

Wicha Singlo 2012: Transformation of Glyphosate Resistance Gene into Physic Nut (*Jatropha curcas* L.). Master of Science (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Sermsiri Chanprame, Ph.D. 76 pages.

*Agrobacterium*-mediated transformation into physic nut callus was optimized using *Agrobacterium tumefaciens* strain EHA105 contained plasmid pCAM-EPSPs 1304 that has  $\beta$ -glucuronidase (*gus*) gene as a reporter gene. Cefotaxime at the concentration of 100-400 mg/l was tested for the optimal concentration that could successfully eliminate *A. tumefaciens*. The effects of transformation parameters were studied including: concentration of *A. tumefaciens* at the dilution ratio ( $OD_{600}=1$  : culture medium) of 1:0, 1:1, 1:10, 1:20, 1:50 and 1:100; inoculation periods of 10, 20, 30 and 40 min and co-cultivation periods of 1, 2, 3 and 4 days. The result demonstrated that 200 mg/l cefotaxime could successfully eliminate *A. tumefaciens* and callus still able to regenerate. The transformation procedure using dilution of *A. tumefaciens* at 1:20, 30 mins inoculation period and co-cultivated for 2 days yielded the highest percentage of transformation with significantly different from other treatments as verified by a GUS histochemical assay.

Transformation of *EPSPs* gene into calli of physic nut using *A. tumefaciens* strain EHA105 containing the pCAM-EPSPs 1304 plasmid with glyphosate resistance (*EPSPs*) gene as a selectable marker. The optimized factors from the earlier study were used for gene transfer and the results showed that 9 clones of calli could survive on selective medium containing glyphosate at the concentration of 1 mM. However, only 3 clones showed PCR positive for *EPSPs* gene. The Southern PCR hybridization also confirmed the presence of *EPSPs* gene in these 3 clones.

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