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Title : Regeneration and Genetic Transformation of *Acacia mangium*
アカシア・マンギウムの個体再生と形質転換に関する研究

Speaker : Md. Mahabubur Rahman (Mission Researcher, RISH, Kyoto University)

Co-workers： Prof. Toshiaki Umezawa (RISH, Kyoto University)
Dr. Takefumi Hattori (RISH, Kyoto University)
Prof. Masahiro Mii (Chiba University)
Dr. Shiro Suzuki (ISS, Kyoto University)

Related RISH mission： Mission 1, 2, and Acacia Intermission

Abstract：

We all know the compelling reasons that our need to reduce the dependence on fossil fuels. The greatest increases in energy demand are declining fossil fuel reserves. Byproducts of fossil fuel burning are steadily increasing the concentration of CO_2 and other greenhouse gases in the atmosphere that one of the cause of global worming and affect to change in weather patterns. Fast-growing trees are one of the best potential sources of biological fuel, they grow in marginal soils that will be not required fertilizer, herbicides, or pesticides; and accumulate biomass density for several years. Molecular breeding is one of the best technology for increasing biomass content and improvement of others agronomical treats. In order to proceed the tree biotechnology, it is prerequisite to establish the efficient genetic transformation and regeneration techniques. *Acacia mangium* and *A. crassicarpa* are important tropical fast-growing plantation trees in Southeast Asia and their research in biotechnology is existing at primary stages. In this project, therefore, we are being attempted to establish protocols of efficient genetic transformation and regeneration in those of *Acacia* species.

The adventitious shoot regeneration through organogenesis and embryogenesis from callus is probably unicellular origin, making them an excellent contender for genetic transformation. In this study, the nodular callus was obtained from leaf (pinnate) segment of *A. mangium* on MS medium supplemented with 5.0 μM TDZ and 1.5 μM

IAA. When this callus was subcultured in MS medium containing 0.1 μM and 0.05 μM TDZ, all cultures produced friable callus and lastly died within 30 day of incubation. The globular and torpedo shaped of embryo-like structures were achieved on liquid MS medium supplemented with 5.0 μM TDZ + 1.5 μM IAA after 2 months of resuspension.

In case of *A. crassicarpa*, the green nodular structures were achieved at cut ends in MS medium supplemented with 2.25 μM TDZ + 2.5 μM NAA after 20 days of culture. The embryogenic callus with globular, heart, and cotyledonary stages obtained in MS medium supplemented with 2.25 μM TDZ + 2.5 μM NAA + 5% coconut water within two months of subculture.

Recently, *Agrobacterium*-mediated genetic transformation of *A. mangium and A. crassicarpa* is being conducted using strain EHA105 harboring the binary vector, pIG121-Hm. Although the concentration of hygromycin at 20 mg/L, G418 at 30 mg/L, and Basta at 20 mg/L in PGR free MS medium optimized for inhibition of growth completely in *A. mangium* seedlings within 40 days.