

Sineethorn Somsueb 2006: Chloroplast Transformation in Tobacco. Master of Science (Agricultural Biotechnology), Major Filed: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Assistant Professor Siriluck Iamtham, Ph. D. 95 pages.
ISBN 974-16-1669-4

The effect of a number of parameters on chloroplast transformation frequency into tobacco using particle bombardment was investigated. These included (1) the use of rupture disk 650 and 1,100 psi (2) three macrocarrier stopping plate to target distance of 6, 9 and 12 cm. The effective spray area of microprojectile to produce transformants is detailed by the diameter of the inner circle (~1 cm) and outer circle (~3 cm). The tobacco leaves pieces were randomly examined histochemically with X-gluc for testing the expression of *uidA* gene. Transient expression of *uidA* gene in tobacco leave pieces under the control of 5' *rrnHv* and 3' *NtpsbA* regions are obtained. The *rrn* promoter from *Hordeum vulgare* is constitutively transcribed in tobacco. The bombardment using 1,100 psi rupture disk at distance 6 cm, macrocarrier stopping plate to target, yield the largest number of blue stain precipitate (76.27%). The spray area of microprojectile at the inner and outer circle showed non-significant result in a number of blue spots per piece (2.57 and 6.11 spots, respectively). Simultaneously, wild type, as negative controls, did not show any *uidA* gene expression. Sample of survival tobacco from selected medium were used to determined the integrates of chloroplast expression cassettes into tobacco chloroplast genome using PCR and Southern blot techniques. The results did not display the integration of chloroplast expression cassette genes into target regions (*rbcL* gene and *accD* gene) of chloroplast genomes.

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Student's signature



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5 / 05 / 06