*) cellulase (Park *et al*. 2003). Overexpression of xyloglucanase in poplar could enhance stem and leaf cell growth (Park *et al*. 2004). Xyloglucanase directly hydrolyzes xyloglucan tethers between microfibrils. Loosening of xyloglucan between cellulose microfibrils in the cell wall could promotes plant growth. Based on the carbohydrate analyses of cell walls, it appears that the petiolule pulvinus and the main vein in the transgenic plants contained less wall-bound xyloglucan than those in the wild-type plants (Hartati *et al*. 2008). The question, therefore, is whether overexpression of wall hydrolases such as cellulase and xyloglucanase, total sugar (hemicelluloses), cellulose and lignin content in sengon and mangium could affect the chemical composition of wood.
The result shown that transgenic sengon and mangium had higher cellulose content than the wild type (Table 1). The effect of transgenic modification on growth response also resulted in the higher stem volume estimation of 1 year old transgenic sengon and 8 months old transgenic mangium. Overexpression of cellulase in sengon resulted in the decrease of hemicelluloses and lignin content. Nevertheless overexpression of xyloglucanase in mangium did not reduce hemicelluloses and lignin content. Transgenic mangium contained xyloglucan 7 times lower than the wild type (Hartati *et al*. 2010). It should be noted genetic modification of sengon and mangium by the overexpressions of cellulase and xyloglucanase could affect not only the growth enhancement but also modification of wood composition. Effect of transgenic modification on wood physical properties needs further research.

Conclusions

Physical and chemical properties analysis showed that two sengon collection that exhibited superior growth and high quality wood. Both of these superior sengon could be used as a genetic source to produce new superior sengon tree generations through seed production or vegetative propagation. Chemical wood composition has been changed by overexpressing wall hydrolases in sengon and mangium i.e. cellulase and xyloglucanase are potential candidates to improve wood quality through transgenic modification.

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N. Sri Hartati and Enny Sudarmonowati
Research Centre for Biotechnology
Indonesian Institute of Sciences,
Jl. Raya Bogor Km 46 Cibinong,
Bogor 16911, Indonesia
Tel. : 62-21-8754587
Fax. : 62-21-8754588
E-mail : Hartati12@com

Widya Fatriasari, Euis Hermiati, and Wahyu Dwianto
Research and Development Unit for Biomaterials
Indonesian Institute of Sciences,
Jl. Raya Bogor Km. 46, Cibinong,
Bogor 16911, Indonesia
Tel. : 62-21-87914509
Fax. : 62-21-87914510

Rumi Kaida, Kei'ichi Baba, and Takahisa Hayashi
RISH, Kyoto University, Uji 611-001, Japan
Tel./Fax. : +81-3-5477-2760
E-mail : t4hayash@nodai.ac.jp