Development of New Selections of *Eucalyptus* Trees Using Genetic Manipulation©

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IMPORTANCE OF EUCALYPTUS TREES

Eucalyptus taxa are polygenus plants comprised of more than 500 species that are native to the Oceania region but predominantly Australia. Many of these *Eucalyptus* plants have excellent growth properties, the ability to adapt to various environments, and a low level of serious insect damage. Since they are also suited industrially to the production of lumber, pulp, and firewood, afforestation of *Eucalyptus* species is conducted in various regions around the world.

GENETIC MANIPULATION

***Eucalyptus* Tissue Culture.** In recent years, genetic modification has become possible in woody plants due to the progress in gene manipulation technology. Today plant breeding is being aggressively conducted through the transformation of useful genes isolated from heterogeneous organisms or the modification of genes in the plant itself; this was not possible with conventional breeding methods such as selection and hybridization breeding. Numerous examples of such breeding are reported in the literature, however, not all woody plants can be transformed with stability at the present time. In order to establish transformation technology using *Agrobacterium tumefaciens* in the target woody plant, it is necessary to develop a method for regenerating the plants from a transformed cell [Japanese Unexamined Patent Publications (JUPP) No. 62-55020], and a method for infecting *A. tumefaciens* into the plant tissue (JUPP No. 63-7720). In order to accomplish this, we have developed methods of regenerating plants from tissue or isolated cells of *E. camaldulensis* (Fig. 1A) by the utilization of shoot primordia (JUPP No. 62-55020, JUPP No. 63-7720, JUPP No. 64-47318, JUPP No. 2-265419, JUPP No. 4-4828, JUPP No. 5-236832, JUPP No. 9-98684, UK Patent No. GB2195656B, US Patent No. 5310673, Ito, et. al.,1996). The rotary culture system (Fig. 1B) is used to induce shoot primordia (Fig. 1C) from shoot tips that have the ability to regenerate shoots and roots with ease (Figs. 1D and 1E).

***Eucalyptus* Transformation.** We have established a method of producing transgenic plants that involves the introduction of a foreign gene by electroporation into the protoplast of a *Eucalyptus* plant according to the regeneration methods in JUPP No. 4-53429. However, in the case of producing transformation by electroporation, not only is considerable time required until a transformation is obtained, but there is also the problem of transformants only being obtained at a low frequency. On the other hand, the *Agrobacterium* method is very popular in plant science. However, *Eucalyptus* transformation was difficult because of the blackening of explants from wounding or co-culturing with *Agrobacterium*. Because of these problems, we investigated different plant tissues and culture conditions suitable for transformation and established a transformation system by infecting cotyledon or hypocotyl tissues derived from seedlings of *Eucalyptus* plants with *A. tumefaciens*

Figure 1. Images showing the sequence of steps in the tissue culture and transformation of *Eucalyptus*.

(JUPP No. 7-203790, JUPP No. 8-89113) (Fig. 1F). These methods made it possible to transform *Eucalyptus* plants for which transformation was previously difficult. However, in the case of infecting segments of the explant from mature trees growing outdoors with *A. tumefaciens* using the same methods, problems occurred that included blackening of segments of the explant by polyphenol induced as a result of wounding or co-culturing with *A. tumefaciens*, thereby preventing the production of transgenic plants. On the other hand, successful gene insertion has been reported in *E. globulus*, however, regeneration of the transgenic plant was not performed (Azmia, et al., 1997; Serrano, et al., 1996). In addition, although successful regeneration of the transgenic plant has been reported in *E. camaldulensis*, due to the low transformation efficiency, this did not lead to a stable transformation system (Mullins, et al., 1997). Moreover, although it has also been reported again in *E. camaldulensis* that a transgenic plant was successfully regenerated using the hypocotyl as the transgenic material, since the hypocotyl is used for the transformation material, transformation is not possible for a specific mature tree (Ho, et al., 1998). However, these systems (including our system) are not applicable to the transformation of mature trees (plus-trees) (Kawazu et al., 1996). We subsequently found that precocious branches (JUPP No. l0-304785) (Fig. 1G) derived from a shoot apex were susceptible to *Agrobacterium* and these precocious branches were utilized as transformation materials (patent pending). This observation allowed us to develop an efficient transformation method for mature *Eucalyptus* trees.

Modification of Lignin Biosynthesis. Lignin is a polymeric constituent of the cell wall that needs to be removed during the paper making process. To improve the pulping properties of *Eucalyptus* trees, cinnamyl alcohol dehydrogenase (CAD) which catalyses using an antisense method the down regulation of lignin biosynthesis was developed. As a result of this transformation, CAD down-regulated *Eucalyptus* trees displayed a red coloration in the outer xylem and this coloration was thought to depend on the accumulation of the aldehyde structure. We expect that depressing CAD activity may be an effective method for improving digestibility in *Eucalyptus* trees. In the future this transformation system will allow the development of new *Eucalyptus* selections that have tolerance to environmental stresses, such as drought, salt, and cold.

LITERATURE CITED

Azmia, A., W. Dewitteb, C. Drevetc, H. Van Onckelenb, P. Landrea, A.M. Boudet, L. Jouaninc, and **D. Chriqui.** 1997. Bud regeneration from *Eucalyptus globulus* clones and seedlings through hormonal imbalances induced by *Agrobacterium tumefaciens* strain 82.139. Plant Sci. 127:81-90.

Ho, C.K., S.H. Chang, J.Y. Tsay, C.J. Tsai, V.L Chiang, and **Z.Z. Chen**.1998. *Agrobacterium tumefaciens*-mediated transformation of *Eucalyptus camaldulensis* and production of transgenic plants. Plant Cell Reports17:675-680

Ito K., K. Doi, Y. Tatemichi, and **M. Shibata.** 1996. Plant regeneration of eucalypts from rotating nodule cultures. Plant Cell Rep. 16:42-45.

Japanese Unexamined Patent Publications No. 4-53429.

Japanese Unexamined Patent Publications No. l0-304785.

Japanese Unexamined Patent Publications No. 5-236832.

Japanese Unexamined Patent Publications No. 9-98684.

Japanese Unexamined Patent Publications No. 4-4828.

Japanese Unexamined Patent Publications No. 7-203790.

Japanese Unexamined Patent Publications No. 8-89113.

Japanese Unexamined Patent Publications No. 64-47318.

Japanese Unexamined Patent Publications No. 62-55020.

Japanese Unexamined Patent Publications No. 2-265419.

Japanese Unexamined Patent Publications No. 63-7720.

Kawazu, T., K. Doi, Y. Tatemichi, K. Ito, and M. Shibata. 1996. Regeneration of transgenic plants by nodule culture system in Eucalyptus camaldulensis. pp. 492-497. In: M.J. Dieters, A C. Matheson, D.G. Nikles, C. E. Harwood, and S. M. Walker (Eds.). Tree improvement for sustainable tropical forestry. Proc. QFRI-IUFRO Conf.

Mullins, KV., D.J. Llewellyn, V.J. Hartney, S. Strauss, and **E.S. Dennis.** 1997. Regeneration and transformation of *Eucalyptus camaldulensis*. Plant cell reports.16:787-791.

Serrano, L., F. Rochange, J.P. Semblat, C. Marque, C. Teulieres, and **A.M. Boudet.** 1996. Genetic transformation of *Eucalyptus globulus* through biolistics: Complementary development of procedures for organogenesis from zygotic embryos and stable transformation of corresponding proliferating tissue. J. Exper. Bot.47:285-290.

UK Patent No. GB2195656B.

US Patent No. 5310673.