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Review

Tree Biotechnology of Tropical *Acacia*

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Abstract To establish a sustainable society, forest biomass or lignocellulosic biomass, which is the most abundantly accumulated biomass resource, plays an important role. Therefore it is critically important to establish systems for sustainable production of forest biomass, especially tropical forest resources. Forest tree biotechnology will play a very important role in such systems. Following the completion of whole genome sequence of *Populus*, tree bioscience and biotechnology have entered into "post-genomic era". However, although tropical *Acacia* is one of the most important tropical plantation tree in Indonesia and Malaysia, its biotechnology is still at a primitive stage. Because the *Acacia mangium* genome sequence data base is far beyond the ability of university laboratories, it is important to construct expressed-sequence tag (EST) data bases for *Acacia*. *A. mangium* EST data bases are quite useful for identifying genes that are possibly involved in the expression of a given trait, and can be exploited to detect *Acacia* genes which are orthologous to those of other model plants that are involved in secondary xylem formation. In addition, establishing efficient systems for *in vitro* regeneration of transformed *Acacia* and identification of genes and metabolites responsible for expression of commercially important traits are indispensable for genetic modification of the species. Importantly, these activities must be done to benefit global environment conservation and social welfare of local communities. In this review, the current status of *Acacia* biotechnology is outlined.

Key words: *Acacia mangium*, *Acacia* hybrid, expressed-sequence tag (EST), tree biotechnology, wood formation.

The fossil resource-based industrial society which was established in the 20th century has provided us with prosperity, especially in developed countries. However it also has brought serious negative impacts on the global environment due to the increase in the atmospheric concentration of carbon dioxide, accompanied by a number of pollution problems. Therefore, it is becoming more and more important to establish a sustainable society that depends on renewable resources; otherwise humans can not survive.

Among renewable resources, it is wood or lignocellulosic biomass that is most abundant, and therefore it has received much attention lately. However, its production and utilization have been conducted based on the paradigm of a fossil resource-based society. Therefore, it is critically important to establish sustainable production and utilization systems for forest resources, so that a sustainable society can be established. This largely depends on innovations in tree and biomass conversion biotechnologies.

Deforestation and large-scale uniform tree plantation

According to the FAO report (FAO 2007, http://www.fao.org/docrep/009/a0773e00.htm), deforestation continues at an alarming rate of approximately 13 million hectares per year. At the same time, forest planting and natural expansion of forests have significantly reduced the net loss of forest area. Over the 15 years from 1990 to 2005, the world lost 3% of its total forest area, an average decrease of some 0.2% per year (FAO 2007, http://www.fao.org/docrep/009/a0773e00.htm). In Asian countries, the region as a whole experienced a net increase in forest area of about 633,000 hectares annually during 2000 to 2005. However, this is largely due to an increase of more than 4 million hectares per year in China, whereas most other countries, especially Southeast Asia experienced the large decline in forest area. The greatest forest loss occurred in Indonesia, almost 1.9 million hectares per year (FAO 2007, http://www.fao.org/docrep/009/a0773e00.htm).

In Indonesia, therefore, the logging of natural forests can not continue, and a number of large plantations of

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Abbreviations: EST, expressed-sequence tag

This article can be found at http://www.jspcmb.jp/

fast-growing trees, especially *Acacia mangium*, have been established. The total acreage of *A. mangium* plantations in Indonesia has been estimated to be approximately 1 million hectares (Yano et al. 2007), and the tree is mostly exploited for pulp. In addition, an *Acacia* hybrid (*A. mangium* × *Acacia auriculiformis*) plantation for structural material production has been established in Sabah, Malaysia (Matsumoto 2002), and its silvicultural management systems are being optimized (Omura et al. 2007; Yoshida et al. 2007).

Importance of tree biotechnology

Large scale, monoculture plantation forests can provide the benefit of reforestation. However, they conflict with an aspect of biodiversity, though they are much better than deforested lands. Hence, it is important to establish forest management systems for sustainable production and utilization of forest biomass resources in harmony with environmental conservation.

To do that, conventional forest operation has been powerful tools. In addition, traditional tree breeding, selection in breeding populations with a broad genetic base, has brought a certain amount of success. However, due to the long life cycles, long generation times, and late sexual maturity, traditional tree breeding programs require very long time intervals. Furthermore, genes responsible for versatile demands in terms of commercially important traits are often not available within the gene populations of the target tree species. For example, taking the world's economic development into account, food production will increase significantly and will occupy rich and superior lands. As a result, inferior lands will be used for biomass production. This will bring about strong demands for improvements in trees' growth and physiological characters such as drought resistance, acid-soil resistance, cold resistance, fast growth, and disease resistance, and so on. In addition, there are also many demands from the users, such as suitability for pulping and bioethanol production, suitability for the use of wood-based materials, e.g. non-taperness, circularness of cross section, less growth stress, less distortion during timber drying, smaller fibril angles, good heartwood coloration, high content of bark tannins, high strength, and high durability, and so on. These improvements require introduction of foreign genes into the target tree species, which can not be achieved by conventional technologies, such as crossing and elite tree selection. Thus, these necessitate better tree improvement programs in which modern biotechnology plays an important role (FAO, 2004).

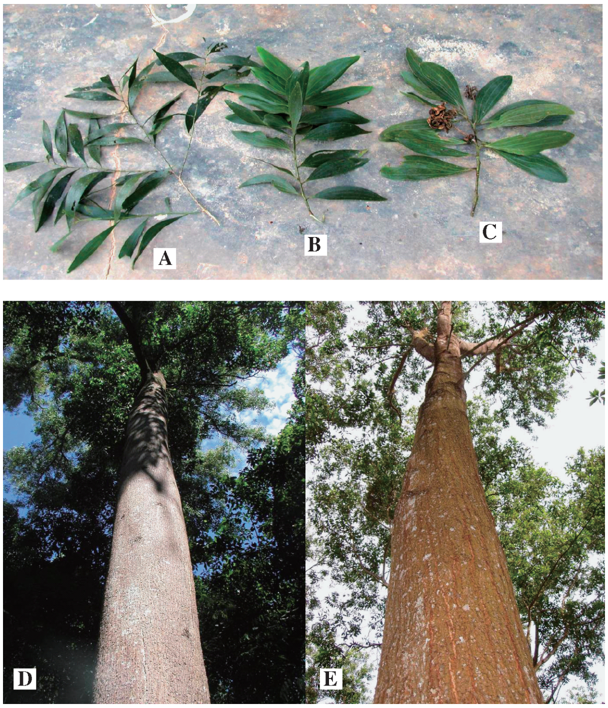

Figure 1. *Acacia mangium*, *Acacia auriculiformis*, and their hybrid. (A), (B), and (C) Phyllodes of *A. auriculiformis*, hybrid, and *A. mangium*, respectively. (D) hybrid, (E) *A. mangium*. Both *A. mangium* and the hybrid have straight trunks. Phyllodes of *A. mangium* are broder, while those of *A. auriculiformis* are falcate. Phyllodes of the hybrid are often intermediate between the parents.

Acacia mangium

A. mangium is endemic in Indonesia (Sula, Ceram, Aru, and Irian Jaya), Papua New Guinea, and Australia (Queensland) (Pinyopusarerk et al. 1993). This tree was originally introduced into Sumatra, Indonesia, and Sabah, Malaysia for environmental reforestation of deforested lands. Later, however, this species was found to be suitable for pulping purposes because of its high density (ca. 0.5) among tropical fast growing trees (Fujimoto et al. 2002) and high straightness of the trunk (Figure 1). Furthermore, this species has many advantages such as very good growth in adverse soil conditions, and a tendency to grow well in humid and hot climate. It also is competitive against other weeds, fixes nitrogen by symbiosis (Pinyopusarerk et al. 1993; Dela Cruz and Yantasath 1993). Therefore, *A. mangium* plantations have expanded rapidly during the last two decades in Southeast Asia especially Indonesia and Malaysia, and now its total acreage in Indonesia is approximately 1 million hectares (Yano et al. 2007). In addition, a plantation of an *Acacia* hybrid (*A. mangium* × *A. auriculiformis*) (Figure 1) is also commercialized in Sabah, Malaysia. *A. auriculiformis* has higher density (ca. 0.56) but its trunk is often not straight, whereas the hybrid inherits good traits from the parents: the density value between the parents (ca. 0.52) and straight tree shape (Figure 1). Hence, the elite trees of the hybrid are suitable for rotary cut veneer production and propagated by petiole cuttings and sterile micropropagation (Matsumoto 2002). As can be seen in Figure 1, the hybrid and their parents can be often distinguished by their phyllodes.

Though it has advantages, *A. mangium* also has a number of drawbacks: high incidence of knots, relatively short fiber length though still acceptable as pulp feedstock, difficulty in drying timbers, low-moderate durability (low resistance to rot and termites), low salt resistance, low drought tolerance, shade intolerance, disease susceptibility (root rot and heart rot), and sensitivety to prolonged low temperature and frost. If these drawbacks are overcome, the species can be exploited more widely for versatile purposes.

Recently, a series of mini reviews covering plantation biodiversity (Yoshimura et al. 2007), chemistry (Umezawa and Suzuki 2007), biology and biotechnology (Kuroda 2007; Suzuki and Umezawa 2007), biomass utilization (Yano et al. 2007; Hata and Joko 2007; Watanabe and Watanabe 2007) of *A. mangium* have been published.

Acacia tree biotechnology

Generally, biotechnology of commercial plants requires several basic resources, such as gene sequence information, transformation and regeneration systems, and isolation of genes responsible for the target traits. However, these basic resources are not yet established for *A. mangium*, and its biotechnology is therefore still at an early stage.

Transformation and regeneration systems

In vitro regeneration of transformed trees is still a technical limitation for many plant species, which is true for *A. mangium* and related species.

Organogenesis and somatic embryogenesis are basic techniques for establishing transformation and regeneration systems. Several papers have been published on organogenesis of *A. mangium* and related species (Nanda et al. 2004; Bhaskar and Subhash 1996; Xie and Hong 2001; Douglas 2000; Saito et al. 1993; Suzuki and Umezawa 2007), and a somatic embryogenesis system for *A. mangium* from immature seeds through embryogenic callus was reported by Xie and Hong (2001) (Suzuki and Umezawa, 2007). However, to our knowledge, only two papers have been published for genetic transformation of *A. mangium* (Xie and Hong 2002) and its hybrid (Kao 2001). Hence, more efficient transformation and regeneration systems for *Acacia* spp. must be established, which are the basis for molecular breeding.

Genome or gene expression data base

A genome project is far beyond powers of a single academic laboratory, while ESTs can be established with a very much smaller budget. Because ESTs are a collection of short sub-sequences of transcribed genes and represent a snapshot of what is expressed in a given tissue at a given developmental stage, this gene-expression information resource is useful for identifying genes that are possibly involved in the expression of a given trait in combination with transcriptomics, especially using microarray techniques.

So far, an *A. mangium* EST data base from developing xylem tissue has not yet been published, though there has been one reported for 1123 flower-specific transcripts (Wang et al. 2005). In this context, we have started to prepare an EST data base for *A. mangium* developing xylem tissue (Suzuki S, Sakurai N, Suzuki H., Shibata D, Umezawa T, unpublished).

Genes responsible for target traits

Many of the commercially important traits, such as good growth, good shape, high-strength, and so on in plants are probably controlled by multi-genes, which are not yet identified. For the time being, these traits are difficult to control by metabolic engineering approaches.

However, there are a number of genes of various plants that have already been identified as being involved in commercially important traits. Using these genes, we can immediately proceed to metabolic engineering of *Acacia* spp. once efficient transformation and regeneration systems are established. For example, down- or up-regulation of the cinnamate/monolignol pathway genes can modify the compositions and contents of lignins in *Acacia* spp. This is expected to result in financial gains from pulp processing improvements. The chance of success in this area are high, because many examples of similar experiments with other species, e.g. *Populus*, have already been reported (Hu et al. 1999; Li et al. 2003).

As for the traits, where the genes responsible have not yet been identified, we must conduct intensive and time-consuming works to identify them by differential analyses of each trait in population of given tree species, as well as in other plants including model plants. To do that, both holistic (or non-targeted) analyses (i.e. genomics, transcriptomics, proteomics and/or metabolomics) and targeted analyses are necessary. In this context, EST database construction is important. *A. mangium* EST data bases are quite useful for identifying genes that are possibly involved in the expression of a given trait in combination with transcriptomics, especially using microarray techniques. Furthermore, an EST data base can be exploited to detect *Acacia* genes which are orthologous to those of other model plants that are involved in, for example, secondary xylem formation or plant defense systems.

In addition, metabolomics or comprehensive analysis of metabolites is a powerful tool to identify compounds that are responsible for important phenotypes, such as disease resistance etc. Although no single technology for metabolomics has been established, two strategies can be adopted. One is a non-targeted and high-throughput analysis with Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS), which can cover a larger number of compounds than any other type of mass spectrometry. However, such machines are expensive and are therefore not widely distributed. Besides, this technique is weak at quantitative metabolic analysis, which is complimented by the targeted analysis. Therefore, when precise metabolite analyses are needed, it is necessary to analyze rather limited but significant numbers of metabolites precisely using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) and with stable isotope-labeled internal standards (Sakakibara et al. 2003; Sakakibara et al. 2007).

Public acceptance and area studies

When the genetic modification approach is used, social acceptance is indispensable. For example, pollen and seed dispersal causes widespread public concern over transgenic trees. One solution might be to use plant triploid trees, which are expected to be sterile. This could be achieved via the production of tetraploids followed by its backcross with diploids. Tetraploid *A. mangium* and *Aacacia dealbata* have been produced by colchicine treatment (Blakesley et al. 2002).

In the practical application of *Acacia* biotechnology, illegal logging has become a pressing issue. For example, the proportion of illegal harvest in 2001 in Indonesia was estimated to be up to 83% (Tacconi et al. 2004). In this context, the target traits must be determined with due consideration for the welfare of local communities. This implies that the tree biotechnology research and development activities must be conducted in collaboration with social sciences, especially tropical area studies.

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References

Bhaskar P, Subhash K (1996) Micropropagation of *Acacia mangium* Willd through nodal bud culture. *Indian J Exp Biol* 34: 590–591

Blakesley D, Allen A, Pellny TK, Roberts AV (2002) Natural and induced polyploidy in *Acacia dealbata* Link. and *Acacia mangium* Willd. *Annal Bot* 90: 391–398

Dela Cruz RE, Yantasath K (1993) Symbiotic associations. In: Awang K, Taylor D (eds) *Acacia mangium Growing and Utilization*. Winrock International and the Food and Agricultural Organization of the United Nations, Bankok, pp 101–111

Douglas GC, McNamara J (2000) Shoot regeneration from seedling explants of *Acacia mangium* Willd. *In Vitro Cellular & Developmental Biology: Plant* 36: 412–415

FAO (2004) *Preliminary review of biotechnology in forestry, including genetic modification. Forest Genetic Resources Working Paper FGR/59E*. Forest Resources Development Service, Forest Resources Division, Rome, pp 1–118

Fujimoto T, Akutsu H, Takizawa T (2002) Several Wood Properties of Plantation-Grown *Acacia* species. *J Hokkaido For Prod Res Inst* 16: 6–10 (in Japanese)

Hata T, Joko Sulistyo (2007) Carbon materials based on tropical fast growing trees. *Seizonken Kenkyu* 3: 59–63 (in Japanese)

Hu WJ, Harding SA, Lung J, Popko JL, Ralph J, Stokke DD, Tsai CJ, Chiang VL (1999) Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees. *Nat Biotechnol* 17: 808–812

Kao YY (2001) Genetic transformation of *Acacia magium* cross *Acacia auriculiformis* and molecular characterization of phenylalanine ammonia-lyase in quaking aspen. *PhD Thesis, Michigan Technological Univ., Houghton, MI, USA* (CAN 137: 366571)

Kuroda H (2007) A note for genus *Acacia*. *Seizonken Kenkyu* 3: 39–40 (in Japanese)

Li L, Zhou Y, Cheng X, Sun J, Marita JM, Ralph J, Chiang VL (2003) Combinatorial modification of multiple lignin traits in trees through multigene cotransformation. *Proc Natl Acad Sci USA* 100: 4939–4944

Matsumoto Y (2002) Report of *Acacia* Hybrid Plantation in Sabah, Malaysia. *Abstract of International Symposium on Sustainable Utilization of Acacia mangium*, pp 124–127

Nanda RM, Das P, Rout GR (2004) *In vitro* clonal propagation of *Acacia mangium* Willd. and its evaluation of genetic stability through RAPD marker. *Annal Forest Sci* 61: 381–386

Omura Y, Takeuchi I, Teraoka Y, Sato Y (2007) Initial growth of *Acacia* hybrid in plantation forest in Borneo, *The proceedings of the 118th annual meeting of the Japanese Forest Society*, p. P1h9, Fukuoka (in Japanese)

Pinyopusarerk K, Liang SB, Gunn BV (1993) Taxonomy, distribution, biology, and use as an exotic. In: Awang K, Taylor D (eds) Acacia mangium *Growing and Utilization*. Winrock International and the Food and Agricultural Organization of the United Nations, Bankok, pp 1–19

Saito Y, Kojima K, Ide Y, Sasaki S (1993) *In vitro* propagation from axillary buds of *Acacia mangium*, a legume tree in the tropics. *Shokubutsu Soshiki Baiyo* 10: 163–168

Sakakibara N, Suzuki S, Umezawa T, Shimada M (2003) Biosynthesis of yatein in *Anthriscus sylvestris*. *Org Biomol Chem* 1: 2474–2485

Sakakibara N, Nakatsubo T, Suzuki S, Shibata D, Shimada M, Umezawa T (2007) Metabolic Analysis of the Cinnamate/Monolignol Pathway in *Carthamus tinctorius* Seeds by a Stable-Isotope-Dilution Method. *Org Biomol Chem* 5: 802–815

Suzuki S, Umezawa T (2007) Recent advances of tree biotechnology in *Acacia mangium* (Fabaceae). *Seizonken Kenkyu* 3: 41–42 (in Japanese)

Tacconi L, Obidzinski K, Agung F (2004) *Learning Lessons to*

Promote Forest Certification and Control Illegal Logging in Indonesia, Center for International Forestry Research, Jakarta, pp 1–81

Umezawa T, Suzuki S (2007) Chemical components of *Acacia mangium* and *Acacia auriculiformis*. *Seizonken Kenkyu* 3: 43–47 (in Japanese)

Wang XJ, Cao XL, Hong Y (2005) Isolation and characterization of flower-specific transcripts in *Acacia mangium*. *Tree Physiol* 25: 167–178

Watanabe T, Watanabe T (2007) Biorefinery of tropical biomass. *Seizonken Kenkyu* 3: 65–71 (in Japanese)

Xie DY, Hong Y (2001) *In vitro* regeneration of *Acacia mangium* via organogenesis. *Plant Cell, Tissue and Organ Culture* 66: 167–173

Xie DY, Hong Y (2001) Regeneration of *Acacia mangium* through somatic embryogenesis. *Plant Cell Rep* 20: 34–40

Xie DY, Hong Y (2002) Agrobacterium-mediated genetic transformation of *Acacia mangium*. *Plant Cell Rep* 20: 917–922

Yano H, Mori T, Umemura K (2007) *Acacia mangium* for building materials. *Seizonken Kenkyu* 3: 49–53 (in Japanese)

Yoshida Y, Matsumoto Y, Sato Y (2007) Properties of *Acacia* hybrid –*Acacia* hybrid plantation in Borneo for structural material production –. *The proceedings of the 118th annual meeting of the Japanese Forest Society* p. P1h10, Fukuoka, Japan (in Japanese)

Yoshimura T, Hattori T, Takematsu Y (2007) Conservation of biodiversity in the tropical plantation forest. *Seizonken Kenkyu* 3: 35–37 (in Japanese)