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 ócleanô and aliveô individuals of all types of explants, especially when followed by surface sterilization using mercury chloride (HgCl2). Treatment with 0.1 % HgCl2 for 15 and 30 seconds yielded the best results for leaf and nodal explants, respectively. Maximum percentage of ócleanô and aliveô seeds was observed when using 0.2 % HgCl2 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 HgCl2 coupled with Benomyl pre-treatment produced a highly efficient sterilization method producing 83 ï 90% ócleanô 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