

## $\gamma$ -Tocopherol methyltransferase Gene Enhances Conversion of $\gamma$ -Tocopherol to $\alpha$ -Tocopherol in Transgenic Perilla Seeds

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### Objectives

Vitamin E(tocopherol) is one of the potent antioxidants occurring in nature. Among the various types of tocopherol( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherol),  $\alpha$ -type maintains the strongest antioxidant activity compared with other types of tocopherol. Meanwhile, Many of plant oils contain more  $\gamma$ -tocopherol than  $\alpha$ -tocopherol. In order to convert  $\gamma$ -tocopherol to  $\alpha$ -tocopherol in perilla seed, we have cloned and characterized  $\gamma$ -tocopherol methyltransferase (*tmt*) gene from *Perilla frutescens*. A binary vector was constructed with bar gene as a selective marker and driven by vicilin promoter for seed specific expression of *tmt*. More than 90% of  $\gamma$ -tocopherol turned into  $\alpha$ -tocopherol in the seed of transgenic perilla.

### Materials and Methods

1. Materials - Plant : *Perilla frutescens* Britt. cv. Yeopsil  
Perilla immature seed cDNA library
2. Methods: cDNA library screening to isolate full-length *tmt* clones *tmt* gene expression in *E. coli* and enzyme activity assay. Plant binary vector construction and perilla transformation by *A.tumefaciens* Southern and Northern blot, HPLC

### Results and Discussion

$\gamma$ -Tocopherol methyltransferase(*tmt*) is an enzyme catalyzing  $\gamma$ -tocopherol into  $\alpha$ -tocopherol at the final step of tocopherol biosynthesis pathway. We cloned *tmt* gene fragment from *P. frutescens* by PCR using the degenerate primer. Full length cDNA clone was selected from perilla immature seed cDNA library by plaque hybridization. To characterize the properties of the *tmt* gene product, we induced the expression of the *tmt* gene in *E. coli* and assayed the  $\gamma$ -tocopherol methyltransferase activity *in vitro*. A vector containing *bar* and *tmt* gene driven by seed specific vicilin promoter was constructed and employed for transformation of perilla via organogenesis. Transformed-plants were confirmed by spraying with 0.3 % Basta<sup>®</sup> and genomic Southern blot analysis. The expression of introduced-*tmt* gene was confirmed in developing seed by Northern blot. As the tocopherol composition in the transgenic perilla seeds was analysed by HPLC, the data showed that more than 90% of  $\gamma$ -tocopherol in the transgenics was converted to  $\alpha$ -tocopherol.