

Increased Expression of Homogentisic Acid Phytoltransferase Enhanced Total Tocopherol Content in Lettuce (*Lactuca sativa* L.)

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Objectives

Tocopherols are typical phytochemicals whose multiple health-promoting effects are demonstrated by plethora of sound scientific evidences. Oriental leaf lettuce is one of the favorite vegetables but it contains low levels of tocopherols. Transgenic approach was attempted to enhance health-promoting quality of lettuce by elevating tocopherols contents via the ectopic expression of a rate-limiting gene involved in tocopherol biosynthesis.

Materials and Methods

1. Materials

Oriental lettuce (cv. Chungchima) was used for genetic transformation. Homogentisic acid phytoltransferase (*ScHPT*) sequence originated from *Synechocystis*. *ScHPT*cDNA sequence was driven by CaMV35S promoter and introduced into *Agrobacterium* (LBA4404).

2. Methods:

ScHPT was stably transformed into lettuce genomic DNA via the *Agrobacterium*-mediated transformation. All the procedures for tissue culture and DNA transformation was conducted as previously described (Kim et al., 2000, *Kor. J. Plant Tissue Culture* 27, 435-439).

Results and Discussion

Total tocopherol content in the transgenic lines expressing *ScHPT* mRNA increased 34% on average compared with that in the nontransgenic plants. The increased total tocopherol content in transgenic plants was contributed by increases in all the tocopherol isoforms. However, the increase rate of each isoform varied significantly. These results indicate that enhanced expression of HPT mRNA results in increase in tocopherol content, supporting that HPT play a rate-limiting role in tocopherol biosynthesis in lettuce. This research was supported by Research Center for Industrial Development of Biofood Materials in Chonbuk National University, Chonju, Korea. The center is designated as a Regional Research Center appointed by the Korea Science and Engineering Foundation (KOSEF), Jeollabuk-do Provincial Government and Chonbuk National University

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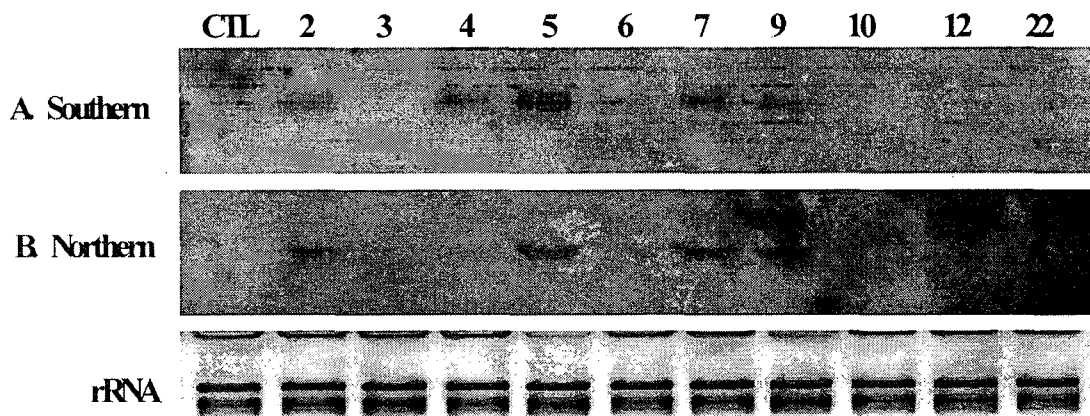


Fig. 1. Presence and expression of *ScHPT* transgene in transgenic lettuce lines. A. Southern blot analysis of genomic DNAs of *ScHPT* transgenic T0 lines. Genomic DNA from the transgenic lines 2-22 digested with cloning site enzyme *Eco* RI. Lane CTL, nontransgenic control plants; each numbered lane indicates *ScHPT* transgenic line. B. Detection of *ScHPT* transgene mRNA in transgenic lettuce lines. The size of the *ScHPT* mRNA detected was about 1.4 kb. Twenty micrograms of total RNA were resolved in a 1.0% (w/v) agarose/formaldehyde gel, transferred to a nylon membrane, then hybridized with the alkaline phosphatase-labeled *ScHPT* DNA probe. Ethidium bromide-stained rRNA bands as an indicator of equal loading (rRNA).

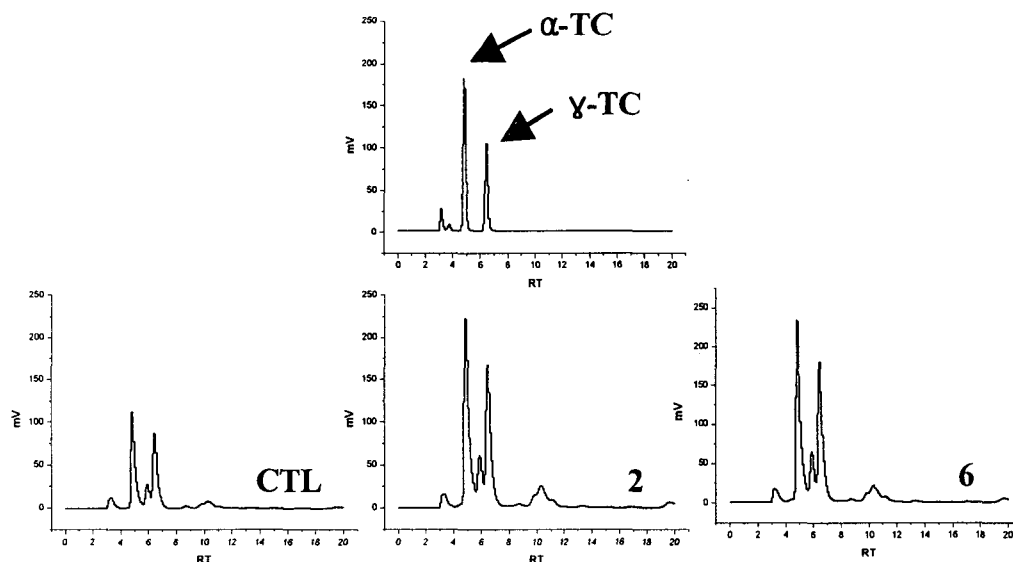


Fig. 2. Detection of tocopherol isoforms in the leaf of *ScHPT* transgenic plants. Nontransgenic control plant (CTL) and transgenic line 2 and 6, respectively. Total tocopherol content was increased by 50 and 44% in the transgenic line 2 and 6, respectively.