

Yaowaphan Sontikun 2014: Gene Transformation into *Tectona grandis* L.f. via *Agrobacterium tumefaciens*. Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Sermsiri Chanprame, Ph.D. 141 pages.

There are several parameters involved in teak transformation. In this study, micropropagation of teak was studied. It was found that callus was successfully induced but plantlets could not be regenerated. Multiple shoots were successfully induced from node tissue cultured on MS solid medium supplemented with 3.0 mg/l BA yielded 5 shoots per node. For gene transformation, the optimal conditions were established. *Agrobacterium tumefaciens* harboring pCAMBIA 1304 which contained  $\beta$ -glucuronidase (*gus*) as reporter gene and hygromycin phosphotransferase (*hpt*) as selectable marker gene was used. The transient expression as evaluated by GUS histochemical assay demonstrated high percentage of transformation when using *A. tumefaciens* strain EHA 105 ( $OD_{600} = 1$ ) to transfer gene into node explants in which wounded by sonication for 10 seconds followed by 5 hours inoculated then co-cultivated for 3 days. The optimal parameters were then applied to the transformation of *EPSPs* gene (*5-enolpyruvylshikimate-3-phosphate synthase*) into teak. The plant vector, pCAM-EPSPS 1304, which contained *EPSPs* gene was used. Explants were transferred onto glyphosate containing medium. After selection, 27 plants survived. The PCR analysis confirmed that 23 putative transgenic lines contained both *gus* and *EPSPs* genes. All putative transgenic lines were multiplied on MS medium containing 3.0 mg/l BA to obtain 109 T<sub>2</sub> planted. The PCR analysis confirmed the existence of the transgenes in 12 out of 109 T<sub>2</sub> plantlets. They were then transplanted in greenhouse and analysed by dot blot hybridization, PCR and Southern blot hybridization. The results confirmed that 4 lines were transgenic but they were not consistency in glyphosate resistance. PCR analysis for the present of the transgenes in these 4 transgenic lines revealed that they were chimeras.

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Thesis Advisor's signature