

Sulforaphane and Total Phenolics Contents and Antioxidant Activity of Radish according to Genotype and Cultivation Location with Different Altitudes

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Abstract. Sulforaphane (SFN) and total phenolics (TPC) contents and antioxidant activity (AA) were analyzed from 13 radish genotypes (*Rhaphanus sativus* L.), cultivated at 3 locations with different altitudes (Gangneung: asl 5 m, Jinbu: asl 550 m, and Daegwallyeong: asl 750 m). SFN varied greatly from 0.1 to 120.5 $\mu\text{g}\cdot\text{g}^{-1}$ in dry weight test and was significantly affected by location ($P\leq 0.001$), genotype ($P\leq 0.001$) and location \times genotype interaction ($P\leq 0.01$). Radishes, cultivated at Daegwallyeong site, showed higher SFN than those of other locations. Among different genotypes, the root of 'Black radish' and leaves of 'Purunmu' of Daegwallyeong had the highest SFN (107.8 and 120.5 $\mu\text{g}\cdot\text{g}^{-1}$, respectively). TPC in root was affected by genotype ($P\leq 0.001$), and location \times genotype interaction ($P\leq 0.01$), but not by location. In leaves, TPC was affected by location ($P\leq 0.01$), genotype ($P\leq 0.001$), and location \times genotype interaction ($P\leq 0.001$). AA expressed as electron donating ability was significantly influenced by location, genotype and location \times genotype interaction and correlated positively with TPC (Pearson's $r=0.897$) in root. These results suggest that radish could be a good source of functional food and high altitude location such as Daegwallyeong has potential for the production of radish with high content of health promoting factors.

Additional key words: Cruciferae, electron donating ability, isothiocyanate, *Rhaphanus sativus*

Introduction

Radish (*Rhaphanus sativus* L.) is a representative and commercially important cruciferous root vegetable, which has been cultivated and consumed as a main component of Kimchi in Korea. As a traditional oriental medicine, radish was used for stimulating digestion and counteracting poison (Kim et al., 2004). It was revealed that extracts of radish inhibited the growth of human lung cancer cell (Yim et al., 2004) and melanoma cell (Kim et al., 2006). Radish also was reported to have a physiological activities such as anti-mutagenic (Yasushi et al., 2001) or antifungal effects (Hwang, 2003). These physiological effects were reported to mainly come from isothiocyanates (ITC), contained in radish (Hamilton and Teel, 1996; Hashem and Saleh, 1999; Hecht, 1999). Radish was known to have more ITC than other vegetables. 4-methylthio-3-butenyl ITC (MTB-ITC), phenethyl ITC and butyl ITC were the main ITCs found in radish and MTB-ITC

was the highest (Yasushi et al., 2001). MTB-ITC had been mainly considered a principal source of pungency in radish. Therefore, most studies on radish were focused on MTB-ITC (Kim and Rhee, 1993; Lee et al., 1996). Sulforaphane (SFN) is a kind of ITC with sulfur, known as 4-methyl sulfinyl-butyl isothiocyanate and (-)-1-isothiocyanato-4-(R)-(methyl- sulfinyl) butane (Zhang et al., 1992). SFN is the hydrolysis product of glucoraphanin (4-methyl sulfinyl-butyl glucosinolate) and is stably maintained in tissues of vegetable commonly. Glucoraphanin, when a tissue is crushed or chewed, is converted into SFN by specific enzymatic reaction with myrosinase (Nakagawa et al., 2006). SFN, in SAGA broccoli, was confirmed as a major inducer of anticarcinogenic protective enzymes and was reported to reduce a number of tumors and to induce cell cycle arrest and apoptosis in human colon or prostate cancer cell (Chiao et al., 2002; Gamet-Payrastrre et al., 2000; Hecht, 1999; Zhang et al., 1992). SFN was also known as the more efficient indirect antioxidant that supported the

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functions of antioxidants such as the tocopherols or co-enzyme Q and that enhanced the synthesis of glutathione, one of the most abundant intracellular direct antioxidants (Fahey and Talalay, 1999). Many studies supported that vegetables containing phytochemicals with antioxidation potential had strong protective effects against major disease risks including cancer (Kaur and Kapoor, 2001; Knekt et al., 1997). Other studies also have shown that the majority of the antioxidant activity may be from phenolic compounds such as anthocyanin or flavones (Kahkonen et al., 1999; Wang et al., 1996). SFN was generally known to be rich in cruciferous vegetables such as broccoli and cabbage. Also, broccoli sprouts were known to have the highest SFN (Fahey et al., 1997; Kim et al., 1997; Liang et al., 2006). Radish was also reported to have SFN like other cruciferous vegetables by Kim et al. (1997) and that SFN level could differ by cultivars or tissues (Kwon et al., 2008; Lee et al., 1996). Further study on SFN, AA, and TPC could be helpful in production of high quality radish as a functional food resource to promote radish consumption. Therefore, we cultivated 13 genotypes of radish at three different locations and investigated growth characteristics together with the content of SFN, TPC, and AA.

Materials and Methods

Locations and cultivars

Radishes were cultivated in open-fields, located in Gangneung (average sea level, hereinafter asl 5 m), Jinbu (asl 550 m), and Daegwallyeong (asl 750 m). We used 13 cultivars (*Raphanus sativus* L.): 'Chungpingsim' (CPH), 'Madoaltari' (MA), 'Winter white' (WW), 'Black radish' (BR), 'Celadon' (CE), 'Redchime' (RC), 'Purunmu' (PRM), 'Backgwang' (BG), 'Yeongdong' (YD), 'YR leaf radish' (YRL), 'Gwandong' (GD), 'Cunggwang' (CG), 'Danchungbackmi' (DCB). A completely randomized design with 3 replications was used for this experiment. Each plot consisted of 13 rows (10 m in length and one cultivar per a row). Between rows was 20 cm for RC and YRL and was 60 cm for others. Spacing within-row were 10 cm (for RC and YRL), 20 cm (for CPH, MA, WW, BR, CE, and PRM), and 30 cm (for BG, YD, GD, CG, and DCB). All cultivars were directly sown on August 1st in Gangneung (the fall cultivation), on June 15th in Jinbu (the summer cultivation), and on June 30th in Daegwallyeong (the summer cultivation).

Chemicals

The standard for SFN ($C_6H_{11}NOS_2$) was purchased from Sigma Chemical Co. (St. Louis, MO.). Methanol, dichloromethane and acetonitrile were HPLC grade. Folin & Ciocalteu's phenol reagent, ferulic acid and dimethylsulfoxide for total phenolic

content, and 1,1-diphenyl-2-picryl hydrazyl (DPPH) for antioxidant activity were analytical grade of Sigma Co.

Sample preparation

Radishes were harvested at 30 (for RC and YRL), 60 (for CPH, MA, WW, BR, CE, and PRM) and 80 days after sowing (for BG, YD, GD, CG, and DCB) at each location. At harvest, ten radishes from the center of each row in each plot were sampled, cleaned, and cut into small pieces. Then pieces were put into a deep-freezer as soon as possible. They were frozen at $-70^{\circ}C$ for 24 hours and then lyophilized at $-50^{\circ}C$ under the vacuum statement using freeze dryer system (EYELA FDU-2100, JAPAN). Dried samples were ground to powder and then stored at $-30^{\circ}C$ until analyzed.

Sulforaphane

SFN was extracted and analyzed by using the methods of Nakagawa et al. (2006) and Liang et al. (2005) with slight modification. Standard calibration curve was prepared in the following concentrations: 5, 10, 25, 50, 100, and $200 \mu g \cdot mL^{-1}$. The calibration curve was made by linear regression and the result represented the average of three curves performed by three injections of each concentration. SFN was analyzed with HPLC system (Waters ZQ-4000, USA), equipped with photodiode array detector and YMC-Pack ODS AM (250×4.6 mm, $5 \mu m$). The solvent program consists of 20% acetonitrile (in water), and changes linearly over 10 min to 60% acetonitrile. Thereafter, the percentage of acetonitrile was raised to 100% over 2 min, and over the next 2 min to 20% acetonitrile. The flow rate was $1 mL \cdot min^{-1}$ and SFN was detected by absorbance at 243 nm wavelength.

Total phenolic content

Total phenolic content (TPC) was determined by the colorimetric reaction with Folin-Ciocalteu reagent, using the method of Mohamed et al. (2004) with minor modification. Crude extract from dried samples by methanol was filtered through the filter paper (Whatman No.42). 1 mL of the filtered extract was mixed with 3 mL distilled water and 1 mL of Folin-Ciocalteu reagent was added. The mixture incubated at $27^{\circ}C$ for 5 min and then 1 mL of sodium carbonate solution was added. After 60 min, the color was measured at 640 nm by UV/VIS spectrophotometer (X-ma 2000, Human Corp.) using ferulic acid (in dimethylsulfoxide) as a standard. The result was expressed as $mg \cdot 100 g^{-1}$ on a dry weight basis of sample.

Antioxidant activity

Antioxidant activity was evaluated by the DPPH method according to the procedure employed by Park et al. (2004).

0.2 mL of methanolic solution of samples was added to a 2.8 mL DPPH solution (4×10^{-4} M). The solution was mixed and left at room temperature for 10 min. The remaining of DPPH was measured at 520 nm using a UV/VIS spectrophotometer. Antioxidant activity was expressed as electron donating ability (EDA), percent of inhibition relative to the control.

Results and Discussion

Growth

Growth characteristics of radishes at three locations are shown in Table 1. Bolting, leaf and root weight, and water content were significantly affected by the location. Jinbu and

Table 1. Growth characteristics of 13 radish cultivars, grown at Gangneung (average sea level 5 m, fall cultivation), Jinbu (asl 550 m, summer cultivation), and Daegwallyeong (asl 750 m, summer cultivation).

Location	Cultivar ^z	Bolting (%)	No. of leaves	Leaf weight (g)	Root weight (g)	Root color		Water content (%)
						Outside	Inside	
Gangneung	CPH	0	17.6	166.1	153.2	White	Red+yellow	91.2
	MA	3	14.8	147.9	270.9	White	White	92.6
	WW	5	16.6	170.8	415.3	Red+white	White	95.0
	BR	0	9.9	77.8	267.5	Black	White	91.4
	CE	3	33.4	291.3	112.9	White	White	93.4
	RC	0	9.4	13.8	49.8	Red	White	94.1
	PRM	0	14.3	180.9	127.3	Pink	White	93.5
	BG	0	19.9	239.8	764.7	Green+white	White	94.8
	YD	0	26.1	903.9	1,624.5	White	White	94.4
	YRL	0	18.9	189.4	123.9	White	White	93.9
	GD	0	23.9	334.1	1,651.4	Green+white	White	94.4
	CG	0	15.7	308.1	531.7	White	White	94.2
	DCB	0	19.4	228.1	827.4	White	White	92.2
Jinbu	CPH	0	18.1	170.0	167.3	White	Red+yellow	90.1
	MA	3	15.2	149.2	103.1	White	White	89.6
	WW	4	17.0	165.1	415.5	Red+white	White	93.9
	BR	2	13.2	88.9	265.3	Black	White	90.3
	CE	5	34.2	287.0	161.6	White	White	89.0
	RC	0	10.0	15.4	56.1	Red	White	92.1
	PRM	0	17.3	183.6	188.1	Pink	White	91.1
	BG	0	16.9	227.3	761.2	Green+white	White	94.2
	YD	0	24.3	976.7	1,630.3	White	White	93.4
	YRL	0	21.9	161.4	109.2	White	White	91.6
	GD	0	25.3	361.7	2,101.0	Green+white	White	91.2
	CG	2	15.6	329.2	772.2	White	White	92.6
	DCB	0	19.3	223.1	779.3	White	White	92.9
Daegwallyeong	CPH	1	17.9	169.1	326.2	White	Red+yellow	89.3
	MA	4	15.9	151.0	257.4	White	White	91.7
	WW	4	17.1	164.3	411.3	Red+white	White	93.3
	BR	0	11.1	89.3	268.7	Black	White	90.2
	CE	3	35.6	288.3	167.5	White	White	91.0
	RC	1	10.1	15.3	55.3	Red	White	92.3
	PRM	0	19.2	183.1	196.6	Pink	White	91.2
	BG	0	17.4	226.1	778.2	Green+white	White	93.3
	YD	0	24.6	979.1	1,632.3	White	White	93.0
	YRL	0	21.4	166.3	110.6	White	White	91.2
	GD	0	25.3	360.1	1,918.9	Green+white	White	93.7
	CG	0	15.6	326.9	698.2	White	White	91.2
	DCB	0	20.1	230.7	770.9	White	White	93.5
Location		**	*	*	**			**
Genotype		**	**	**	**	-	-	**
Location×Genotype		**	*	**	**			**

^zCPH, 'Chungpihongsim'; MA, 'Madoaltari'; WW, 'Winter white'; BR, 'Black radish'; CE, 'Celadon'; RC, 'Redchime'; PRM, 'Purunmu'; BG, 'Backgwang'; YD, 'Yeongdong'; YRL, 'YR leaf radish'; GD, 'Gwandong'; CG, 'Cunggwang'; DCB, 'Danchungbackmi'.

^{NS}, *, **Non-significant or significant at $P \leq 0.05$ or 0.01 , respectively.

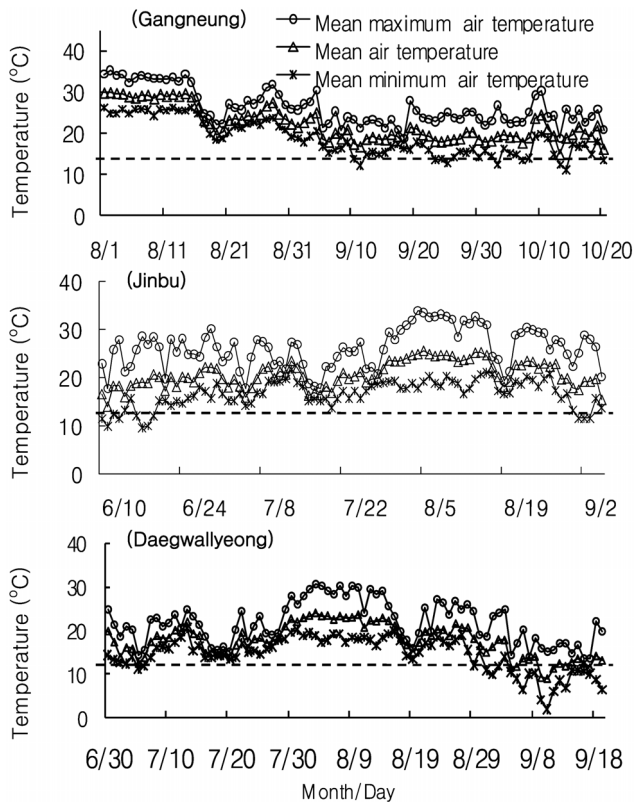


Fig. 1. Changes of temperature during the growing period of radish at Gangneung (average sea level 5 m, fall cultivation), Jinbu (asl 550 m, summer cultivation), and Daegwallyeong (asl 750 m, summer cultivation). The dotted line on a graph indicates 13°C.

Daegwallyeong showed higher bolting rate than Gangneung. Bolting was generally known to be sensitive to low temperature (Yoon et al., 1983). In this study, Daegwallyeong and Jinbu showed much lower mean air temperature, ranging from 7°C to 25°C in summer cultivation, than Gangneung showing from 17 to 30°C in the fall (Fig. 1).

Sulforaphane

HPLC chromatogram of SFN standard are shown at Fig. 2A. A peak of SFN standard was detected at a retention time of 7.4 min in 243 nm. The peak ascribed to SFN in radish root and leaf is also shown at Fig. 2B and C, respectively. SFN content as shown at Table 2 differed significantly among locations ($P \leq 0.01$ in root and $P \leq 0.001$ in leaf) and among genotypes ($P \leq 0.001$ in both root and leaf). Also, there was a significant location \times genotype interaction ($P < 0.001$ in both root and leaf). Mean SFN content was higher in Daegwallyeong, and Jinbu and Gangneung followed in order, and this pattern was same in both root and leaf. Most genotypes consistently showed 2-fold to 33-fold higher SFN contents in Daegwallyeong (the summer cultivation) than in Gangneung (the fall cultivation) although some genotypes did not show any difference by location. High SFN radishes

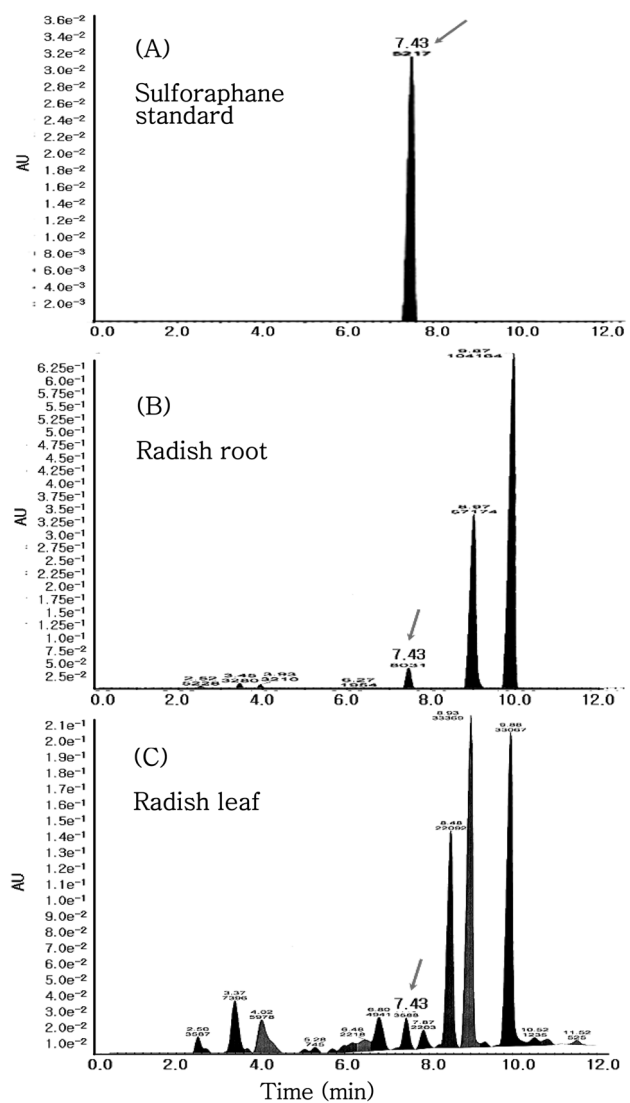


Fig. 2. HPLC chromatograms of sulforaphane standard (A), radish root (B), and radish leaf (C). The arrow indicates the peak of sulforaphane.

(over 100 $\mu\text{g}\cdot\text{g}^{-1}$) were CPH (101.1 $\mu\text{g}\cdot\text{g}^{-1}$ in leaf), BR (107.8 $\mu\text{g}\cdot\text{g}^{-1}$ in root, 102.5 $\mu\text{g}\cdot\text{g}^{-1}$ in leaf) and PR (120.5 $\mu\text{g}\cdot\text{g}^{-1}$ in leaf) grown in Daegwallyeong. Previous studies reported that glucosinolate (a precursor of SFN) content and myrosinase (a hydrolysis enzyme of glucosinolate) activity were influenced by environmental factors such as temperature, ultraviolet ray, or cultivation seasons (Craig and Carl, 2004; Rosa et al., 1996; Vallejo et al., 2003). Vallejo et al. (2003), especially, reported that glucosinolate concentration could be higher when an environment factor gave stress to vegetables. It was known that Daegwallyeong had higher UV (A and B) and light intensity, and more severe temperature difference between day and night than Gangneung (NIHA, 2005). In this study, the temperature or strong ultraviolet ray of Daegwallyeong could be heavier stress-inducing factors to radish than that of Gangneung. Result of this study also suggests that there

Table 2. Content of sulforaphane, total phenolics, and electron donating ability in root and leaves taken from 13 cultivars of radish, grown at Gangneung (average sea level 5 m, fall cultivation), Jinbu (asl 550 m, summer cultivation), and Daegwallyeong (asl 750 m, summer cultivation).

Location	Cultivar ^z	Sulforaphane ($\mu\text{g}\cdot\text{Eg}^{-1}$ dry wt)		Total phenolic content ($\text{mg}\cdot\text{E100 g}^{-1}$ dry wt)		Electron donating ability (%)	
		Root	Leaf	Root	Leaf	Root	Leaf
Gangneung	CPH	17.0 \pm 1.7 ^y	4.7 \pm 0.5	1,198.5 \pm 132.8	825.7 \pm 112.4	56.3 \pm 0.9	74.5 \pm 1.1
	MA	1.9 \pm 0.2	4.2 \pm 0.2	764.3 \pm 68.6	1,609.5 \pm 120.1	32.8 \pm 0.7	82.6 \pm 2.0
	WW	20.4 \pm 3.3	3.9 \pm 0.7	822.0 \pm 106.9	1,494.4 \pm 79.1	45.9 \pm 1.1	84.6 \pm 0.5
	BR	10.8 \pm 1.4	26.6 \pm 1.8	834.8 \pm 104.7	1,272.9 \pm 56.6	43.7 \pm 0.4	87.9 \pm 1.4
	CE	17.1 \pm 0.2	52.6 \pm 1.4	894.9 \pm 58.4	1,624.1 \pm 110.7	35.2 \pm 0.5	82.8 \pm 0.8
	RC	42.1 \pm 2.7	0.1 \pm 0.1	1,338.1 \pm 122.8	1,297.1 \pm 111.2	69.9 \pm 1.3	82.4 \pm 1.0
	PR	10.5 \pm 2.6	50.6 \pm 3.5	1,656.9 \pm 211.7	1,030.4 \pm 110.6	85.8 \pm 1.1	84.6 \pm 1.4
	BG	13.8 \pm 1.3	3.7 \pm 0.8	846.3 \pm 56.6	1,428.6 \pm 120.7	47.3 \pm 0.5	85.2 \pm 0.5
	YD	0.7 \pm 0.4	6.1 \pm 0.2	709.7 \pm 64.1	1,541.9 \pm 113.8	32.1 \pm 0.3	84.8 \pm 1.1
	YRL	3.7 \pm 1.6	17.9 \pm 0.3	600.4 \pm 11.3	1,688.0 \pm 111.8	37.1 \pm 0.6	80.6 \pm 2.4
	GD	0.2 \pm 0.1	3.5 \pm 0.6	743.1 \pm 103.1	1,472.5 \pm 69.2	33.2 \pm 0.3	86.8 \pm 1.6
	CG	24.9 \pm 0.6	8.1 \pm 0.6	718.8 \pm 92.0	1,828.7 \pm 115.2	36.6 \pm 0.3	84.6 \pm 2.1
	DCB	0.1 \pm 0.0	12.9 \pm 1.1	594.3 \pm 88.4	1,006.6 \pm 108.9	31.3 \pm 0.2	59.5 \pm 0.3
Jinbu	CPH	20.3 \pm 0.9	50.3 \pm 6.1	1,346.0 \pm 120.5	753.5 \pm 101.3	68.2 \pm 1.0	50.8 \pm 0.7
	MA	16.2 \pm 2.1	20.5 \pm 1.3	1,039.4 \pm 110.1	1,152.1 \pm 131.7	51.5 \pm 0.5	86.0 \pm 1.4
	WW	21.9 \pm 1.2	19.4 \pm 2.0	1,192.2 \pm 110.6	1,796.8 \pm 100.5	56.6 \pm 0.8	88.1 \pm 1.1
	BR	23.4 \pm 2.0	64.0 \pm 4.5	749.2 \pm 105.8	1,311.5 \pm 112.3	43.9 \pm 0.1	60.5 \pm 0.9
	CE	47.0 \pm 1.8	15.7 \pm 0.8	1,074.0 \pm 162.8	1,433.6 \pm 110.6	48.4 \pm 0.3	51.3 \pm 0.7
	RC	38.6 \pm 1.6	58.6 \pm 2.1	1,335.1 \pm 112.5	1,328.9 \pm 104.7	85.7 \pm 1.3	81.4 \pm 0.9
	PR	22.9 \pm 1.1	54.5 \pm 0.4	1,089.2 \pm 80.3	1,213.8 \pm 99.7	64.8 \pm 0.7	70.6 \pm 1.3
	BG	5.6 \pm 0.3	21.4 \pm 1.0	685.4 \pm 59.5	777.8 \pm 111.4	46.6 \pm 0.6	50.6 \pm 0.8
	YD	1.2 \pm 0.3	6.1 \pm 0.4	485.1 \pm 39.0	1,429.8 \pm 125.6	38.0 \pm 0.1	84.7 \pm 1.3
	YRL	5.7 \pm 1.1	14.8 \pm 0.6	773.5 \pm 70.1	1,601.0 \pm 120.6	46.6 \pm 0.2	63.1 \pm 1.0
	GD	3.7 \pm 0.6	4.3 \pm 0.6	536.7 \pm 31.6	1,324.7 \pm 89.4	40.1 \pm 0.3	54.4 \pm 0.9
	CG	5.9 \pm 0.7	8.0 \pm 0.5	639.9 \pm 34.3	1,667.1 \pm 132.4	41.6 \pm 0.1	54.9 \pm 0.9
	DCB	5.3 \pm 0.7	19.5 \pm 1.0	595.9 \pm 93.9	1,241.6 \pm 79.2	35.1 \pm 0.1	44.6 \pm 0.3
Daegwallyeong	CPH	9.0 \pm 1.7	101.1 \pm 12.0	1,751.0 \pm 109.8	953.6 \pm 104.4	88.3 \pm 1.0	60.8 \pm 0.9
	MA	7.2 \pm 0.8	54.4 \pm 1.1	1,288.9 \pm 222.1	1,565.6 \pm 128.0	50.4 \pm 0.7	86.0 \pm 1.5
	WW	21.8 \pm 1.5	26.9 \pm 0.7	1,196.7 \pm 119.7	1,806.8 \pm 149.3	52.2 \pm 0.7	88.1 \pm 0.6
	BR	107.8 \pm 10.2	102.5 \pm 4.4	652.0 \pm 49.2	1,430.4 \pm 112.7	40.8 \pm 0.9	86.8 \pm 0.5
	CE	48.5 \pm 1.5	21.6 \pm 0.4	1,154.2 \pm 111.9	1,562.0 \pm 131.5	48.0 \pm 0.1	87.7 \pm 1.3
	RC	18.7 \pm 1.3	65.2 \pm 1.0	1,368.5 \pm 160.4	1,585.7 \pm 113.0	67.5 \pm 1.0	88.3 \pm 1.0
	PR	20.4 \pm 1.2	120.5 \pm 5.2	652.0 \pm 47.4	1,222.2 \pm 124.1	42.8 \pm 0.3	86.0 \pm 1.1
	BG	27.0 \pm 0.9	62.9 \pm 1.3	825.1 \pm 109.0	873.2 \pm 24.3	51.1 \pm 1.3	69.0 \pm 0.5
	YD	8.7 \pm 0.6	7.0 \pm 0.4	659.7 \pm 61.8	1,551.0 \pm 146.6	36.4 \pm 0.9	88.1 \pm 1.3
	YRL	8.9 \pm 0.7	17.2 \pm 0.9	917.0 \pm 59.5	1,644.1 \pm 131.6	51.8 \pm 1.1	84.8 \pm 2.1
	GD	6.6 \pm 0.4	7.6 \pm 0.5	642.6 \pm 36.1	1,536.4 \pm 87.9	38.3 \pm 0.6	88.5 \pm 1.3
	CG	33.2 \pm 1.9	8.2 \pm 0.4	579.2 \pm 40.6	1,757.5 \pm 53.7	36.1 \pm 0.7	87.0 \pm 2.0
	DCB	28.4 \pm 2.4	26.8 \pm 0.5	843.4 \pm 37.3	1,593.0 \pm 37.0	42.7 \pm 0.6	88.1 \pm 0.6
Location		**	***	NS	**	**	***
Genotype		***	***	***	***	***	***
Location \times Genotype		***	***	**	***	***	***

^zCPH, 'Chungpihongsim'; MA, 'Madoaltari'; WW, 'Winter white'; BR, 'Black radish'; CE, 'Celadon'; RC, 'Redchime'; PRM, 'Purunmu'; BG, 'Backgwang'; YD, 'Yeongdong'; YRL, 'YR leaf radish'; GD, 'Gwandong'; CG, 'Cunggwang'; DCB, 'Danchungbackmi'.

^yEach value represents the mean \pm SD of three replicates per accession.

^{NS}, **, ***Non-significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

was genetic variation in SFN accumulation and the genotype responded differently to different environments of cultivation locations. However more research is needed to determine how environmental factors affect SFN content. SFN levels, meanwhile, were different between the sampling parts and were highly varied (from 0.1 to 107.8 $\mu\text{g}\cdot\text{g}^{-1}$ on a dry weight basis in root and from 0.1 to 120.5 $\mu\text{g}\cdot\text{g}^{-1}$ in leaf). Leaf showed higher SFN than root, and SFN accumulation varied in root and leaf by locations in the same genotype. This result could be explained by Craig and Carl (2004) who reported that environmental factors (such as temperature, photosynthetic photon flux and photoperiod) differently affected glucosinolate content and myrosinase activity in leaves, stems, and roots.

Total phenolic content

Total phenolic content (TPC) of root ranged widely from 485.1 to 1,751.0 $\text{mg}\cdot 100\text{ g}^{-1}$ on a dry weight basis. It was varied significantly by genotype ($P\leq 0.001$) and by interactions between location and genotype ($P\leq 0.01$), but not by location. Leaf showed average 1,444.1 $\text{mg}\cdot 100\text{ g}^{-1}$ of TPC, 530 $\text{mg}\cdot 100\text{ g}^{-1}$ more than that of root. Leaf sample of Daegwallyeong had the highest TPC (1,488.0 $\text{mg}\cdot 100\text{ g}^{-1}$) and there were significant differences among locations ($P\leq 0.01$), genotypes ($P\leq 0.001$) and location \times genotype interaction ($P\leq 0.001$). Several studies reported similar results. Xiangfei et al. (2007) reported that TPC were different by cultivar types and by harvest time in lettuce. Oomah et al. (1996) also reported that the variation in phenolics was mainly influenced by cultivar, seasonal effects and cultivar \times season interaction. Therefore, TPC could be differed by several factors, such as cultivation method, environment, and genotypes, and identification of the effective pattern of environmental factors that lead to increase TPC of radish is needed.

Antioxidant activity

Antioxidant activity, analyzed by using electron donating ability (EDA) in Table 2, showed significant variation by 3 locations ($P\leq 0.01$ in root, $P\leq 0.001$ in leaf), by 13 genotypes ($P\leq 0.001$ in both root and leaf) and by location \times genotype interactions ($P\leq 0.001$ in both root and leaves). Yu et al. (2003) reported that the environmental factors, such as solar radiation, altered antioxidant properties together with TPC. Wang and Zheng (2001) revealed that the highest difference between day and night temperatures showed the greatest production of phenolics and antioxidant activities. Highland area such as Daegwallyeong generally could have stronger solar radiation due to the high elevation and big difference between the day and night temperature. Therefore, highland areas can be good places for the production of radish containing

higher functional compounds.

Correlations

The relationships between root weight and water content (WTC), SFN, TPC, EDA are shown in Table 3. Correlations of root weight and WTC (*Pearson's* $r=0.396$, $P\leq 0.01$), TPC (*Pearson's* $r=-0.518$, $P\leq 0.001$), and EDA (*Pearson's* $r=-0.463$, $P\leq 0.01$) were significant, but correlations between root weight and SFN were non-significant. The relationship of TPC and EDA in root showed a high positive linear correlation (*Pearson's* $r=0.897$, $P\leq 0.001$). The increase of EDA with the increase of TPC was known as the result of polyphenols exerting their antioxidant action by donating hydrogen atoms to free radicals (Kaur and Kapoor, 2001; Madsen et al., 2000). Poly-phenolics were characterized as phenolic acids that had been deeply involved with natural antioxidants in fruits and vegetables (Kahkonen et al., 1999). In this study, the correlation between TPC and EDA in root suggested that 89.6% of the antioxidant capacity of radish was originated from the contribution of phenolic compounds. On the other hand, antioxidant activity of radish root might not be limited to phenolics and could come from other antioxidants of the secondary metabolites such as carotene and vitamins, which might contribute to about 11% of the antioxidant activity in radish root (Cao et al., 1996; Palace et al., 1999; Stahelin et al., 1991; Wang et al., 1996; Willett, 1994). SFN did not show any correlation with EDA in this study. ITCs such as SFN actually are not direct antioxidants. There is, however, growing evidence that ITC group including SFN play a role as efficient indirect antioxidants. SFN is a potent inducer of Phase 2 enzymes such as glutathione transferase and NAD(P)H:quinone reductase, which detoxify any residual electrophilic metabolites generated by Phase 1 enzymes and thereby destroy their ability to damage DNA (Zhang and Talalay, 1994). SFN also raised the levels of tissue GSH, prototype antioxidant which protects cells from toxins such as free radicals (Pompella et al., 2003), by stimulating the

Table 3. Correlation among radish root weight (RW), water content (WC), sulfuraphane content (SFN), total phenolic content (TPC), and electron donating ability (EDA), based on n=39 means from 13 radish cultivars.

Variable	RW	WC	SFN	TPC	EDA
RW	1.0	0.396**	-0.302 ^{NS}	-0.518***	-0.463**
WC		1.0	-0.138 ^{NS}	-0.116 ^{NS}	-0.138 ^{NS}
SFN			1.0	0.150 ^{NS}	0.164 ^{NS}
TPC				1.0	0.897***
EDA					1.0

^{NS,*,***} Non-significant or significant at $P\leq 0.05$, 0.01, or 0.001, respectively.

antioxidant response elements in the 5'-upstream region of the gene for the heavy sub-unit of G-glutamyl cysteine synthetase (Mulcahy et al., 1997). Therefore, SFN supports the function of naturally-occurring, direct-acting antioxidants such as tocopherols and co-enzyme Q and elevates enzymes that can cope with a wide variety of types of oxidants (Fahey and Talalay, 1999). From these studies, SFN, despite of low correlations with EDA, might indirectly contribute to about 11% of antioxidant activity of radish root together with vitamins and carotenoids. To explain more clearly the low correlation between SFN and EDA in radish, however, it would be necessary to study the antioxidative mechanism of SFN and physiological interactions between SFN and antioxidants of radish. In conclusion, this study suggests that radish could be a good dietary source of natural phenolic antioxidants and health promoter such as SFN. Also, to extend radish consumption through the way of health promotion, it would be important to research radish genotypes that contain high phytochemicals, such as SFN with health benefits and phenolics with high antioxidant activity.

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재배지 고도에 따른 무 품종별 설포라판, 총페놀함량 및 항산화 특성

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초 록. 무 13종을 고도가 다른 3지역(강릉-해발5m, 진부-해발550m, 대관령-해발 750m)에서 재배하여 설포라판함량, 총페놀함량, 항산화성을 조사하였다. 설포라판함량은 재배지역과 품종에 따라 0.1-120.5 $\mu\text{g}\cdot\text{g}^{-1}$ 의 큰 차이가 있었으며 재배지역($P\leq 0.001$), 품종($P\leq 0.001$), 그리고 두 요소의 교호작용($P\leq 0.01$)에 의해 영향을 받은 것으로 나타났다. 특히, 대관령에서 재배된 무 품종들은 다른 두 지역보다 설포라판함량이 높았으며, 품종들 중에는 검정무(근부, 107.8 $\mu\text{g}\cdot\text{g}^{-1}$)와 푸른무(엽부, 120.5 $\mu\text{g}\cdot\text{g}^{-1}$)가 가장 높았다. 총페놀함량은 근부의 경우 품종별($P\leq 0.001$) 큰 차이가 있었으며, 품종 및 지대의 교호작용($P\leq 0.01$)에 의해서도 영향을 받았으나, 지역에 따른 유의적 차이는 없었다. 엽에서는 근부와 달리 지역($P\leq 0.01$)에 따라 차이가 있었으며, 품종별($P\leq 0.001$) 그리고 지대와 품종의 교호작용($P\leq 0.001$)에 의해서도 영향을 받았다. 전자공여능으로 분석한 항산화능은 재배지대와 품종, 그리고 두 요소의 교호작용에 의해 차이가 났다. 한편, 주요 시식부위인 무의 근부에서 총페놀함량과 항산화능의 상관성은 매우 높은 정의 상관(Pearson's $r=0.897$)을 보였으나 설포라판과 총페놀함량 및 설포라판과 항산화능은 상관성이 낮았다. 본 연구에서 무는 일반식품뿐만 아니라 기능성 식품의 원료로도 가치가 있음이 확인되었다. 또한, 건강기능성을 목적으로 한 무의 생산을 위해서는 품종과 재배지대의 선택이 중요하며, 지대가 높은 고랭지가 유리한 것으로 판단되었다.

추가 주요어 : 배추과, 전자공여능, 아이소치오시아네이트, *Raphanus sativus*