

Research Article



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## 저온처리가 케일(*Brassica oleracea*)잎 내 Carotenoid에 미치는 영향

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### Effect of Cold Stress on Carotenoids in Kale Leaves (*Brassica oleracea*)

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Received: 17 June 2017 / Revised: 21 June 2017 / Accepted: 23 June 2017

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#### Abstract

**BACKGROUND:** Kale (*Brassica oleracea*) biosynthesizes various phytochemicals including glucosinolates, flavonoids, and carotenoids. Phytochemicals of plants are influenced by light, temperature, carbon dioxide, and growing conditions. Specifically, carotenoids are affected by temperature, light, and oxygen. The aim of this study was to investigate the effect of cold stress (day/night: 25°C/20°C, 20°C/15°C, 15°C/10°C) on carotenoids in kale leaves.

**METHODS AND RESULTS:** Kale was grown in pots for up to 50 days after sowing (DAS) in a greenhouse. For cold acclimation experiments, kale grown in growth chambers for 3 days and was subjected to low temperature for 4 days. The conditions maintained in the growth chambers were as follows: photoperiod, 12/12 h (day/night); light, fluorescent; and relative humidity, 60%. Carotenoid (lutein,  $\alpha$ -carotene, zeaxanthin,  $\beta$ -carotene) contents were analyzed by high-performance liquid chromatography (HPLC). The total carotenoid content gradually increased during cold acclimation for 3 days. When kale was subjected to cold stress, the total carotenoid content was high at 25°C/20°C treatment, but low at 15°C/10°C treatment. The total carotenoid content of kale leaves continuously grown in

greenhouse decreased from 50 to 57 DAS (1,418 and 1,160 mgkg<sup>-1</sup> dry wt., respectively). The lutein,  $\alpha$ -carotene, and  $\beta$ -carotene contents were very low and the zeaxanthin contents were very high at 15°C/10°C treatment. When kale was subjected to cold stress, the ratio of individual to the total carotenoid contents of kale leaves was 4553% for  $\alpha$ -carotene and 210% for zeaxanthin.

**CONCLUSION:** The  $\beta$ -carotene and zeaxanthin contents in kale leaves indicate their sensitiveness toward cold stress.

**Key words:** Carotenoid, Cold stress, HPLC, Kale

#### 서론

(abiotic stress environment)

(Alonso-

Blanco *et al.*, 2009).

가

(Sanghera *et al.*, 2011).

( , , )

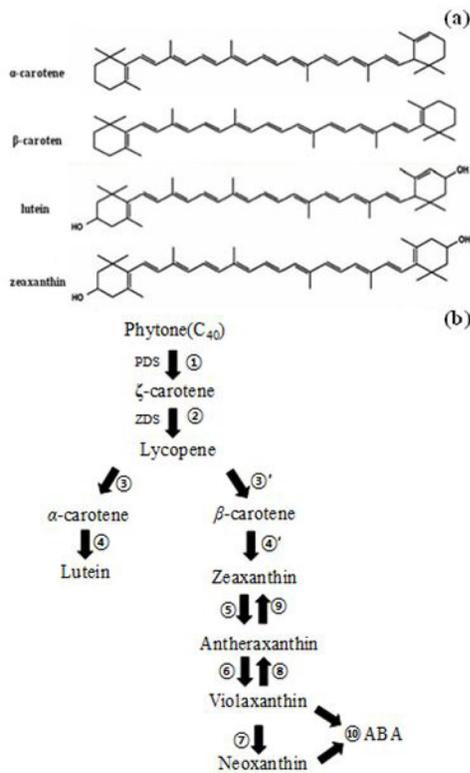
(phytochemical)

(Davies, 1995).

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**Fig. 1. Various carotenoid structure (a) and their biosynthesis pathway (b) (modified from Ha et al., 2012; Demming-Adams and Adams, 2002). PDS, phytyl desaturase; ZDS,  $\zeta$ -Carotene desaturase.**

(Stephen, 1999; Voutilainen et al., 2006).  
 (Lee et al., 2015).  
 (phenolic compounds), (terpenes), (betalains), (organosulfides)/ (sulfur compounds), (glucosinolates), (other organic acids)  
 (Chang et al., 2016).  
 $C_{40}$   
 가 600 가 (Fig. 1a).  
 (Fig. 1b) 40 phytone  
 phytone desaturase (PDS)  
 $\zeta$ - ( $\zeta$ -carotene) (1).  $\zeta$ -carotene desaturase (ZDS)

(lycopene) (2).  
 ,  $\alpha$   $\beta$   
 (3). (xanthophylls)  
 ,  $\alpha$  가 가  
 (lutein) (4)  $\beta$ -  
 (zeaxanthin) (4).  
 (antheraxanthin) (5)  
 (violaxanthin) (6)  
 (neoxanthin) (7).  
 (8) (9)  
 (abscisic acid, ABA)  
 (10)(Ha et al., 2003; Howitt and Pogson, 2006). 가

$\alpha$ - (Fig. 1a).  
 (Mc Graw et al., 2006).  
 $\beta$ - retinol ( A)  
 A , retinoid retinal ( ),  
 retinoic acid (Fraser and Bramley, 2004).  $\alpha$   
 - A  $\beta$

$\beta$ - (Krinsky and Johnson, 2005).  
 (Brassica oleacea var.acephala)  
 $\beta$ - 가 (B. oleracea)  
 가 가  
 (Halvorsen et al., 2002).  
 , , 가  
 , 15 cm  
 가 (A, B, C), ,  
 ( . , )  $\beta$ -  
 , , ,  
 가  
 가 . 2  
 가  
 가 (Matinez-Ballesta, 2013).  
 15°C /9°C 가 12 h ,  
 gluconasturtiin 가 21°C /15°C  
 가 24 h glucoiberin 51% 가  
 sinigrin 65% 가 (Steindal et al., 2014).  
 9 5  
 quercetin isorhamnetin 가 kaempferol  
 , 9 ‘Arsis’ 가 9.

7°C 0.3°C 713 molm<sup>-2</sup>s<sup>-1</sup> 172 molm<sup>-2</sup>s<sup>-1</sup> 가 (Schmidt, 2010). 가 (Spinacia oleracea) 1.5 가 가 (Lefsrud *et al.*, 2005), (35°C) β (Hamazu *et al.*, 1998), 가

**재료 및 방법**

**시약**

HPLC-grade ethanol (C<sub>2</sub>H<sub>6</sub>OH), methanol (CH<sub>3</sub>OH), ethyl acetate (CH<sub>3</sub>COOC<sub>2</sub>H<sub>5</sub>) Fisher Scientific Korea Ltd. (Seoul, Korea) . Potassium hydroxide (KOH) DAEJUNG CHEMICALS & METALS Co., Ltd. (Shiheung, Korea) . Hexane (C<sub>6</sub>H<sub>14</sub>) J.T Baker Inc. (Phillipsburg, NJ, USA) , dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) Merck-Millipore (Darmstadt, Germany)

**시료 채취 및 재배 환경**

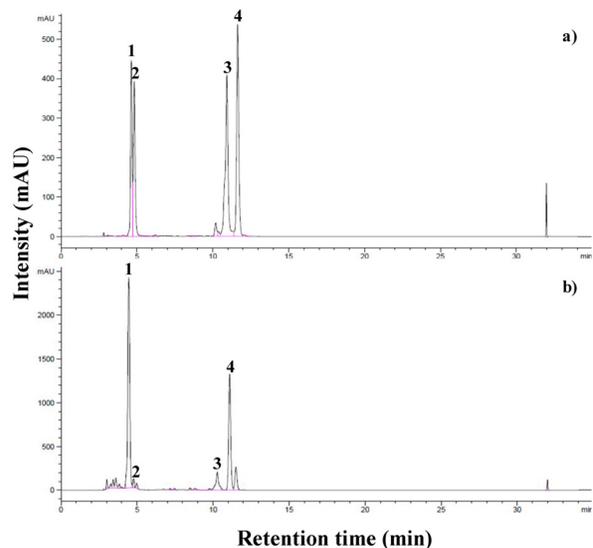
(Asia seed Co., Ltd, Seoul, Korea) TBC 2015 3 2 ( plug tray, 72 ) (High, Punong, Gyeongju, Korea) 7 95% , 21 (DAS, day after sowing) 가 (15×15×19 cm) . 42 DAS (N-P-K, 12-10-9%; ( ), ) 2 g , 2 300 ml . 50 DAS ( ) (SJ-503PL, SEJONG SCIENTIFIC Co., Bucheon, Korea) ( , 165 molm<sup>-2</sup>s<sup>-1</sup>), / 12 h/12 h, 60% , 3 (20°C/15°C) 1 (53 DAS) , 3가 (25°C/20°C, 20°C/15°C, 15°C/10°C) 4 (57 DAS) . -70°C (SFDSF 12, Samwon Freezing Engineering Co., Busan, Korea)

**Carotenoid 추출**

500 mg 50.0 mL-Falcon tube ethanol (5.0 mL) , (75°C) 5 . 80% KOH (1.5 mL) 10 . (2.5 mL) 5 (2.5 mL) vortex (3,000 rpm, 3 min) (Hexane ) (Hexane ) (40°C) dichloromethane: methanol= 50:50 (v/v) 1.0 mL sonicator (POWERSONIC 410, Hwashin Technology Co., Incheon, Korea) 0.45 m hydrophilic PTFE syringe filter ( 13mm) , HPLC vial

**HPLC 분석**

YMC carotenoid C30 column (250×4.6 mm I.D., particle size 3.0 μm) 1200 series HPLC system (Agilent Technologies, CA, USA) (column temperature) 40°C, (detection wavelength) 454nm, (flow



**Fig. 2.** HPLC chromatogram of four carotenoids at 57 DAS in kale leaves. (a), carotenoid 4 STDs; (b), under cold stress (15 C/10 C); Peak 1, lutein; 2, zeaxanthin; 3, -carotene;4, -carotene.

**Table 1. Plant growth ( $n=3$ ) of kale leaves under cold stress**

	Green house		Cold acclimation (53 DAS)	Cold stress (57 DAS, day/night)		
	50 DAS	57 DAS		25°C /20°C	20°C /15°C	15°C /10°C
Leaf number	11±0.0d <sup>a)</sup>	15±0.6a	12±1.0cd	14±0.6abA <sup>b)</sup>	13±0.6bcA	13±0.6bcA
leaf length (cm)	16.2±1.3b	19.0±0.9a	17.5±0.5ab	18.3±0.6aA	17.7±0.8abA	17.7±0.3abA
leaf width (cm)	12.8±1.3b	15.3±0.8a	13.9±1.4ab	14.3±0.3abA	14.8±0.6abA	13.8±0.3abA
Fresh weight (g)	27.9±3.1d	60.0±0.8a	36.5±3.4c	44.9±5.2bA	41.3±1.7bcA	44.4±0.8bcA
Dry weight (g)	3.1±0.4d	8.6±0.7a	4.3±0.5c	5.0±0.3cB	4.9±0.4cB	6.3±0.3bA
Water content (%)	91.8±5.1a	85.7±1.2b	88.1±1.0ab	88.8±0.6abA	88.1±0.6abA	85.8±0.4abB

<sup>a),b)</sup>Within each column, values followed by the same small/capital letters are not significantly different at  $P \leq 0.05$ , using Tukey's multiple range test ( $n=3$ ). Mean±standard deviation.

**Table 2. Carotenoid contents ( $\text{mg} \cdot \text{kg}^{-1}$  DW) in kale leaves**

No. <sup>a)</sup>	RT <sup>b)</sup>	Trivial names	Green house		Cold acclimation (53 DAS)	Cold stress (57 DAS, day/night)		
			50 DAS	57 DAS		25°C /20°C	20°C /15°C	15°C /10°C
1	4.50	Lutein	478.0±21.6a <sup>c)</sup>	404.0±31.3b	462.3±13.1a	467.7±5.68aAd)	468.2±20.6aA	390.4±11.0bB
2	4.83	Zeaxanthin	27.84±0.47b	61.45±35.2b	28.45±5.87b	35.28±0.35bB	20.47±1.52bB	132.1±29.1aA
3	10.53	$\alpha$ -Carotene	187.0±13.4bc	152.8±18.1c	212.3±14.2ab	237.8±14.0aA	223.2±17.5abAB	189.6±15.9bcB
4	11.39	$\beta$ -Carotene	725.1±23.8a	542.4±54.9b	771.6±72.0a	829.0±42.4aA	779.8±20.9aA	591.8±33.9bB
<b>Total</b>			<b>1,418.0±42.6ab</b>	<b>1,160.6±69.7c</b>	<b>1,474.6±94.6ab</b>	<b>1,569.7±54.3aA</b>	<b>1,491.7±56.7aA</b>	<b>1,303.8±69.7bcB</b>
Ratio		$\alpha$ -Carotene/Lutein	0.39±0.62bc	0.38±0.58c	0.46±1.08abc	0.51±2.47aA	0.48±0.85abA	0.49±1.44aA
		$\beta$ -Carotene/Zeaxanthin	26.04±50.12abc	8.83±1.56cd	27.12±12.27ab	23.50±119.63bcB	38.10±13.77aA	4.48±1.16dC

<sup>a)</sup>NO., the HPLC elution order of carotenoid.

<sup>b)</sup>RT, retention time (min).

<sup>c),d)</sup>Within each column, values followed by the same small/capital letters are not significantly different at  $P \leq 0.05$ , using Tukey's multiple range test ( $n=3$ ). Mean±standard deviation.

rate) 1.0 mL/min, (injection volume) 10 L

A[water: metanol=25:75(v/v)]

B[ethyl acetate]

20 70% 가 25 100% 가

25.1 0% 35 10

( 35 ). 4

( , ,  $\alpha$  ,  $\beta$  )(Fig. 2)  
(Wako Pure Chemical Industries, Ltd.

Osaka, Japan) HPLC (area)  
( $\text{mgkg}^{-1}$  DW)

통계분석

' TBC' 3 , HPLC

Microsoft Office Excel 2010

(average) (SD, standard deviation)

IBM SPSS Statistics (21 version)

(P) 0.05

Tukey

## 결과 및 고찰

### 케일의 생장

16.2 cm, 12.8 cm, 27.92 g , 50 DAS 11 ,

15 , 19 cm, 15.3 cm, 60.0

g (Table 1).

8 cm, 4 cm 16cm, 14

cm

(Cristian *et al.*, 2005).

50 DAS가 91.7 % 57 DAS (85.7 %)

( ) 14

가 가 17.9

cm 25°C /20°C (18.3)가 가

가 16.0 cm

43.6

g 25°C /20°C (44.9)가 20°C /15°C

15°C /10°C

87.5 % 가

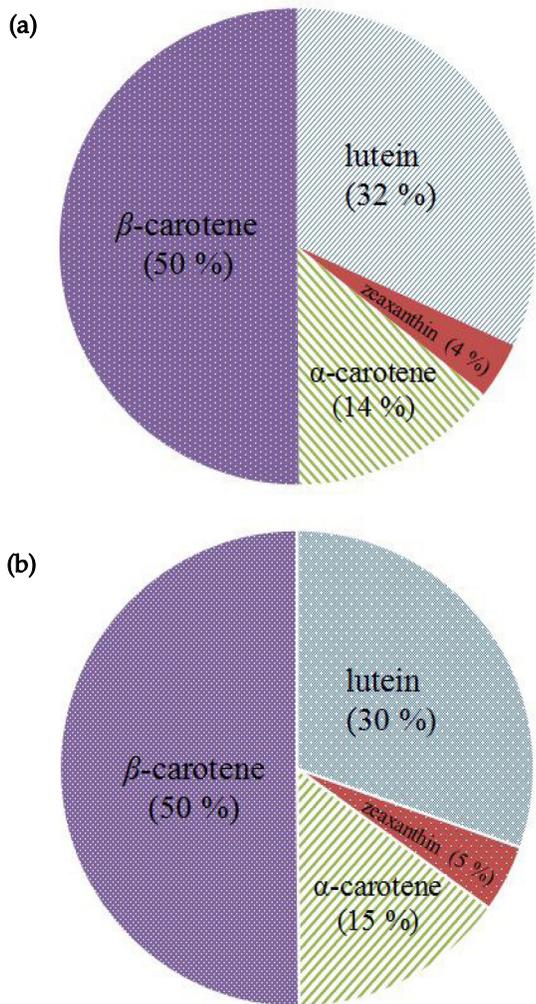


Fig. 3. The ratio (%) of individual carotenoids contents in kale leaves. (a), sum of all data; (b), only sum of the data under cold stress at 57 DAS. These data are recalculated from Table 2 or Table 3.

40% (Raquel *et al.*, 2014).

저온처리에 따른 카로티노이드 함량 ( , )가 .

360~478,  $\alpha$ - 152~237, 20~132,  $\beta$ - 542~828 mgkg<sup>-1</sup>DW (Table 2).

(50.1)> (31.9)> $\alpha$ - (14.2)>  $\beta$ - (3.83%)가 (Fig. 3).

$\beta$ - (Christian *et al.*, 2005; Rachel *et al.*, 2015).

50 DAS (1,418) 57 DAS (1,160 mgkg<sup>-1</sup>DW) 18% 15%, 18% , 2.2 가 25% .

53 DAS 50 DAS 가 . (57 DAS) 25°C/20°C (1,570) 가 가 0°C 가 .

neoxanthin, violaxanthin, lycopene (Park *et al.*, 2014). 20°C/15°C 가 25°C/20°C 15°C/10°C 1.2 .

(Spinacia oleracea) 가 가 1.5 가 (Lefsrud *et al.*, 2005).  $\beta$ - 2 5°C/20°C (15.1%) 가 가 (Table 3). 15°C/10°C 가 20°C/15°C 6.5 ,

Table 3. Individual carotenoid proportions (%) of the total carotenoid content in kale leaves

No. <sup>a)</sup>	Trivial names	Green house		Cold acclimation (53 DAS)	Cold stress (57 DAS, day/night)		
		50 DAS	57 DAS		25 C/20 C	20 C/15 C	15 C/10 C
1	Lutein	33.7±1.3a <sup>b)</sup>	34.8±0.6a	31.4±1.5ab	29.8±1.2bA <sup>c)</sup>	31.4±0.7abA	30.0±2.1bA
2	Zeaxanthin	1.9±0.1b	5.4±3.3b	1.9±0.3b	2.3±0.1bB	1.4±0.1bB	10.1±1.7aA
3	$\alpha$ -Carotene	13.2±0.6b	13.1±0.8b	14.4±0.8ab	15.1±0.4aA	15.0±0.6aA	14.5±0.5abA
4	$\beta$ -Carotene	51.1±1.1a	46.7±1.9b	52.3±1.9a	52.8±0.9aA	52.3±0.7aA	45.4±1.1bB

<sup>a)</sup>NO., the HPLC elution order of carotenoid.

<sup>b),c)</sup>Within each column, values followed by the same small/capital letters are not significantly different at  $P \leq 0.05$ , using Tukey's multiple range test ( $n=3$ ). Mean±standard deviation.

25°C / 20°C 3.7 가 . β  
 25°C/20°C (829.0 mg/ kg dry wt.) 가  
 가 .  
 - 20°C 가 10°C  
 1.6 가 (Lefsrud *et al.*, 2005),  
 (Lycopersicon esculentum) β 20°C  
 가 가  
 (Krumbein *et al.*, 2006). β  
 , β  
 가 .  
 (Pisum  
 sativum L. cv Lincoln) , β  
 1.5 ,  
 4.8 가 (Iturbe-Ormaetxe *et al.*, 1998).  
 , β  
 (Hamazu *et al.*, 1998)  
 β  
 가 .

### Acknowledgment

This research was supported by Golden Seed Project (311022-05-5-SB020), Ministry of Agriculture, Food and Rural Affairs, Ministry of Oceans and Fisheries, Rural Development Administration and Korea Forest Service.

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