

***Agrobacterium*-MEDIATED TRANSFORMATION OF *CryIAb* GENE INTO *Tectona grandis* L.(TEAK)**

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ABSTRACT

A binary vector pCAMBIA1302-Ab containing *CryIAb* and *hygromycin phosphotransferase* (*hpt*) genes was used in the transformation of *in vitro* teak nodes and multiple shoots clumps. The explants were submerged in suspension culture of *Agrobacterium tumefaciens* strain EHA105 (OD₆₀₀=1.2) then were sonicated for 3 min in sonicator bath followed by vacuum-infiltration for 5 min. The co-cultivation was performed for 3 days on MS medium containing 100 µM acetosyringone. Two months after selection on 50 mgL⁻¹hygromycin containing medium, calli were formed. Total RNA was extracted from these calli and was subjected to first-stranded RT-PCR analysis of *CryIAb* gene. The result indicated the presence of *CryIAb* transcript in all selected calli. A single shoot developed from a multiple shoots clump cultured on selective medium. The 4-weeks-old putative transformed shoot also showed positive results of *CryIAb* and *hpt* genes by PCR analysis. However, chimera tissue was observed in this shoot. The *CryIAb* protein in transgenic tissue was also determined using Bt-*CryIAb*/1Ac ELISA Kit. The expressed *CryIAb* protein in putative transformed shoot was confirmed in the leaves that contained *CryIAb* and *hpt* genes.

Keywords: Genetic transformation, tree, insect resistance gene, *Agrobacterium*

INTRODUCTION

Teak (*Tectona grandis* L.) is one of the highly demanded timber-producing trees of the tropical zone. The demands for teak wood and wood products are continuously increasing throughout the world but its natural resources are rapidly depleting (Pandey and Brown, 2000). Thus, the large scale plantations are promoted beyond its native countries in Asia (i.e. Thailand, Indonesia, Malaysia) and Africa (i.e. Ivory Coast, Congo, Nigeria), and Latin America (i.e. Brazil, Costa Rica, Panama and Honduras) (White, 1991). However, mono-cropping plantation system of teak is confronted with many problems, and the big problem is infestation of various pest species. The teak defoliator (*Hyblaea puera* Cramer) and teak bee-hole borer (*Xyleutes ceramicus* Walker) are serious insect pests in teak plantation. Low growth rate and low quality of wood normally occurred when these two insect pests come into the plantation (Nair *et al.*, 1984). There are many approaches that tried to solve these problems such as spraying with *Bacillus thuringiensis* (*Bt*) on the leaves. The main limitation of this technique, however, is the poor coverage on plant surfaces which cannot reach the teak bee-hole borer