

SEMINAR- 4

METABOLIC ENGINEERING FOR BIOSYNTHESIS OF TOCOTRIENOL IN *PERILLA FRUTESCENS* (L.) BRITTON

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Summary

A. Metabolic engineering in plants

Metabolic engineering can be defined as the directed improvement of product formation or cellular properties through the modification of specific biochemical reactions or introduction of new ones with the use of recombinant DNA technology. This redirection can result in an increase in the concentration of an existing compound or novel accumulation of a desired product.

B. What are tocotrienols ?

Tocotrienols are fraction of vitamin E family of lipid soluble antioxidants in plants. It comprises one of the two groups of structurally related substances (vitamers) belonging to the vitamin E family, the other group being the tocopherols. Similar to tocopherols, tocotrienols are plastid localized molecules that consist of a polar chromanol head group linked to a long-chain hydrocarbon tail (Figure 1). Naturally, tocotrienols exist in four different forms or isomers, named α -, β -, γ - and δ - tocotrienols

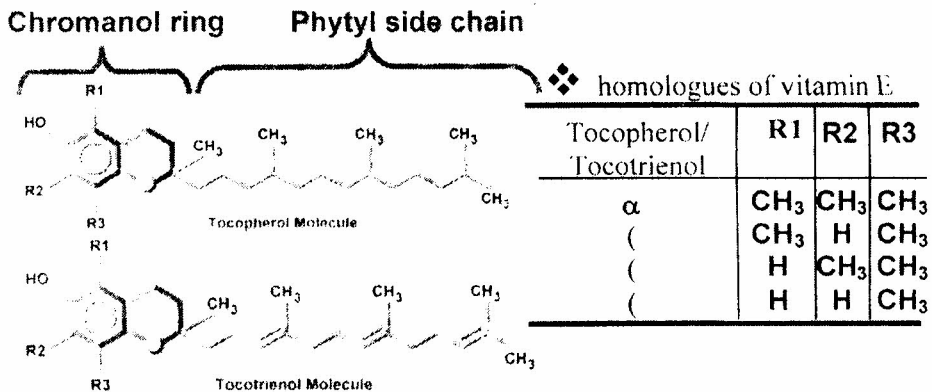


Figure 1. Chemical structure of α -, β -, γ -, δ - tocopherols and tocotrienols.

C. *Perilla frutescens* (L.) Britton

Perilla is a semi-hardy annual herb native to China belonging to the family Labiatae, and broadly distinguished by the red to green colored species of the crop. On the basis of their morphology and use, the well-known species includes the *Perilla frutescens*

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(L) Briton var. *frutescens* and *Perilla frutescens* (L) Briton var. *crispa* belongs to the categories green leaved *Perilla*, called *Perilla* Green and the purple leaved one, named *Perilla* Red respectively. This short-day annual crop is called as "Wild sesame" in English; "BanTulsi" in Bengali; Deulggae / Kkaennip in Korean.

D. Background and out-put of the study

Following a recent report on metabolic profiles, with special reference to vitamin E levels in some Korean crops, opened a fact that *Perilla* were absent with tocotrienol but contained adequate amount (40.9mg/100g of dry weight) of tocopherol although the crop is much popular for her efficiencies in several pharmacological areas supported by the modern medicine. Therefore, remodelling of the metabolic pathway of vitamin E biosynthesis in *Perilla* for tocotrienol production could have gained a significant nutritional benefit to human health. It has already been demonstrated that the tocotrienol is produced by the enzymatic activity of homogentisic acid geranylgeranyl transferase (HGGT) and also showed that a single heterologous expression of this enzyme resulted with accumulation of tocotrienols in *Arabidopsis thaliana*. So, the cDNA encoding homogentisic acid geranylgeranyl transferase (HGGT) in Dongjin, a Japonica cultivar rice of Korea, was isolated and was found 100% identical to a known HGGT gene in another Japonica rice (Taichung 65) deposited in the Genebank (AY222862) database in both the nucleotide and amino acid levels. The cDNA was functionally characterized *in vivo* and confirmed to produce tocotrienol in the *Nicotiana benthamiana* (L.) leaves using the Agroinfiltration technique. A reproducible *Agrobacterium tumefaciens* mediated transformation protocol for leafy *Perilla* was established with an average of 3.18% transformation efficiency using apical buds. The constitutive expression of the HGGT gene led to accumulation of tocotrienol, as a novel form of vitamin E in transgenic leafy *Perilla*, which was not found in the non-transformed one. The α - form of tocotrienol was detected by GC-FID analysis and confirmed by the GC-Mass spectrometry. Apparently, the genetically improved *Perilla*, as demonstrated in this study, can be used for the production of leaves co-existed with tocopherol and tocotrienol forms of vitamin E. In addition, the seed oil could be co-extracted with tocotrienols which could improve shelf life and oxidative ability for cooking and in other applications.

