

Katanyutita Damchuay 2011: Genetic Transformation of (*E*)- β -farnesene Synthase from Mint (*Mentha cordifolia* Opiz.) into Tobacco (*Nicotiana tabacum*). Master of Science (Genetic Engineering), Major Field: Genetic Engineering, Interdisciplinary Graduate Program. Thesis Advisor: Mr. Srimek Chowpongpan, Ph.D. 82 pages.

The genetic transformation of encoded (*E*)- β -farnesene synthase (*TSPA11*) which catalyzes the synthesis of (*E*)- β -farnesene in mint that mimics the aphid alarm pheromone was transformed into tobacco plant. *TSPA11* was isolated from total RNA extracted from mint *via* cDNA using One Step RT-PCR with specific primers FarX1 and Far2B that contained the *Xba*I and *Bam*HI recognition sites, respectively. The PCR product was cloned into PCR[®] 8/GW/ TOPO[®] cloning vector followed by DNA sequencing. The cloned gene contained 1,662 bp and 553 deduced amino acids, and its nucleotide sequence was similar to *TSPA11* existed in GenBank. The inserted *TSPA11* was excised from cloning vector by *Xba*I and *Bam*HI digestion and ligated into binary vector pCAMBIA 2311 under the control of CaMV35S promoter and NOS terminator. The recombinant was named pCAMBIA23Far. The resulting construct was introduced into *Agrobacterium tumefaciens* AGL-1 for transferring the *TSPA11* into tobacco. Leaf explants were cocultivated for 3 days and selected on MS medium supplement with naphthaleneacetic acid (NAA) 0.1 mg/L, benzyladenine (BA) 1 mg/L, cefotaxime 200 mg/L and geneticin 50 mg/L. Shoots regeneration appeared within eight weeks on selective media containing geneticin. Forty seven transformation lines were achieved with 30% (47/181) transformation rate. The *TSPA11* transgene integration into tobacco genome was determined by PCR and DNA dot blot hybridization. The result showed that all transgenic lines were positive for *TSPA11*. The expression level was evaluated by real-time qRT-PCR, and shown that *TSPA11* expression was variously expressed. The information from this study will be used to apply the property of *TSPA11* from mint to other important economic crops enabling the production of (*E*)- β -farnesene that repels aphids, the major insect vectors of many plant viruses minimizing the yield losses.

Student's signature

Thesis Advisor's signature