

## **An improved surface sterilization technique for introducing leaf, nodal and seed explants of *Aquilaria malaccensis* from field sources into tissue culture**

### **ABSTRACT**

A critical stage in the introduction of plants into tissue culture is to obtain cultures free from microbial contamination. This study investigated different sterilization regimes for leaf and nodal explants from *Aquilaria malaccensis* grown in the shade house under natural environmental conditions, and for seeds from wild mature trees. We found that pre-sterilization using 0.2% Benomyl for 15 minutes improved the number of 'clean and alive' individuals of all types of explants, especially when followed by surface sterilization using mercury chloride (HgCl<sub>2</sub>). Treatment with 0.1 % HgCl<sub>2</sub> for 15 and 30 seconds yielded the best results for leaf and nodal explants, respectively. Maximum percentage of 'clean and alive' seeds was observed when using 0.2 % HgCl<sub>2</sub> for 12 minutes. Treatment with Clorox® bleach (5.25% sodium hypochlorite as the active ingredient) even at high concentration (50% Clorox®) alone was not sufficient to control fungal and bacterial contamination in the explants. We conclude that HgCl<sub>2</sub> coupled with Benomyl pre-treatment produced a highly efficient sterilization method producing 83 ó 90% 'clean' leaf, nodal and seed explants of *A. malaccensis* from natural sources after fourteen days in culture.

**Keyword:** Agarwood; Endangered tree; In vitro; Microbial contamination; Thymelaeaceae