Carotenoid accumulation and characterization of cDNAs encoding phytoene

synthase and phytoene desaturase in garlic (Allium sativum)

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Objectives

In this study, we analyzed the expression of PSY and PDS by real-time PCR and measured the carotenoid content with high-performance liquid chromatography (HPLC) to characterize the mechanism of carotenoid biosynthesis in *A. sativum*.

Materials and Methods

- Plant Material: Garlic (*A.sativum*)wasgrownfrombulbsinagreenhouse at the experimental farm of Chungnam National University (Daejeon, Korea). Plant materials were excised from mature plants and dissected into bulbils, scapes, leaves, stems, bulbs, and roots.

- Real-Time Polymerase Chain Reaction Analysis

- Extraction and High Performance Liquid Chromatography Analysis of Carotenoids

Results

Phytoene synthase (PSY) and phytoene desaturase (PDS) which catalyze the first and second steps of the carotenoid biosynthetic pathway, respectively, are key enzymes for the accumulation of carotenoids in many plants. We isolated 2 partial cDNAs encoding PSY (*AsPSY-1* and *AsPSY-2*) and a partial cDNA encoding PDS (*AsPDS*) from *Allium sativum*. They shared high sequence identity and conserved motifs with other orthologous genes. Quantitative real-time PCR analysis was determined the expression levels of AsPSY1, AsPSY2, and AsPDS in the bulbils, scapes, leaves, stems, bulbs, and roots of garlic High-performance liquid chromatography demonstrated that carotenoids were not biosynthesized in the underground organs (rootsandbulbs), but were very abundant in the photosynthetic organs (leaves) of *A. sativum*. A significantly higher amount of β -carotene (73.44 μ g · g⁻¹) was detected in the leaves of *A. sativum* than in the other organs.

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Table 1. Evaluation of carotenoid content from different organs of *A. sativum* ($\mu g \cdot g^{-1} dry$ weight)(n=3). Statistical significance of the differences between treatments was determined using ANOVA followed by paired-group comparisons. The letters (a and b) indicate significance at P(0.05).

Compound	Bulbils	Scapes	Leaves	Stems	Bulbs	Roots
a-carotene	0.34 ± 0.00	ND	1.84 ± 0.15	ND	ND	ND
lutein	7.65 ± 0.24 b	$2.22 \pm 0.30 \text{ b}$	$179.8 \pm 4.65 a$	$8.2 \pm 0.13 \text{ b}$	ND	ND
β-carotene	2.85 ± 0.08 b	$0.60 \pm 0.03 \text{ b}$	73.44 ± 2.51 a	$1.49 \pm 0.03 \text{ b}$	ND	ND
zeaxanthin	1.03 ± 0.01	ND	5.13 ± 0.11	ND	ND	ND

ND = not detected.

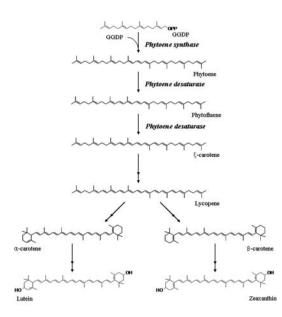


Figure 1. Activity of phytoene synthase and phytoene desaturase in the biosynthesis of carotenoids. GGDP, geranylgeranyl diphosphate.

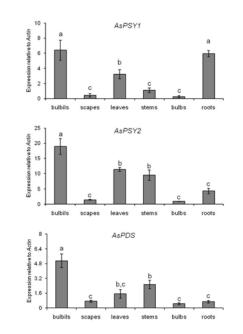


Figure 2. Expression of AsPSY1, AsPSY2, and AsPDS in different organs of *A. sativum*. The values and the error bars represent the average and standard error from 3 independent reactions, respectively. The statistical significance of the differences between treatments was determined using ANOVA followed by paired-group comparisons. The different letters (a, b, and c) indicate significance at P<0.05.