Plant Omics Journal POJ

POJ 5(2):143-148 (2012) ISSN:1836-3644

Carotenoids accumulation and expression of carotenogenesis genes during seedling and leaf development in Chinese cabbage (*Brassica rapa* subsp. *pekinensis*)

Pham Anh Tuan[1,*], Nam Il Park[2,*], Woo Tae Park[1], Yeon Bok Kim[1], Jae Kwang Kim[3], Jonghoon Lee[4], Suh-hee Lee[4], Tae-Jin Yang[4,5,†] and Sang Un Park[1,†]

[1]Department of Crop Science, College of Agriculture & Life Sciences, Chungnam National University, 79 Daehangno, Yuseong-gu, Daejeon, 305-764, Korea
[2]Wildlife Genetic Resources Center, National Institute of Biological Resources, 42 Nanji-ro, Seo-gu, Incheon, 404-708, Korea
[3]National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Korea
[4]Department of Plant Science, Plant Genomics and Breeding Institute, [5]Research Institute for Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul, 151-921, Korea

[*]PA Tuan and NI Park Contributed equally.
***Corresponding author:T.J. Yang - E-mail: tjyang@snu.ac.kr or S.U. Park - E-mail: supark@cnu.ac.kr**

Abstract

To investigate the regulation mechanisms of carotenoid biosynthesis in Chinese cabbage (*Brassica rapa* subsp. *pekinensis*), carotenoids accumulation and carotenogenesis genes (BrPSY, BrPDS, BrZDS, BrLCYB, BrLCYE, BrCHXB, BrZEP, and BrNCED) involved in carotenoids biosynthesis were analyzed during seedling and leaf development using real-time PCR. For seedling, some of carotenogenesis genes were showed similar to expression pattern between under dark and light condition. Carotenogenesis genes reached the maximum transcription at 8 days after sowing and this was similar to the highest contents of most detected carotenoids. Carotenoids contents (mainly lutein and β-carotene) in light-grown seedlings were several time higher than those in dark-grown ones. For leaf development, direct lighted–outer leaves contain a 30-fold higher carotenoid amount compared to inner and middle leaves. However, expression level of carotenogenesis genes in outer leaves did not show a big different with those in inner and middle leaves. These results suggest a considerable role of light in carotenoids biosynthesis during seedling and leaf development.

Keywords: Carotenoid, *Brassica rapa*, Chinese cabbage, carotenogenesis genes, gene expression.
Abbreviations: DEPC, diethylpyrocarbonate; DW, dry weight; HPLC, high-performance liquid chromatography; DAS, days after sowing; GGDP, geranylgeranyl diphosphate; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ξ-carotene desaturase; LCYB, lycopene β-cyclase; LCYE, lycopene ε-cyclase; CHXB, β-ring carotene hydroxylase; CHXE, ε-ring carotene hydroxylase; ZEP, zeaxanthin epoxidase; NCED, 9-cis epoxycarotenoid dioxygenase; I-leaves, inner leaves; M-leaves, middle leaves; O-leaves, outer leaves.

Introduction

Carotenoids are a diverse group of over 600 structures and consist of 40 carbon molecules. Carotenoids are synthesized in all type of plastid in plant where they fulfill in many important processes. As pigment, carotenoids contribute to yellow, orange, red color of flower and fruit attracting pollinator and seed dispersal agents (Howitt and Pogson, 2006). Carotenoids participate in light harvesting and protect against photo-oxidation during photosynthesis (Bartley and Scolnik, 1995). Carotenoids are precursors of flavors, abscisic acid and other derivatives involved in plant growth and development (Auldridge et al., 2006; Simkin et al., 2004). Moreover, carotenoids have been considered as essential nutrition for animal and human. Provitamin A and a higher intake of them can reduce the risks of some cancers, macular degeneration, and cataracts (Giovannucci, 1999; Mayne, 1996), however, the deficiency of carotenoids leads to xerophthalmia, blindness and premature death (IMFNB, 2000; Mayne, 1996). Because human lack the ability to synthesize carotenoids so they must obtain them by diet, enhancing carotenoids in crop have attracted the interest of many researchers for a long time. Golden Rice is an excellent example for metabolic engineering of carotenoids in crop (Ye et al., 2000). Carotenoids biosynthetic pathway takes place in plastid and have been extensively studied in most of the species. The first step of carotenoids biosynthetic pathway is the condensation of two geranylgeranyl diphosphate (GGDP) to produce phytoene, catalyzed by phytoene synthase (PSY) (Fig. 1) (Cong et al., 2009). Phytoene desaturase (PDS) and ξ-carotene desaturase (ZDS) introduce four double bonds that convert phytoene to lycopene via ξ-carotene (Guo et al., 2009; Yan et al., 2011). Cyclization of lycopene is a branching point in the pathway: one branch leads to α-carotene catalyzed by the co-ordination action of lycopene β-cyclase (LCYB) and lycopene ε-cyclase (LCYE), one branch leads to β-carotene catalyzed by single action of LCYB (Mendesa et al., 2011). Subsequently, hydrolation of α-carotene and β-carotene produce lutein by both β-ring carotene hydroxylase (CHXB), ε-ring carotene hydroxylase (CHXE) and zeaxanthin by CHXB alone, respectively (Tian et al., 2003). Epoxidation of zeaxanthin yields violaxanthin

catalyzed by zeaxanthin epoxidase (ZEP) (Audran et al., 1998). Violaxanthin is used as a substrate for 9-cis epoxycarotenoid dioxygenase (NCED) to synthesis plant hormone abscisic acid (Martínez-Andújar et al., 2011). To date, almost all carotenogenesis genes which involved in carotenoids biosynthesis have been identified and characterization in various plants (Cunningham, 2002; Dobrowolska, 2006). The transcription of carotenogenesis genes is a highly regulated process by many factors. Among them, light is proved to play a considerable role in induce the expression of carotenogenesis genes in some plants (Pizarro and Stange, 2009; Römer and Fraser, 2005). The genus *Brassica* includes many vegetable crops such as broccoli, cabbage, Chinese cabbage, cauliflower, mustard, rape, kale, and turnip. *Brassica rapa* is widely cultivated as a leafy vegetable. One of the subspecies of Chinese cabbage, namely *B. rapa* subsp. *pekinensis*, is one of the most important vegetable crops in Asia and, although it originated in China, is now increasingly popular in Europe as well as the United States since they contain rich sources of glucosinolates as well as high concentrations of flavonoids, carotenoids, and chlorophylls (Artemyeva and Solovyeva, 2006; Krumbein et al., 2005). In this study, carotenoids accumulation and expression of carotenogenesis genes were analyzed during seedling and leaf development of Chinese cabbage (*B. rapa* subsp. *pekinensis*). The effect of light on carotenoids biosynthesis in seedling was also investigated.

Results

Expression of carotenogenesis genes during seedling development under dark and light condition

The seedlings of Chinese cabbage frown for 10 days after sowing (DAS) under light or dark condition were shown in Fig 2A. The seedlings grown in dark condition were higher than those grown in light. The cotyledons of dark-grown seedling were small and yellow while the cotyledons of light-grown sprout were bigger and pale green. The expression level of carotenogenesis genes were examined during seedling development under light or dark condition (Fig 2B). Begin the carotenoids biosynthetic pathway; expressions of *BrPSY* under light or dark condition are similar each other in pattern. Transcription of *BrPSY* was almost the same during first 6 DAS, then increased on 8 DAS and dropped on 10 DAS. Except *BrLCYE*, this expression pattern was continued in next 4 genes (*BrPDS*, *BrZDS*, *BrLCYB* and *BrCHXB*). Unlike the lycopene β-cyclase (BrLCYB), lycopene ε-cyclase (BrLCYE) expressed differently under light or dark condition. Under light condition, expression of *BrLCYE* was reduced from 2 to 8 DAS and recovered slightly on 10 DAS. Under dark condition, expression of *BrLCYE* was reduced from 2 to 6 DAS, increased strongly on 8 DAS and finally decreased on 10 DAS. The transcription level of *BrZEP* was relatively low in 2, 4, 6 DAS, higher in 8 DAS under both light and dark condition, and then followed by a decrease in 10 DAS under dark condition or an increase in 10 DAS under light condition. *BrNCED* expression rose from 2 to 8 DAS and fell on 10 DAS under both dark and light condition. According to the relative quantities of transcription to actin gene, carotenogenesis genes in seedlings under dark condition did not show big difference with those under light condition. In general, carotenogenesis genes share a similar expression pattern during seedling development with the highest transcriptrion detected on 8 DAS.

Analysis of carotenoids biosynthesis during seedling development under dark and light condition

To evaluate the relation between carotenogenesis genes and carotenoids accumulation, 8 carotenoids (lycopene, α-carotene, lutein, β-carotene, β-cryptoxanthin, zeaxanthin, antheraxanthin, and violaxanthin) were identified from 1 g of seedlings during development by HPLC (Table 2). Under dark condition, most of carotenoids were synthesized poorly during seedling development and reached the maximum level on 8 DAS. Except lutein and β-carotene, there was also no remarkable content of other carotenoids found in seedling under light condition. From 4 to 10 DAS, significant amounts of lutein (ranging from 34.09 to 59.72 μg/g) and β-carotene (ranging from 105.77 to 118.49 μg/g) were detected. Under dark condition, total carotenoids content increased from 2 DAS (15.94 μg/g) to 4 DAS (33.84 μg/g) and then kept stably on 6 DAS (30.58 μg/g), 8 DAS (36.37 μg/g), and 10 DAS (31.46 μg/g). Under light condition, total carotenoids contents increased drastically from 48.12 μg/g in 2 DAS to 203.53 μg/g in 8 DAS and decreased to 178.56 μg/g in 10 DAS. Total carotenoids content in seedlings under light condition was several times higher than that under dark condition because of the abundant content of lutein and β-carotene, which are essential for photosynthesis (Yamamizo et al., 2010). It is supposed that lutein and β-carotene mainly contribute to the photosynthetic processes in Chinese cabbage.

Expression of carotenogenesis genes during leaf development

A similar pattern in expression of upstream carotenogenesis genes during leaf development was observed in Fig 3. Transcription of *BrPSY*, *BrPDS*, *BrZDS*, *BrLCYB*, *BrLCYE*, *BrCHXB* reduced from inner leaves to middle leaves and recovered in outer leaves. Two genes, *BrZEP* and *BrNCED*, involved in ABA biosynthesis (Thompson et al., 2000) show a different expression pattern with upstream carotenogenesis genes. Expression level of *BrZEP* and *BrNCED* increased from inner leaves to middle leaves, and in case of *BrNCED*, the level of expression decreased in outer leaves while *BrZEP* kept increasing.

Analysis of carotenoids biosynthesis during leaf development

Changes in accumulation of 3 carotenoids (α-carotene, β-carotene and lutein) during leaf development were showed in Table 3. In inner leaves, 9.2 μg/g lutein and 2.56 μg/g β-carotene were found. Middle leaves contained lower amount lutein (8.6 μg/g) and β-carotene (2.48 μg/g) than those in inner leaves. The level of lutein, β-carotene in outer leaves are 73 μg/ g and 260.51, respectively and a trace amount of α-carotene (1.1 μg/g) also existed in this stage. Carotenoids accumulation had an increase of approximately 30-fold during the development from inner to outer leaves. In Chinese cabbage, outer leaves cover inner and middle leaves and contact to light directly. The abundance content of carotenoids in outer leaves compared to inner and middle leaves probably relate to photosynthesis which mostly occur in outer leaves.

Table 1. Primers used for real-time PCR.

Name	Sequence (5' to 3')	Amplicon (base pair)
BrPSY_RT F	GCTATCTACGTTTGGTGCAGAAGAA	189
BrPSY_RT R	AAATGGCTGAATATCGACAGGGTAT	
BrPDS_RT F	GAGCTCGAGGATGATGGTACTGTTA	175
BrPDS_RT R	TAACTGGCACACCAACTAGCTTCTC	
BrZDS_RT F	CCTTCTTGTCAAAGACCACACTCAT	160
BrZDS_RT R	AGCTAGTGAGTTCCTCAGCTTGTCA	
BrLCYB_RT F	AAGATATCCAAGAGAGGATGGTTGC	180
BrLCYB_RT R	CCACCATGTAACCTGTAGAAGGATG	
BrLCYE_RT F	ATGGATGAACAGTCTAAGCTCGTTG	185
BrLCYE_RT R	ACACCGTAGTTGTTTGTGAAAGGAA	
BrCHXB_RT F	CAGAGAAAACAAGCTCTCTGGACAC	185
BrCHXB_RT R	CATCTGCCAAGAGAATCGGTAGTAA	
BrZEP_RT F	AGACTTAAGCGCCATAAGAGGAGAA	185
BrZEP_RT R	ACTTGACATACCAAGTGCCAGAGAC	
BrNCED_RT F	CACATCCTCTGTTTTGTTCACGAC	171
BrNCED_RT R	AAGAGTTTGTTCCTGGAGTTGTTCC	
BrActin_RT F	TAGTGTTGTTGGTAGGCCAAGACAT	188
BrActin_RT R	GGAGCTCGTTGTAGAAAGTGTGATG	

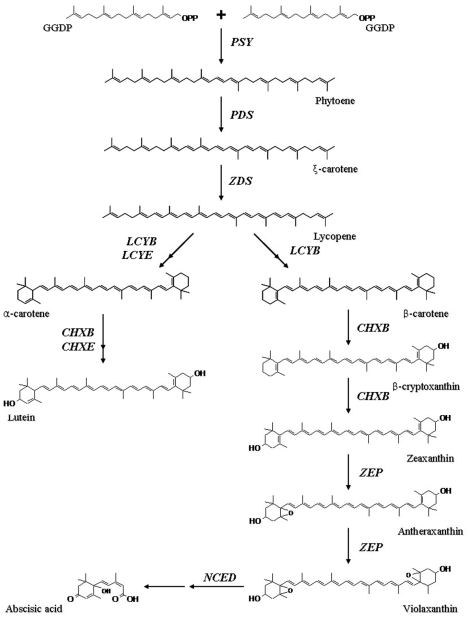

Fig 1. Carotenoids biosynthetic pathway. GGDP, geranylgeranyl diphosphate; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ξ-carotene desaturase; LCYB, lycopene β-cyclase; LCYE, lycopene ε-cyclase; CHXB, β-ring carotene hydroxylase; CHXE, ε-ring carotene hydroxylase; ZEP, zeaxanthin epoxidase; NCED, 9-cis epoxycarotenoid dioxygenase.

Discussions

Most of carotenogenesis genes have a similar expression pattern during seedling development with the highest expression at 8 DAS correlated to the highest contents of most detected carotenoids. The transcription of carotegenesis genes were not big different in seedling between dark and light condition but the carotenoids content under light condition were several times higher than that under dark condition (Table 2). Likewise, light was found to increase the carotenoid content of seedlings in tomato but has little effect on PDS and PSY transcription (Giuliano et al., 1993). Similar to the case of seedling development, co-expression of upstream genes involved in carotenoid biosynthesis was investigated in leaf development; this suggested that an existence of transcription factors regulate these genes in Chinese cabbage. In *Arabidopsis*, the AtRAP2.2 transcription factor was proved to bind to the cis-acting element ATCTA, which is found in both the PSY and PDS promoters (Welsch et al., 2007). Abundant accumulation of 3 detected carotenoids (α-carotene, β-carotene and lutein) were found in outer leaves of Chinese cabbage (Table 3) while outer leaves did not show a significant increase of carotenoid biosynthetic genes expression like in inner and middle leaves. Because there are more carotenoids available, it suggests that several specific carotenoids except 3 detected one in this study were synthesized in each stage of leaf development and further analysis is needed to have a better view of carotenoids

Table 2. Carotenoid composition in seedlings of Chinese cabbage during 10 days after sowing (μg g^{-1} dry weight). Results are expressed as mean ± standard error of mean (n = 3).

Light condition					
Carotenoids	2 days	4 days	6 days	8 days	10 days
Lycopene	3.26 ± 0.42	4.62 ± 0.62	2.23 ± 0.35	1.76 ± 0.17	1.06 ± 0.07
α-carotene	0.73 ± 0.05	2.39 ± 0.17	2.55 ± 0.18	3.04 ± 0.21	2.43 ± 0.07
Lutein	7.63 ± 0.77	34.09 ± 0.82	40.84 ± 4.57	59.72 ± 5.16	52.22 ± 7.12
β-carotene	26.97 ± 2.11	106.61 ± 3.11	107.95 ± 4.67	118.49 ± 9.71	105.77 ± 1.26
β-cryptoxanthin	2.62 ± 0.19	3.33 ± 0.15	2.91 ± 0.26	3.52 ± 0.27	2.92 ± 0.29
Zeaxanthin	1.94 ± 0.16	4.47 ± 0.17	4.05 ± 0.38	5.66 ± 0.33	5.07 ± 0.97
Antheraxanthin	0.73 ± 0.06	2.15 ± 0.03	2.04 ± 0.27	2.01 ± 0.10	1.68 ± 0.21
Violaxanthin	4.25 ± 0.53	15.57 ± 0.21	11.21 ± 1.30	9.33 ± 1.02	7.42 ± 0.60
Total	48.12 ± 0.79	173.22 ± 5.29	173.78 ± 11.99	203.53 ± 16.97	178.56 ± 10.59
Dark condition					
Carotenoids	2 days	4 days	6 days	8 days	10 days
Lycopene	1.58 ± 0.07	8.19 ± 1.11	9.95 ± 0.64	10.61 ± 0.99	10.42 ± 0.15
α-carotene	0.43 ± 0.00	0.87 ± 0.09	0.78 ± 0.04	0.93 ± 0.06	0.88 ± 0.00
Lutein	2.83 ± 0.06	4.29 ± 0.11	4.60 ± 0.25	6.18 ± 0.44	5.41 ± 0.02
β-carotene	7.16 ± 0.50	13.58 ± 1.48	7.88 ± 0.42	10.16 ± 1.34	7.30 ± 0.02
β-cryptoxanthin	1.07 ± 0.02	2.59 ± 0.17	3.71 ± 0.20	4.84 ± 0.47	4.77 ± 0.04
Zeaxanthin	0.52 ± 0.01	0.66 ± 0.03	0.96 ± 0.05	1.23 ± 0.09	1.09 ± 0.01
Antheraxanthin	0.41 ± 0.04	0.73 ± 0.05	0.74 ± 0.10	0.80 ± 0.01	0.66 ± 0.08
Violaxanthin	1.94 ± 0.09	2.94 ± 0.11	1.96 ± 0.29	1.60 ± 0.08	0.92 ± 0.33
Total	15.94 ± 0.79	33.84 ± 3.14	30.58 ± 2.00	36.37 ± 3.5	31.46 ± 0.64

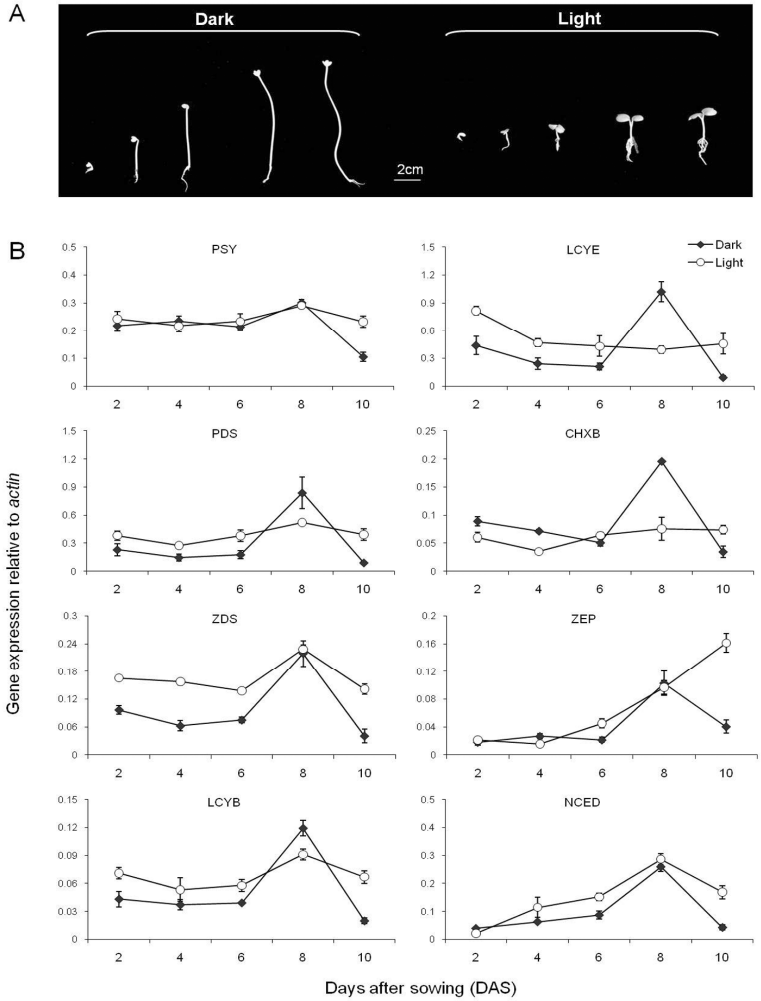

Fig 2. Carotenogenesis genes expression during seedling development of Chinese cabbage. (A) Photograph of Chinese cabbage seedling for 2, 4, 6, 8, 10 days after sowing under dark and light condition. (B) Expression of carotenogenesis genes during seedling development of Chinese cabbage. Units on the horizontal axis indicate the number of days after sowing. The values and the error bars represent the average and standard error from three independent reactions.

Table 3. Carotenoid composition in different stage of Chinese cabbage leaves (μg g^{-1} dry weight). Results are expressed as mean ± standard error of mean (n = 3). N.D., not detected; I-leaves, inner leaves; M-leaves, middle leaves; O-leaves, outer leaves.

Carotenoids	I-leaves	M-leaves	O-leaves
α-carotene	N.D	N.D	1.1 ± 0.06
Lutein	9.2 ± 0.54	8.6 ± 0.27	73.68 ± 0.91
β-carotene	2.56 ± 0.15	2.48 ± 0.06	260.51 ± 4.73
Total	11.76 ± 0.70	11.1 ± 0.34	335.33 ± 5.72

N.D = not detected

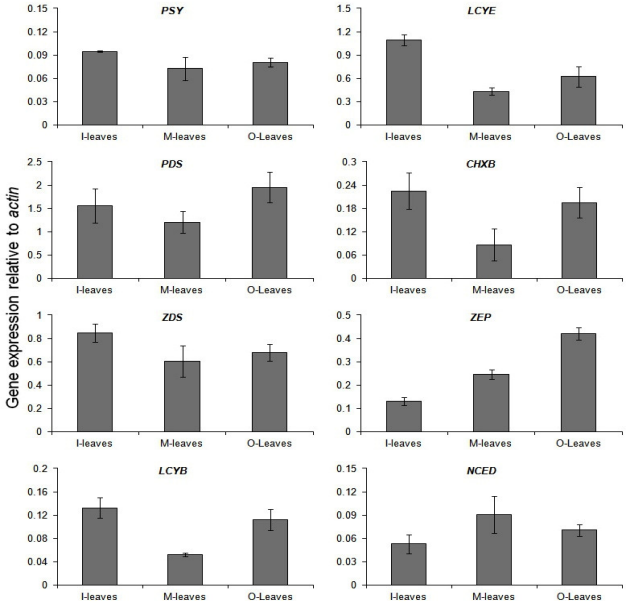

Fig 3. Expression of carotenogenesis genes in different stage of Chinese cabbage leaves. The values and the error bars represent the average and standard error from three independent reactions. I-leaves, inner leaves; M-leaves, middle leaves; O-leaves, outer leaves.

biosynthesis in leaves. Changes in carotenoid composition of leaves at different developmental stages were also observed in white clover (Yoo et al., 2003). Moreover, higher carotenoids content found in direct lighted leaves (outer leaves) and seedling under light condition suggest a proposed role of light in biosynthesis of carotenoids for seedling and leaf development. To date, some researches proved that carotenoid biosynthesis in plants is highly regulated by light, but these processes involved have not yet been identified (Giovannoni, 2004; Pizarro and Stange, 2009). This study might be helpful in understanding the mechanism controlling carotenoids biosynthesis in Chinese cabbage. It will support the process of engineering carotenoids biosynthetic pathway in Chinese cabbage, a popular vegetable in Eastern Asia.

Materials and methods

Plant materials

For seedling development, seeds of Chinese cabbage were soaked in water for 24h before sowing. Seeds were sown in plastic boxes containing the hormone-free half MS medium gelled by agar (8g/L). The seedlings were grown in a growth chamber at 25°C under standard cool white fluorescent tubes with a flux rate of 35 μmol s^{-1} m^{-2}. Two different conditions [light condition (16h light/8h darkness) and dark condition (24h/day darkness)] were used. The seedlings (2, 4, 6, 8 and 10 days after sowing) were collected. For leaf development study, Chinese cabbage was grown in a greenhouse at the experimental farm of Chungnam National University (Daejeon, Korea) for 3 months. Leaves were separated inner, middle, and outer leaves from mature Chinese cabbage.

Expression analysis by real-time PCR

Total RNA was extracted separately from each sample of Chinese cabbage. The single strand cDNA was synthesized using ReverTra Ace-α- (Toyobo, Osaka, Japan) kit according to the manufacturer's protocol. A 25-fold dilution of the resulting cDNA was used for real-time PCR. Using information of carotenogenesis genes of Chinese cabbage published previously [Genbank FJ227935 (BrPSY),FJ606826 (BrPDS), FJ606827 (BrZDS), FJ606828 (BrLCYB), FJ606829 (BrLCYE), GQ178285 (BrCHXB), FJ606830 (BrZEP), and AAV35466 (BrNCED)], specific primers for real-time PCR were designed by Primer 3 (http://frodo.wi.mit.edu/primer3) (Table 1). Actin gene (*BrActin*: FJ969844) was used as an housekeeping gene. Real-time PCR was carried out in a 20 μL reaction volume including 0.5 μM of each primer and 1× of SYBR Green Real time PCR master mix (Toyobo). Real-time PCR reaction was repeated independently three times and analyzed by the MiniOpticon system (Bio-Rad Laboratories; Hercules, CA, USA). The PCR conditions were: 94°C for 5 min; 94°C for 15 seconds, annealing temperature 56°C for 15 seconds and 72°C for 20 seconds for 40 cycles.

Extraction and analysis of carotenoids

Carotenoids were extracted from Chinese cabbage samples (1 g) with 30 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 L 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. Then, the extracts were freeze-dried under a stream of nitrogen gas and resuspended in 50:50 (v/v) dichloromethane/methanol. The extraction method used for carotenoid analysis was similar to that described (Howe and Tanumihardjo, 2006). For high performance liquid chromatography analysis (HPLC), the carotenoids were separated on an Agilent 1100 HPLC system with a C_{30} YMC column (250 × 4.6 mm, 3 m; Waters Corporation, Milford, MA) and detected with a photodiode array (PDA) 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 (MTBE). 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. Identification and peak assignment of carotenoids were primarily based on comparison of their retention time and UV-visible spectrum data with that of standards, and with guidelines previously presented (Fraser et al., 2000; Howe and Tanumihardjo, 2006).

Acknowledgments

This research was supported by Technology Development Program for Agriculture and Forestry (No. 2011-1607), Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea and in part, this research was supported by Molecular and Analytical Service Center for Quality Improvement of Crops (No. 609001-5), Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

References

Artemyeva AM, Solovyeva AE (2006) Quality evaluation of some cultivar types of leafy *Brassica rapa*. Acta Horticulturae 706:121-128

Audran C, Borel C, Frey A, Sotta B, Meyer C, Simonneau T, Marion-Poll A (1998) Expression studies of the Zeaxanthin epoxidase gene in *Nicotiana plumbaginifolia*. Plant Physiol 18:1021-1028

Auldridge ME, McCarty DR, Klee HJ (2006) Plant carotenoid cleavage oxygenases and their apocarotenoid products. Curr Opin Plant Biol 9:315-321

Bartley GE, Scolnik PA (1995) Plant carotenoids: Pigments for photoprotection, visual attraction, and human health. Plant Cell 7:1027-1038

Cong L, Wang C, Chen L, Liu H, Yang G, He G (2009) 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 57:8652-8660

Cunningham FX (2002) Regulation of carotenoid synthesis and accumulation in plants. Pure Appli Chem 74:1409-1417

Dobrowolska A (2006) Molecular view on the carotenogenesis in plant - a mini review. Pol J Food Nutr Sci 15:385–389

Fraser PD, Pinto MES, Holloway DE, Bramley PM (2000) Application of high-performance liquid chromatography with photodiode array detection to the metabolic profiling of plant isoprenoids. Plant J 24:551-558

Giovannoni JJ (2004) Genetic regulation of fruit development and ripening. Plant Cell 16:170-180

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

Giuliano G, Bartley GE, Scolnik PA (1993) Regulation of carotenoid biosynthesis during tomato development. Plant Cell 5:379-387

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

Howe JA, Tanumihardjo SA (2006) Evaluation of analytical methods for carotenoid extraction from biofortified maize (*Zea mays* sp.). J Agric Food Chem 54:7992-7997

Howitt CA, Pogson BJ (2006) Carotenoid accumulation and function in seeds and non-green tissues. Plant Cell Environ 29:435–445

IMFNB (2000) Beta-carotene and other carotenoids. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. National Academy Press 325-400

Krumbein A, Schonhof I, Schreiner M (2005) Composition and contents of phytochemicals (glucosinolates, carotenoids and chlorophylls) and ascorbic acid in selected brassica species (*B juncea*, *B. rapa* subsp. *nipposinica* var. *chinoleifera*, *B. rapa* subsp. *chinensis* and *B. rapa* subsp. *rapa*). J Appl Botany Food Quality 79:168-174

Martínez-Andújar C, Ordiz MI, Huang Z, Nonogaki M, Beachy RN, Nonogaki H (2011) Induction of 9-cis-epoxycarotenoid dioxygenase in *Arabidopsis thaliana* seeds enhances seed dormancy. PNAS 108:17225-17229

Mayne ST (1996) Beta-carotene, carotenoids, and disease prevention in humans. FASEB J 10:690-701

Mendesa AFS, Chenb C, Gmitter Jr FG, Moorec GA, Costa MGC (2011) Expression and phylogenetic analysis of two new lycopene β-cyclases from *Citrus paradisi*. Physiologia Plantarum 141:1-10

Pizarro L, Stange C (2009) Light-dependent regulation of carotenoid biosynthesis in plants. Cien Inv Agr 36:143-162

Römer S, Fraser PD (2005) Recent advances in carotenoid biosynthesis, regulation and manipulation. Planta 221:305-308

Simkin AJ, Schwartz SH, Auldridge M, Taylor MG, Klee HJ (2004) The tomato carotenoid cleavage dioxygenase 1 genes contribute to the formation of the flavor volatiles *β*-ionone, pseudoionone, and geranylacetone. Plant J 40:882−892

Thompson AJ, Jackson AC, Parker RA, Morpeth DR, Burbidge A, Taylor IB (2000) Abscisic acid biosynthesis in tomato: regulation of zeaxanthin epoxidase and 9-*cis*-epoxycarotenoid dioxygenase mRNAs by light/dark cycles, water stress and abscisic acid. Plant Mol Biol 42:833-845

Tian L, Magallanes-Lundback M, Musetti V, DellaPenna D (2003) Functional analysis of beta- and epsilon-ring carotenoid hydroxylases in *Arabidopsis*. Plant Cell 15:1320-1332

Welsch R, Maass D, Voegel T, DellaPenna D, Beyer P (2007) Transcription factor RAP2.2 and its interacting partner SINAT2: Stable elements in the carotenogenesis of *Arabidopsis* leaves. Plant Physiol 145:1073-1085

Yamamizo C, Kishimoto S, Ohmiya A (2010) Carotenoid composition and carotenogenic gene expression during *Ipomoea* petal development. J Exp Bot 61:709-719

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

Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000) Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287:303-305

Yoo SD, Greer DH, Laingc WA, McManus MT (2003) Changes in photosynthetic efficiency and carotenoid composition in leaves of white clover at different developmental stages. Plant Physiol Biochem 41:887-893