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Carotenoid Accumulation and Characterization of cDNAs Encoding Phytoene Synthase and Phytoene Desaturase in Garlic (*Allium sativum*)

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ABSTRACT: 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 used to determine 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 (roots and bulbs), but were very abundant in the photosynthetic organs (leaves) of *A. sativum*. A significantly higher amount of β-carotene (73.44 $\mu g \cdot g^{-1}$) was detected in the leaves of *A. sativum* than in the other organs.

KEYWORDS: *Allium sativum*, phytoene synthase, phytoene desaturase, gene expression, carotenoids

INTRODUCTION

Carotenoids, the second most abundant pigment in nature, consist of over 750 members.[1] In plants, carotenoids play a number of crucial roles in photosynthesis, photomorphogenesis, and photoprotection.[2,3] Carotenoids contribute to the red, orange, and yellow colors of flowers and fruit that attract pollinating and seed dispersal agents.[4] Moreover, carotenoids are the precursor of abscisic acid, an important plant hormone.[5]

In humans and animals, some carotenoids serve as precursors for vitamin A and as antioxidants that can prevent degenerative diseases.[6] A diet containing carotenoid-rich vegetables and fruit can protect against some cancers, heart disease, cataracts, and UV-induced skin damage.[7] Due to their health benefits, many nutritional products and medicines containing carotenoids have been developed.

The main carotenoid biosynthetic pathway has been extensively studied and is well characterized in the majority of species (Figure 1). The pathway begins with the synthesis of C40 phytoene by the head-to-head condensation of 2 molecules of geranylgeranyl diphosphate, which is catalyzed by phytoene synthase (PSY).[8,9] Two desaturases, phytoene desaturase (PDS) [10] and ξ-carotene desaturase,[11] introduce 4 double bonds to the colorless phytoene molecule to produce the pink-colored lycopene. At this point, the pathway is branched because lycopene can be converted to α-carotene and lutein or β-carotene and zeaxanthin, which leads to the production of abscisic acid.[12] PSY and PDS, which catalyze the first and second steps of the pathway, respectively, are the key regulators of carotenoid biosynthesis in many plants. For example, overexpression of PSY in tomato significantly increases the total accumulation of carotenoids,[13] and disruption of PDS is responsible for the dwarf phenotype in *Arabidopsis* as a result of reduced carotenoid biosynthesis.[14] Due to their important role in carotenoid biosynthesis, PSY and PDS genes have been cloned from yeast, bacteria, algae, and plants.

Garlic (*Allium sativum*), with over 4,000 years of use by humans, has been considered to be one of the best disease-preventive foods. A number of investigations have reported that garlic extract has a wide range of health benefits, e.g., against some forms of cancer and cardiovascular disorders [15] and as an antioxidant;[16,17] furthermore, garlic can reduce blood glucose levels.[18,19] Recently, besides sulfur-containing compounds, which are mostly believed to have health-related functions, there are very few data describing the health benefits of non-sulfur compounds in garlic.

Here, we report the isolation of cDNAs encoding PSYs and PDS (GenBank accession number HQ630601, HQ630602, and HQ630600, respectively) in *A. sativum*. In addition, 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 Materials. Garlic (*A. sativum*) was grown from bulbs in a greenhouse 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. The plant materials were frozen in liquid nitrogen and then stored at −80 °C for RNA isolation or freeze-dried for HPLC analysis.

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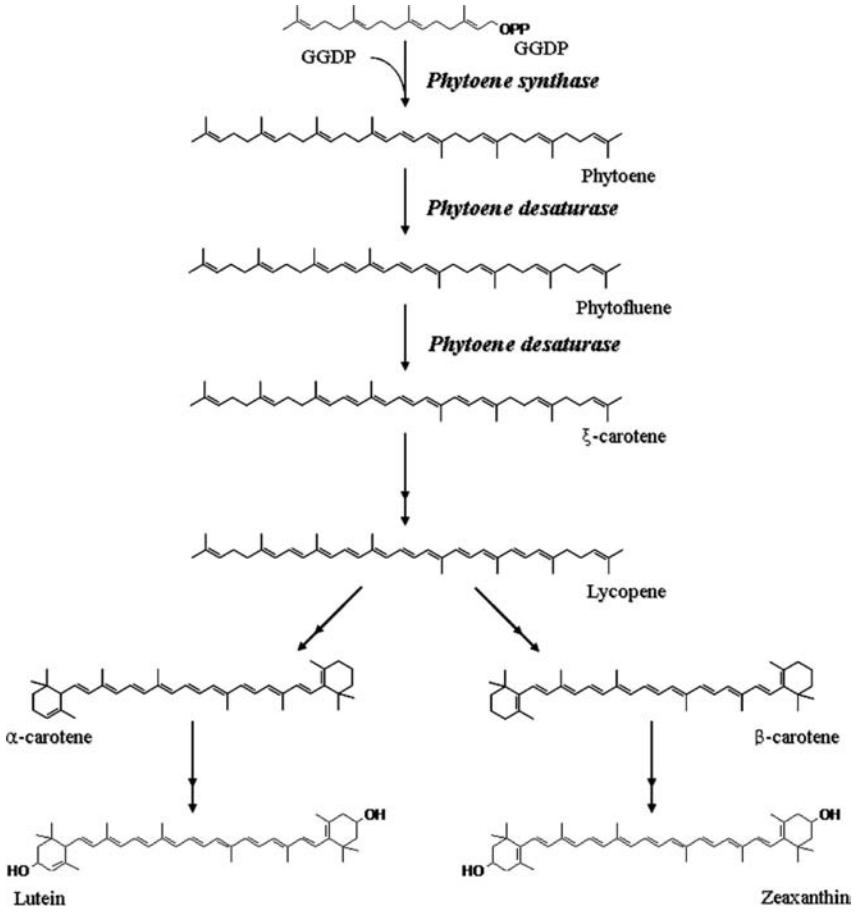

Figure 1. Activity of phytoene synthase and phytoene desaturase in the biosynthesis of carotenoids. GGDP, geranylgeranyl diphosphate.

RNA Isolation and cDNA Synthesis. Total RNA was extracted from different organs separately using the Plant Total RNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instruction. The quality and concentration of the extracted total RNA were measured by agarose gel electrophoresis and spectrophotometer analysis, respectively. High quality total RNA was used to synthesize first-strand cDNA using the GeneRacer Kit (Invitrogen, Carlsbad, CA, USA), while a 10-fold dilution of the resulting cDNA was used as a template for rapid amplification of cDNA ends (RACE) PCR. For quantitative real-time PCR, cDNA was synthesized from 1 μg of total RNA using the ReverTra Ace-α Kit (Toyobo, Osaka, Japan).

Cloning of the cDNAs Encoding Phytoene Synthase and Phytoene Desaturase. The amino acid sequences of the previously cloned PSY and PDS cDNAs from GenBank were aligned. Regions with the highest conservation were identified and conserved primers for PSY and PDS were designed to these regions (Table 1). Using the obtained partial sequences, gene-specific primers were designed to generate information for the 3′-ends of PSY and PDS from garlic. We performed 3′-RACE PCR according to the manufacturer's protocol. The expected PCR products were purified and cloned into the T-blunt vector (SolGent, Daejeon, Korea) for sequencing.

Sequence Analysis. The sequences were analyzed for homology using BLAST at the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/BLAST). Multiple sequence alignments were generated using the MultAlin program (http://bioinfo.genotoul.fr/multalin/multalin.html) and modified with BioEdit Sequence Alignment Editor, version 5.0.9 (Department of Microbiology, North Carolina State University, Raleigh, NC).

Table 1. Primers Used for the Isolation and Expression Analysis of AsPSY1, AsPSY2, and AsPDS

primer	sequence (5′ to 3′)	amplicon (base pairs)
	Use: Partial Sequences	
PSY_F	AGRMGRACWGAYGARCTKGTDGATGG	400
PSY_R	CTYCCYCTTCTNGCRTCYTCKCCWAC	
PDS_ F	TTRAAYMGRTTTCTTCAGGARAA	360
PDS_ R	TGRTCRTATGTRTTSTTCAGTTT	
	Use: 3′-RACE PCR	
AsPSY1_3	TGAATCTAAGGCTACAACCACAACCATT	590
AsPSY2_3	AGAGTCCTTAGCAAGTACAGAAAGCGTC	760
AsPDS_3	CCAGAGAGGCTATGCATGCCAATAGCTG	970
	Use: Real-Time PCR	
AsPSY1 F	ATTGACATTCAGCCATTCAAGGA	154
AsPSY1 R	TCAGGTGCAATACCCATAACTGG	
AsPSY2 F	GCCGGTATGGGCATCACTATTAT	186
AsPSY2 R	CATGCTTTCGTTAGGTTCGTTTG	
AsPDS F	TGCCAATAGCTGAACACATTCAA	161
AsPDS R	GTCAACAGGAGCAGCAACTACG	
AsActin F	TGTTTCCTAGTATTGCTGGTAGA	193
AsActin R	AGCTCGTTGTAGAAAGTGTGAT	

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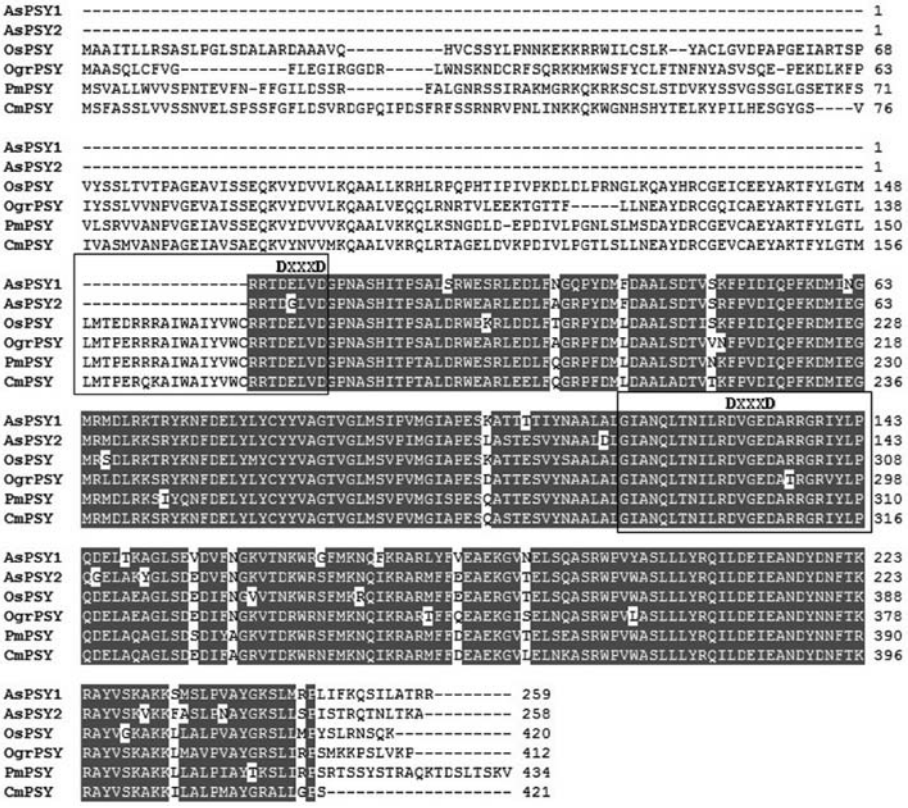

Figure 2. Comparison of the deduced amino acid sequences of AsPSY1 and AsPSY2 from *A. sativum* with PSY proteins from other plants. The black boxes are the 2 conserved domains in the PSY genes. DXXXD, where X encodes for any amino acid, represents 2 aspartate-rich regions. OsPSY, *O. sativa* PSY (AJ715786); OgrPSY, *O. cv. 'Gower Ramsey'* PSY (AY496865); PmPSY, *P. mume* PSY (AB253628); and CmPSY, *C. melo* PSY (GU361622).

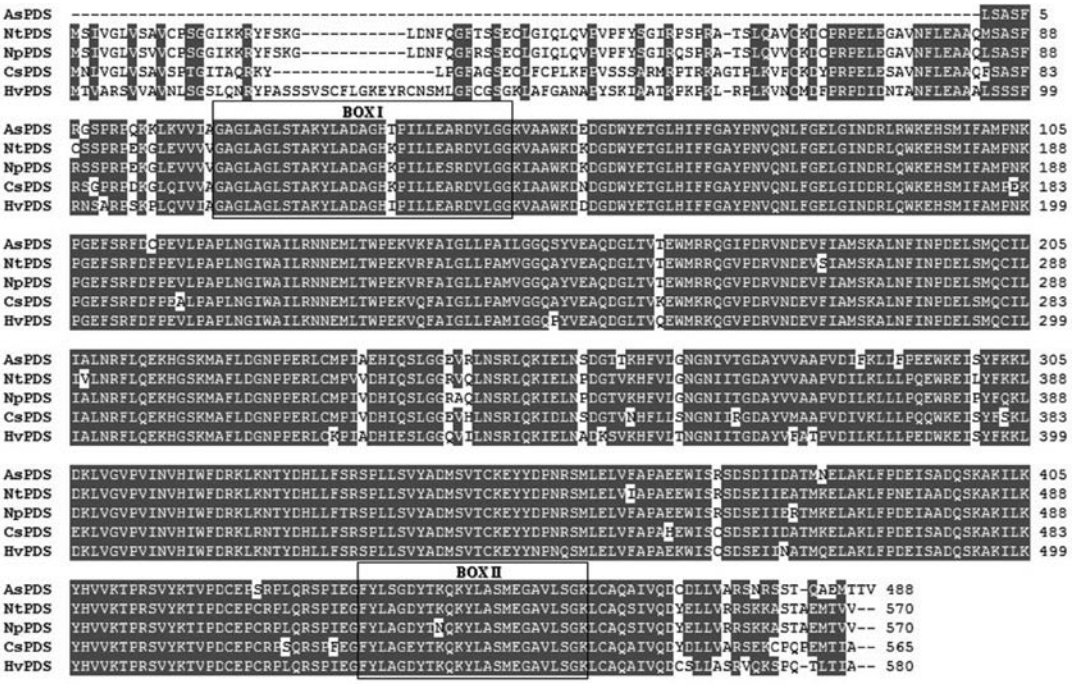

Figure 3. Comparison of the deduced amino acid sequence of AsPDS from *A. sativum* with PDS proteins from other plants. Box I indicates the dinucleotide-binding domain, and Box II indicates the carotenoid-binding domain. NtPDS, *N. tazetta var. chinensis* PDS (EU138883); NpPDS, *N. pseudonarcissus* PDS (X78815); CsPDS, *C. sativus* PDS (AY183118); and HvPDS, *H. verticillata* PDS (AY639658).

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Real-Time PCR. Real-time PCR primers specific to the coding sequence of PSY and PDS from garlic were designed using the Primer3 Web site (http://frodo.wi.mit.edu/primer3/) (Table 1). The garlic actin gene (GenBank accession number: AY821677), amplified with the primers AsActin F and AsActin R, was used as an internal reference. Real-time PCR reactions were carried out in a 20 μL reaction mixture containing 5 μL of template cDNA, 10 μL of 1× SYBR Green Real-time PCR Master Mix (Toyobo), 0.5 μL of each primer (10 μM), and diethyl pyrocarbonate (DEPC) water. The thermal cycling conditions were as follows: 95 °C for 5 min and 40 cycles of 95 °C for 15 s, 56 °C for 15 s, and 72 °C for 20 s. PCR products were analyzed with the MiniOpticon system (Bio-Rad Laboratories; Hercules, CA, USA). Three replications for each sample were used for real-time analysis.

Extraction and High Performance Liquid Chromatography Analysis of Carotenoids. Carotenoids were extracted from *A. sativum* samples (0.1 g) with 3 mL of ethanol containing 0.1% ascorbic acid (w/v). This mixture was vortexed for 20 s and then incubated in a water bath at 85 °C for 5 min. Subsequently, 120 μL of potassium hydroxide (80% w/v) was added to saponify any potentially interfering oils. After vortexing and incubating at 85 °C for 10 min, the samples were placed on ice and 1.5 mL of cold deionized water and 0.05 mL of β-apo-8′-carotenal (12.5 $\mu g \cdot mL^{-1}$), an internal standard, were added. Next, the carotenoids were extracted twice with 1.5 mL of hexane and centrifuged at 1200*g* each time to separate the layers. The extracts were freeze-dried under a stream of nitrogen gas and resuspended in 50:50 (v/v) dichloromethane/methanol. For HPLC analysis, the carotenoids were separated on an Agilent 1100 HPLC system with a C_{30} YMC column (250 × 4.6 mm, 3 μm; Waters Corporation, Milford, MA, USA) and detected with a photodiode array detector at 450 nm. Solvent A consisted of methanol/water (92:8 v/v) with 10 mM ammonium acetate. Solvent B consisted of 100% methyl *tert*-butyl ether. The flow rate was maintained at 1 $mL \cdot min^{-1}$, and samples were eluted with the following gradient: 0 min, 83% A/17% B; 23 min, 70% A/30% B; 29 min, 59% A/41% B; 35 min, 30% A/70% B; 40 min, 30% A/70% B; 44 min, 83% A/17% B; and 55 min, 83% A/17% B.

Statistical Analysis. Data were analyzed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by pairwise comparisons was performed with the post hoc Tukey's honestly significant difference test, with significance set at $P < 0.05$.

RESULTS AND DISCUSSION

Cloning of PSY and PDS from *A. sativum*. A 400-bp fragment was amplified using the primers PSY_F and PSY_R. The sequence of the fragment revealed 2 distinct PSY genes from garlic, AsPSY1 and AsPSY2, which showed similarity with other PSY genes by a BLAST search. On the basis of the partial sequence, 3′-RACE PCR was carried out to isolate the 3′-ends of AsPSY1 and AsPSY2. AsPSY1 was 777 nucleotides long, encoding 259 amino acids, and AsPSY2 was 774 nucleotides long, encoding 258 amino acids. The primers PDS_F and PDS_R generated a 360-bp fragment with similarities to other PDS genes by a BLAST search. The 3′-end of AsPDS was obtained by 3′-RACE PCR and was 1464 nucleotides long, encoding 488 amino acids.

Sequence Analyses of AsPSY1, AsPSY2, and AsPDS. The partial deduced amino acid sequences of AsPSY1 and AsPSY2 were 81% identical. Multiple alignment analysis indicated that AsPSY1 and AsPSY2 had a highly homology to PSY from other plants (Figure 2). AsPSY1 and AsPSY2 share 83% and 84% identity with *Oryza sativa* PSY; 82% and 84% with *Oncidium cv. 'Gower Ramsey'* PSY; 83% and 85% with *Prunus mume* PSY; and 83% and 87% with *Cucumis melo* PSY, respectively. The conserved *trans*-IPPS-HH (*trans*-isoprenyl diphosphate synthase, for the head-to-head condensation reaction) domains are shown as 2 black boxes in Figure 2.[20] Two aspartate-rich regions (DXXXD, where X encodes any amino acid) were found in AsPSY1 and AsPSY2.[21]

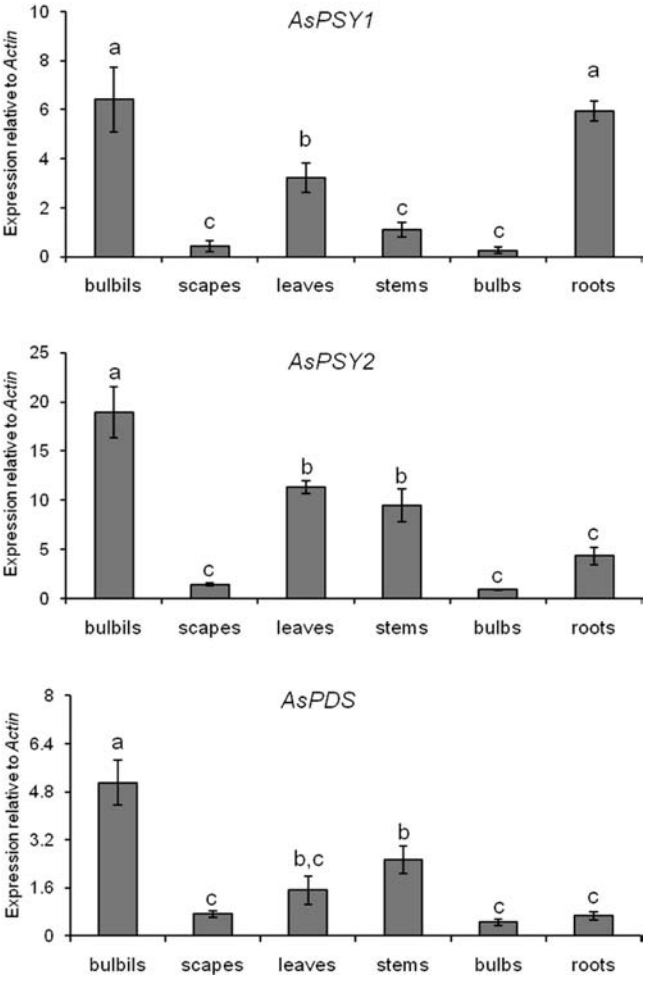

Figure 4. Expression of AsPSY1, AsPSY2, and AsPDS in different organs of *A. sativum*. The values and the error bars represent the average and standard error from 3 independent reactions, respectively. The statistical significance of the differences between treatments was determined using ANOVA followed by paired-group comparisons. The different letters (a, b, and c) indicate significance at $P < 0.05$.

The alignment of the deduced amino acid sequences of AsPDS and PDS proteins from other plants is shown in Figure 3. It shows that AsPDS shares 89% identity and 95% similarity with *Narcissus tazetta var. chinensis* PDS, 89% identity and 94% similarity with *N. pseudonarcissus* PDS, 88% identity and 93% similarity with *Crocus sativus* PDS, and 89% identity and 95% similarity with *Hydrilla verticillata* PDS. The deduced AsPDS contains a conserved dinucleotide-binding motif ($GXGX_2GX_3AX_2LX_3GX_6EX_5GG$) and a carotenoid-binding domain (Figure 3).[10,22]

Expression Levels of the *PSY* and *PDS* Genes in Different Organs of *A. sativum*. Quantitative real-time PCR analysis was used to determine the expression levels of *AsPSY1*, *AsPSY2*, and *AsPDS* in the bulbils, scapes, leaves, stems, bulbs, and roots of garlic (Figure 4). *AsPSY1* and *AsPSY2* were expressed in all organs examined and shared a similar expression pattern. *AsPSY1* and *AsPSY2* were expressed at the highest level in the bulbils, where their relative rate of transcription (RQ value) to the actin

Table 2. Evaluation of Carotenoid Content from Different Organs of *A. sativum* ($\mu g \cdot g^{-1}$ dry weight) ($n = 3$)[a]

compound	bulbils	scapes	leaves	stems	bulbs	roots
α-carotene	0.34 ± 0.00	nd[b]	1.84 ± 0.15	nd	nd	nd
lutein	7.65 ± 0.24 b	2.22 ± 0.30 b	179.8 ± 4.65 a	8.2 ± 0.13 b	nd	nd
β-carotene	2.85 ± 0.08 b	0.60 ± 0.03 b	73.44 ± 2.51 a	1.49 ± 0.03 b	nd	nd
zeaxanthin	1.03 ± 0.01	nd	5.13 ± 0.11	nd	nd	nd

[a] Statistical significance of the differences between treatments was determined using ANOVA followed by paired-group comparisons. The letters (a and b) indicate significance at $P < 0.05$. [b] Not detected.

gene was 6.42 and 18.9, respectively. In the root, the RQ of *AsPSY1* was 5.96, higher than the 4.35 of *AsPSY2*. *AsPSY1* was expressed at a moderate level in the leaves (RQ 3.23) and was weakly expressed in the scapes (RQ 0.44), stems (RQ 1.1), and bulbs (RQ 0.26). *AsPSY2* was abundantly expressed in the leaves (RQ 11.33) and stems (RQ 9.48), whereas it was expressed at a low level in the scapes (RQ 1.42) and bulbs (RQ 0.92). According to these RQ values, *AsPSY1* was expressed at a higher level than *AsPSY2* in garlic. Like *AsPSY1* and *AsPSY2*, AsPDS was expressed constitutively: with the highest levels in the bulbils (RQ 5.1) and the lowest levels in the bulbs (RQ 0.46). The RQ of *AsPDS* in the scapes, leaves, stems, and roots was 0.73, 1.52, 2.54, and 0.66, respectively.

Analysis of Carotenoid Content from *A. sativum*. Carotenoids were identified from 1 g of tissue from different organs of *A. sativum* by HPLC (Table 2). In bulbils, we observed 0.34 $\mu g \cdot g^{-1}$ dry weight α-carotene, 7.65 $\mu g \cdot g^{-1}$ lutein, 2.85 $\mu g \cdot g^{-1}$ β-carotene, and 1.03 $\mu g \cdot g^{-1}$ zeaxanthin. Only small amounts of lutein and β-carotene were found in the scapes and stems, while α-carotene and zeaxanthin were not detected in the scapes and stem. Lutein and β-carotene, which are essential carotenoids for photosynthesis, were abundant in the leaves.[23] Specifically, the levels of lutein and β-carotene in the leaves were 179.6 $\mu g \cdot g^{-1}$ and 73.44 $\mu g \cdot g^{-1}$, respectively, much higher than the 1.84 $\mu g \cdot g^{-1}$ of α-carotene and 5.13 $\mu g \cdot g^{-1}$ of zeaxanthin in this tissue. In contrast to the leaves, carotenoids were not found in the underground organs (roots and bulbs).

AsPSY1, *AsPSY2*, and *AsPDS* genes from *A. sativum* were expressed constitutively with the highest levels in the bulbils and the lowest levels in the bulbs. HPLC analysis demonstrated that carotenoids were not biosynthesized in the underground organs (roots and bulbs), but were very abundant in the photosynthetic organs (leaves) of *A. sativum*. These results suggest a role for light in the accumulation of carotenoids in garlic. Surprisingly, a significant amount of β-carotene was found in the leaves of *A. sativum*, 73.44 $\mu g \cdot g^{-1}$, higher than observed in some common sources of β-carotene, e.g., carrot (65 $\mu g \cdot g^{-1}$), ripe tomato (6.2 $\mu g \cdot g^{-1}$), sweet potato (18.7 $\mu g \cdot g^{-1}$), and pumpkin (11.6 $\mu g \cdot g^{-1}$).[24] Historically, β-carotene, which is converted into vitamin A, has been considered to be an important chemical for humans with the capacity to reduce the risk of stroke, heart disease, and cancer.[25,26]

We discovered that the leaves of garlic have the highest concentration of carotenoid compounds compared with other organs; however, the most commonly used part of garlic is the bulb. As a result, we need to increase consumer awareness of the quality and usefulness of other parts of garlic to maximize the health benefits of carotenoids from this source. For example, immature scapes have a mild taste and can be stir-fried or eaten raw. Garlic leaves are also a popular vegetable in many parts of Asia.

In conclusion, the sequences of PSY and PDS together with the carotenoid analysis presented in this report provide important data to investigate the contribution of carotenoids to the medicinal benefits of *A. sativum*. Furthermore, our research might be helpful to engineer carotenoid biosynthesis in *A. sativum*, a daily foodstuff and a potential medicinal plant.

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ABBREVIATIONS USED

ANOVA, analysis of variance; DEPC, diethylpyrocarbonate; DW, dry weight; HPLC, high-performance liquid chromatography; PDS, phytoene desaturase; PSY, phytoene synthase; RACE, rapid amplification of cDNA ends; ZDS, ξ-carotene desaturase

REFERENCES

(1) Britton, G.; Liaaen-Jensen, S.; Pfander, H. *Carotenoids. Handbook*. Birkhäuser Verlag: Basel, Boston, Berlin, 2004.

(2) Bartley, G. E.; Scolnik, P. A. Plant carotenoids: pigments for photoprotection, visual attraction, and human health. *Plant Cell* **1995**, *7*, 1027–1038.

(3) Peñuelas, J.; Munné-Bosch, S. Isoprenoids: an evolutionary pool for photoprotection. *Trends Plant Sci.* **2005**, *10* (4), 166–169.

(4) Howitt, C. A.; Pogson, B. J. Carotenoid accumulation and function in seeds and non-green tissues. *Plant, Cell Environ.* **2006**, *29* (3), 435–445.

(5) Hirschberg, J. Carotenoid biosynthesis in flowering plants. *Curr. Opin. Plant Biol.* **2001**, *4* (3), 210–218.

(6) Giovannucci, E. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *J. Natl. Cancer Inst.* **1999**, *91* (4), 317–331.

(7) Botella-Pavia, P.; Rodríguez-Concepcion, M. Carotenoid biotechnology in plants for nutritionally improved foods. *Physiol. Plant.* **2006**, *126* (3), 369–381.

(8) Cong, L.; Wang, C.; Chen, L.; Liu, H.; Yang, G.; He, G. Expression of *phytoene synthase1* and carotene desaturase *crtI* genes result in an increase in the total carotenoids content in transgenic elite wheat (*Triticum aestivum* L.). *J. Agric. Food Chem.* **2009**, *57* (18), 8652–8660.

(9) Arango, J.; Wüst, F.; Beyer, P.; Welsch, R. Characterization of phytoene synthases from cassava and their involvement in abiotic stress-mediated responses. *Planta* **2010**, *232* (5), 1251–1262.

(10) Yan, P.; Gao, X.; Shen, W.; Zhou, P. Cloning and expression analysis of phytoene desaturase and ζ-carotene desaturase genes in *Carica papaya*. *Mol. Biol. Rep.* **2011**, *38* (2), 785–791.

(11) Guo, X.; Yang, L.; Hu, H.; Yang, L. Cloning and expression analysis of carotenogenic genes during ripening of autumn olive fruit (*Elaeagnus umbellata*). *J. Agric. Food Chem.* **2009**, *57* (12), 5334–5339.

(12) Livingstone, K.; Anderson, S. Patterns of variation in the evolution of carotenoid biosynthetic pathway enzymes of higher plants. *J. Hered.* **2009**, *100* (6), 754–761.

(13) Fraser, P. D.; Romer, S.; Shipton, C. A.; Mills, P. B.; Kiano, J. W.; Misawa, N.; Drake, R. G.; Schuch, W.; Bramley, P. M. Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99* (2), 1092–1097.

(14) Qin, G.; Gu, H.; Ma, L.; Peng, Y.; Deng, X. W.; Chen, Z.; Qu, L.-J. Disruption of phytoene desaturase gene results in albino and dwarf phenotypes in *Arabidopsis* by impairing chlorophyll, carotenoid, and gibberellin biosynthesis. *Cell Res.* **2007**, *17*, 471–482.

(15) Bhagyalakshmi, N.; Thimmaraju, R.; Venkatachalam, L.; Murthy, K. N. C.; Sreedhar, R. V. Nutraceutical applications of garlic and the intervention of biotechnology. *Crit. Rev. Food Sci. Nutr.* **2005**, *45* (7), 607–621.

(16) Bozin, B.; Mimica-Dukic, N.; Samojlik, I.; Goran, A.; Igic, R. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). *Food Chem.* **2008**, *111* (4), 925–929.

(17) Gorinstein, S.; Leontowicz, M.; Leontowicz, H.; Najman, K.; Namiesnik, J.; Park, Y.-S.; Jung, S.-T.; Kang, S.-G.; Trakhtenberg, S. Supplementation of garlic lowers lipids and increases antioxidant capacity in plasma of rats. *Nutr. Res. (N.Y.)* **2006**, *26* (7), 362–368.

(18) Ohaeri, O. Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. *Biosci. Rep.* **2001**, *21* (1), 19–24.

(19) Patumraj, S.; Tewit, S.; Amatyakul, S.; Jariyapongskul, A.; Maneesri, S.; Kasantikul, V.; Shepro, D. Comparative Effects of Garlic and Aspirin on Diabetic Cardiovascular Complications. *Drug Delivery* **2000**, *7* (2), 91–96.

(20) Rasid, O. A.; Syuhada, W. S. W. N.; Hanin, A. N.; Masura, S. S.; Zulqarnain, M.; Ho, C. L.; Suhaimi, N.; Sambanthamurthi, R. RT-PCR amplification and cloning of partial DNA sequence coding for oil palm (*Elaeis oleifera*) phytoene synthase gene. *Asia Pac. J. Mol. Biol. Biotechnol.* **2008**, *16*, 17–24.

(21) Ohnuma, S.-i.; Nakazawa, T.; Hemmi, H.; Hallberg, A.-M.; Koyama, T.; Ogura, K.; Nishino, T. Conversion from farnesyl diphosphate synthase to geranylgeranyl diphosphate synthase by random chemical mutagenesis. *J. Biol. Chem.* **1996**, *271* (17), 10087–10095.

(22) Zhu, Y.-H.; Jiang, J.-G.; Yan, Y.; Chen, X.-W. Isolation and characterization of phytoene desaturase cDNA involved in the β-carotene biosynthetic pathway in *Dunaliella salina*. *J. Agric. Food Chem.* **2005**, *53* (14), 5593–5597.

(23) Yamamizo, C.; Kishimoto, S.; Ohmiya, A. Carotenoid composition and carotenogenic gene expression during Ipomoea petal development. *J. Exp. Bot.* **2009**, *61* (3), 709–719.

(24) Bhaskarachary, K.; Rao, D. S. S.; Deosthale, Y. G.; Reddy, V. Carotene content of some common and less familiar foods of plant origin. *Food Chem.* **1995**, *54* (2), 189–193.

(25) Mayne, S. T. Beta-carotene, carotenoids, and disease prevention in humans. *FASEB J.* **1996**, *10* (7), 690–701.

(26) Kritchevsky, S. B. beta -Carotene, carotenoids and the prevention of coronary heart disease. *J. Nutr.* **1999**, *129* (1), 5–8.