HABITAT INFLUENCES COMPOSITION OF VOLATILE CONSTITUENTS IN ALLIUM VICTORIALIS VAR. PLATYPHYLLUM

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Abstract

The composition of volatile constituents from tissues of Korean wild garlic, *Allium victorialis* var. *platyphyllum* collected from different habitats of Ulleung Island, Korea, was determined. The concentrations of total volatile constituents in garlic bulbs and stems were variable. The most abundant volatile compound in *A. victorialis* bulb extract was alanine (47–65%), followed by dimethyl tridisulfide (5.1–10.3%) and allyl disulfide (0.1–12.1%). Dimethyl trisulfide (5.2–10.3%), diallyl disulfide (0.1–12.1%) and dimethl pentasulfide (0–0.3%) were abundant in stem tissues. Very significant differences in the composition of volatile sulfides in stems and bulbs of *A. victorialis* were noticed. Finally principal component analysis (PCA) was adopted to know the chemical-population structure of the garlic extracts. Habitat of garlic plant appreciably affected the proportions of alk(en)yl group (methyl, m-propyl and allyl radicals) in the of bulb and stem tissues.

Introduction

Genus Allium comprises of more than 600 different species with wide distribution throughout North America, North Africa, Europe, and Asia (Ozturk *et al.*, 2012). Most Allium species, such as garlic and onion, are edible and possess characteristic aromas (Lancaster & Boland, 1999; Khan *et al.*, 2005; Ouzounidou *et al.*, 2011; Nabi *et al.*, 2013). The leaves and bulbs have been used not only as mountain-edible herbs but also as functional foods for controlling gastritis and heart failures (Moon, 1984; Fatima *et al.*, 2011).

Many investigations have been conducted on the nature of volatile constituents in *Allium* sp., particularly garlic and onion, for their biochemical actions and flavor production. It is a well known fact that the volatiles are produced by enzymatic splitting of the nonvolatile precursors like *S*-alk(en)yl-L-cysteine sulfoxides when the plants are crushed. The alk(en)yl group volatile sulfides are mainly a combination of propyl, 1-propenyl, allyl, and methyl groups, depending on the species. In these studies varying composition of volatile constituents has been noticed in *Allium* sp. (Block, 1991; Kubec *et al.*, 2000).

The reports on the composition of volatile components of native Korean wild garlic (*Allium victorialis* var. *platyphyllum*) are scanty. This species is a perennial herb, widely distributed in the wild on Mt. Chiri, Mt. Odae and Ulleung Island of Korean peninsula. *A. victorialis* has been traditionally used as an edible and medicinal herb for the treatment of gastritis and heart disease in Korea (Likens & Nickerson, 1964; Moon, 1984). It is reported to contain carbohydrates and ascorbic acid in its leaf and sulfur-containing substances in bulb (Lawson *et al.*, 1991).

Populations of *A. victorialis* in Korea are mainly located at 1,200-1,400m above sea level, especially on $10-35^{\circ}$ slopes at nearby ridges of Mt. Horyeong and Mt. Dongdae in South Korea. The plants are grown under relatively cool (8.2-20.1°C) and humid (71-74%)

conditions. The Ulleung Isalnd, research site selected for the study is about 120 km east of the Korean Peninsula. The island is of volcanic origin, the rocky steep-sided, lying at the top of a large strato volcano, which rises from the seafloor, reaching a maximum elevation of 984 metres at Seongin Peak (Park *et al.*, 2009). Although Ulleung Island is narrow, a large diversity of growing conditions such as soil, rainfall, altitude, etc., exist in the area. It is also a biodiversity hot spot, harboring approximately 700 species of vascular plants with a high endemism (Oh *et al.*, 2010).

Plant species interact with environmental factors, such as drought, and modify their production of secondary metabolites (Mattson & Haack, 1987; Waterman & Mole, 1989; Azhar *et al.*, 2011). The variation of the profiles of secondary metabolites due to environmental factors in higher plants including *Allium* sp., has not been well investigated (Block, 1991). Hence a systematic study on the volatile metabolite profiling of *A. victorialis* var. *platyphyllum* based on varying habitats was undertaken and this manuscript reports the differential profiling of volatile constituents of *A. victorialis*.

Materials and Methods

Plant material and sampling sites: *A. victorialis* var. *platyphyllum* was collected during August 2009 from four naturally growing populations (Jungmaewha valley, Teawha pass, Nari basin, and Sungin peak) in Ulleung Island, Kyungbuk Province, Korea (Fig. 1). Details of sampling sites for each sampled plant are presented in Table 1. The voucher specimens are deposited in the Herbarium of Korea Forest Research Institute in Jinju, Korea.

The plants were divided into two parts; the stem including leaf and the bulb. Each part was dried avoiding direct sunlight and pulverized for further extraction of volatile constituents. The fresh leaves were preserved in refrigerator at -10°C and used within 2 days.

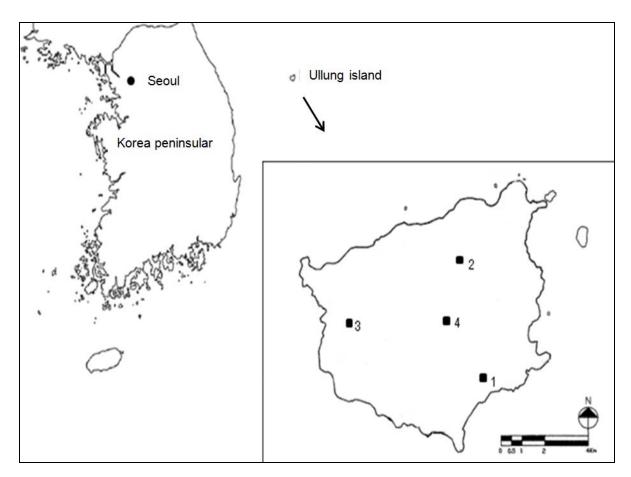


Fig. 1. Location map of the studied area for *A. victorialis* var. *platyphyllum* populations at Ulleung Island. 1 = Jungmaewha valley (10 sites), 2 = Nari basin (8 sites), 3 = Taewha pass (9 sites) and 4 = Sungin peak (17 sites).

	Taewha pass	Nari basin	Seongin peak	Jeongmaehwa valley
Elevation (m)	449 (387-474)	404 (309-521)	866 (768-972)	279 (238-310)
Slope degree (°)	26 (5-40)	16 (3-40)	30 (15-47)	34 (20-52)
Topography (No.) ^z	U6, R2, L1	M4, L2, T1, U1	U17	M5, U3, L1, V1
Soil exposure rate (%)	7 (0-10)	14 (0-70)	14 (0-70	16 (5-40)
Litter layer (cm)	12 (2-16)	3 (1-5)	5 (1-15	6 (2-10)
Tree layer cover degree rate (%)	85 (75-95)	76 (70-80)	84 (70-90)	87 (80-90)
Herb layer cover degree rate (%)	64 (50-75)	85 (50-95)	86 (70-95)	75 (55-90)
Tree layer height (m)	14 (13-17)	16 (12-21)	14 (9-18)	15 (3-15)
DBH of tree layer (cm)	42 (14-66)	32 (22-56)	30 (18-42)	25 (20-30)
No. of present species	26 (18-33)	22 (16-35)	24 (16-35)	23 (15-37)
No. of samples	9	8	17	10

Table 1. Ecological characteristics for A	. victorialis var.	<i>platyphyllum</i> ha	abitat of Ulleung Island.

 ^{z}U = upper slope area, R = ridge area, L = lower slope area, M = middle slope area, V = valley area, DBH = diameter at breast height.

Sample preparation: 10 g of each fresh bulb and stems were harvested, homogenized and transferred to Likens-Nickerson apparatus (Lim *et al.*, 1996), with 1 L of distilled water. Blended 25mL ether and 25mL n-pentane were injected into extraction flask. Both flasks were heated on heating mantle for 1hr. Extract layer of blended ether and n-pentane was collected after repeated cooling. The extract was then dried overnight with sodium sulfate (Na₂SO₄) and decanted, resulting extract was subjected to

rotary evaporation at 30°C under nitrogen gas (N_2) to obtain concentrated extract.

Determination of volatile constituents by GC-MS analysis: The extracts were subjected to gas chromatography (HP5890 SERIES II) (GC) –mass spectrometer (MS) (HP 5971 SERIES MSD) for metabolite profiling. The GC column was HP-1 fused silica capillary column 60 mm \times 0.25 mm ID \times 0.25 µm

film thickness. The GC was operated as follows: injector temperature, 250°C; column temperature, isothermal at 50°C for 5 min, then programmed to 240°C at 3 min and held at this temperature for 10 min; ion source temperature, 230°C. Helium was used as the carrier gas at the rate of 1 mL·min⁻¹. The effluent of the GC column was directly fed into MS. The MS Spectra was recorded in EI mode at 70eV ionization energy. The sector mass analyzer was set to scan from 50 to 800 u for 2s. The volatile constituents were identified by comparison with retention times and mass spectra obtained with the authentic standards in the GC-MS system used for analysis. When an authentic sample was not available, the identification was carried out by comparison of mass spectra with those in the mass spectra library (The Wiley Registry of Mass Spectral Data, 6th Ed). The concentration of volatile constituents in bulbs and stem tissues were determined from calculated peak areas with at least three independent replicate analysis.

Statistical analysis: The statistical analysis of the data was carried out by factor analysis (FA) using principal components analysis (PCA) (Benzecri, 1980). The PCA data was subjected to statistical software program 'XLSTAT 2010 (Addinsoft)'-www.xlstat.com for analysis. Unweighted pair group method with averaging (UPGMA) cluster analysis of SPSS 12K was also employed (SPSS Inc., Chicago, IL.).

Results and Discussion

Profiling of volatile constituents in *A. victorialis:* The volatile constituents of Korean wild garlic were obtained by simultaneous steam and solvent distillation. The extract was subjected to GC-MS analysis (Fig. 2). The identities of volatile constituents of Korean wild garlic are listed in Table 2. Highest abundance of aliphatics, aromatics and terpenes were detected in bulb and stem. Numbers of volatile constituents in bulb extract were comparable with stem tissue extract. Numbers of volatile constituents in bulb extract were 36 and 34 respectively. Although the number of compounds in different groups did not vary much among the habitats, the amount of volatile constituents varied drastically. Numbers of volatile constituents in bulb extract were less, but their individual amounts were high.

Among the volatile constituents, sulfur containing compounds are contained much compared to other constituents as mono, sesqui terpenes and etc. Number of methyl groups in stem and bulb extracts were high in alk(en)yl groups (Table 3). Although number of allyl group volatiles were fewer than those of propyl and methyl group, its amounts were comparable with other alk(en)yl groups. Highest contents of volatile constituents belonged to other types like terpene, aromatics, etc.

The most abundant volatile constituent of Korean wild garlic stem extract was alanine (47-65%), followed by dimethyl tridisulfide (5.1-10.3%) and allyl disulfide (0.1-12.1%) (Table 2). Dimethyl tridisulfide and allyldisulfide composition varied according to habitats represented in Table 1. Alanine, and aliphatic amino acid

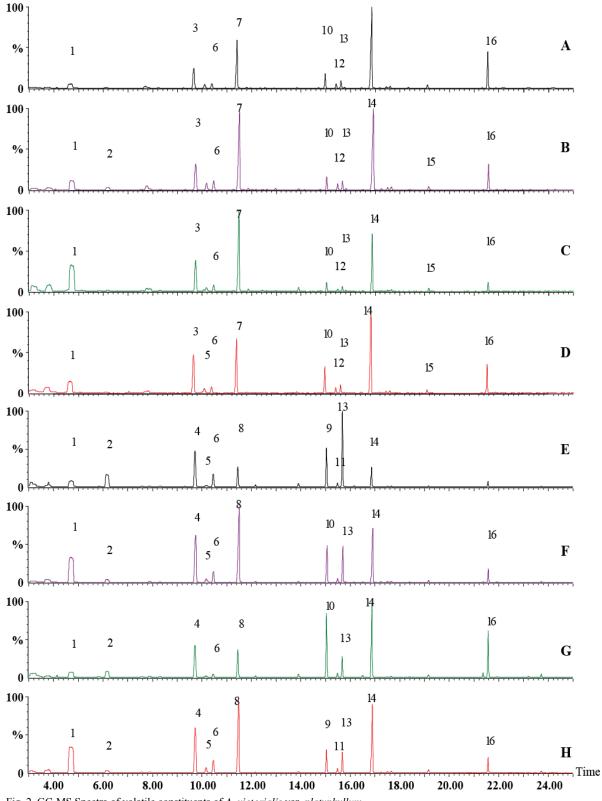
were found in all habitats. In Korean wild garlic bulb extracts, the most abundant volatile constituents were alanine (47–65%), followed by dimethyl tridisulfide (5.1–10.3%) and allyl disulfide (0.1–12.1%) (Table 2).

The variations among 36 significant volatile constituents in stem were determined by principal component analysis (PCA) (Fig. 3). After submitting the normalized data matrix to PCA, it was possible to obtain some variation patterns for the compounds. The distribution of the stem volatile constituents on the correlation circle defined by the three first principal components, allowed the establishment of three groups of compounds. Groups I and II are highly representative of the first principal type component and present a high negative correlation between the two groups. Groups I and III are highly representative of the second principal type component and show a high negative correlation between the two groups. It is important to notice that within each group I-III, compounds have some structural analogy (Fig. 3): compounds in group I are composed of both methyl and alk(en)yl constituents, other volatile constituents; allyl, alkenyl and other volatile constituents are in group II, and group III is composed of other volatile constituents.

Variation of volatile sulfides in plant parts: Volatile sulfides determined in the stem and bulbs of Korean wild garlic revealed that no significant differences are found in the proportions of methyl, propyl and allyl radicals between the bulb and stem. However, large and significant differences were recorded in the concentration of volatile sulfides (Table 2; Fig. 2). Dimethyl trisulfide, diallyl disulfide and dimethl pentasulfide were abundant in stem tissues, but comprised of allylthiol, methyl trisulfide diallyl disulfide, diallyl tetrasulfide, 1-allyl-3-methyltrisulfane and diallyl trisulfide in the bulbs.

Plant habitat based variation of volatile constituents: Habitat of Korean wild garlic influenced the composition of volatile constituents. Among volatile constituents; alk(en)yl group substances were variable. One compound a propyl based constituent was noted only in three populations except Jungmaewha valley. Allyl related constituents were found in plants from all habitats. The diversity and concentrations of these constituents varied depending upon the habitat. Methyl related compounds also showed small differences due to habitat. However, number of allyl related constituents was lower than those of methyl group, and the concentrations of these were high compared to other compounds in all habitats. Interestingly, although number and concentration of volatile constituents did not differ much, a large difference in composition of compounds was dependant on the habitats

Volatile constituents from 4 different habitats of Ulleung Island could be divided into 2 populations by unweighted pair group method with averaging (UPGMA) cluster analysis (Fig. 4). Among these habitats, volatile constituents in Nari basin differed from the habitats in Sungin peak, Taehwa pass, and Jungmaehwa valley.



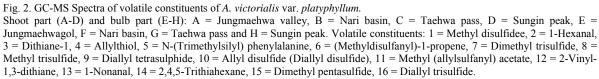


Table 2. Volatile constituents of A. victorialis var. platyphyllum from different habitats

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		Area content, % ^z							
Compounds	RT	Jungmaehwa valley		Nari basin		Taehwa pass		Sungin peak	
Compounds	KI								
		Stem	Bulb	Stem	Bulb	Stem	Bulb	Stem	Bulb
Hydrourea	1.746	64.58	_ ^y	47.03	67.19	60	53.47	57.6	48.17
à-Alanine $(1)^{x}$	1.765	64.58		47.03	-	60	-	57.6	-
Methyl sulfide	2.021	-	-	-	10.38	-	9.63	-	1.93
á-Methylbutanal (2)	3.192	0.13	-	1.64	1.32	0.83	0.93	0.79	0.56
Methyl allyl sulfide (3)	3.77	0.54	3.31	1.8	0.32	2.08	1.03	0.92	1.31
Ethylic acid (4)	4.046	0.07	0.01	-2)	0.11	-	0.01	-	0.07
Methanecarboxylic acid (5)	4.07	-	-	-	-	-	-	0.06	-
Methyl disulfide (6)	4.688	1.9	7.94	7.21	1.18	3.74	0.09	3.68	10.26
2,3-Dithiabutane (7)	4.877	0.09	-	-	-	0.09	-	0.12	-
Methyl vinyl sulfoxide (8)	5.08	-	-	0.36	-	0.13	-	-	-
3-Methylbutanol (9)	5.33	-	-	-	-	-	-	0.06	-
2,3-Butanolone (10)	5.831	-	-	0.19	-	0.06	-	-	-
Dihydroxydimethylsilane	5.12	-	0.11	-	0.04	-	0.08	-	0.02
Mercapto-2-[mercaptomethyl]propionic acid	5.285	-	-	-	-	-	-	-	0.04
Pentanol	5.337	-	0.45	-	0.06	-	-	-	0.02
2-(Hydroxymethyl)cyclopentanol	5.884	-	0.36	-	-	-	0.06	-	-
1-Hexanal (11)	6.125	0.23	11.79	0.2	1.02	0.17	0.5	0.74	0.91
2,3-Butanediol (12)	7.036	-	-	0.05	-	0.12	-	-	-
(4E)-4-Heptenal	7.579	-	0.36	-	0.09	-	0.1	-	0.01
Allyl sulfide (13)	7.756	0.62	0.52	0.75	0.17	0.54	0.17	1.11	0.18
Hexanol (14)	8.251	0.07	0.49	0.17	0.09	0.1	0.04	0.06	0.06
2-Thiophenecarboxylic acid	9.291	-		-	-	-	-	-	-
Dithiane-1,3 (15)	9.696	3.46	0.22	2.99	0.02	4.61	0.02	4.04	0.02
Allylthiol	9.727	-	17.02	-	2.72	-	6.57	-	7.45
N-(Trimethylsilyl)phenylalanine	10.165	-	1.03	-	0.16	-	-	-	0.66
1-(Methyldisulfanyl)-1-propene (16)	10.352	1.32	5.53	1.04	-	1.38	-	2.21	1.5
2-Heptenal	11.133	-	-	-	-	-	0.01	-	-
Benzoic aldehyde	11.261	-	0.1	-	0.01	-	-	-	0.01
Dimethyl trisulfide (17)	11.394	6.7	-	6.4	-	5.16	-	10.32	-
Methyl trisulfide	11.474	-	7.57	-	1.83	-	7.99	-	10.32
3-Octenol (18)	11.829	0.1	0.07	0.15	0.02	0.07	-	0.13	0.01
Vinyl hexoate (19)	12.028	0.02	-	-	-	-	-	0.05	-
2-Amylfuran	12.163	-	0.57	-	0.07	-	0.04	-	0.09
6-Methyl-3-heptanol (20)	12.418	0.04	-	0.12	-	0.04	-	0.04	-
1-Octanal (21)	12.613	-	-	-	-	-	-	0.05	-
Trimethyl-3-hexene	12.633	-	0.04	-	-	-	0.01	-	-
Cyclopentanecarboxaldehyde (22)	12.969	0.02	-	-	-	-	-	0.13	-

à-Toluic aldehyde (23)	13.876	0.05	1.19	0.31	0.22	0.11	0.12	0.1	0.08
Diallyl tetrasulphide (24)	14.969	1.47	20.41	-	-	1.87	-	-	3.72
Allyl disulfide (Diallyl disulfide) (25)	15.042	12.07	10.81	0.11	3.51	7.39	0.03	11.98	0.01
Methyl (allylsulfanyl)acetate	15.483	-	1.17	-	-	-	-	-	0.38
2-Vinyl-1,3-dithiane (26)	15.544	0.75	-	0.14	-	0.58	-	1.34	-
1-Nonanal (27)	15.798	0.07	0.15	0.35	0.01	0.05	0.01	0.099	0.02
2,2-Dimethyl-1,3-dithiane	16.15	-	-	-	-	-	0.01	-	-
2,4,5-Trithiahexane	16.324	-	0.48	-	0.19	-	0.04	-	0.08
Benzoic aldehyde	17.235	-	0.06	-	-	-	0.04	-	-
3-Mercapto-2-[mercaptomethyl]propionic acid (28)	17.462	0.12	0.21	-	-	0.11	0.09	0.166	0.06
1-Allyl-3-methyltrisulfane (29)	17.629	0.19	5.57	0.19	4	0.16	6.08	0.25	6.42
3-Vinyl-3,6-dihydro-1,2-dithiine (30)	18.351	0.09	0.01	-	0.03	0.06	0.03	0.053	0.03
Dimethyl pentasulfide (31)	19.139	-	-	0.21	-	0.26	-	0.307	-
Dimethyl tetrasulfide (32)	19.161	-	0.31	-	-	-	0.27	-	-
Methyl (methylsulfinyl)methyl sulfide	21.364	-	0.02	-	-	-	0.02	-	0.05
Diallyl trisulfide	21.56	3.97	-	0.49	2.26	2.1	1.14	2.355	1.21
i-Butyl isothiocyanate (33)	21.89	0.03	-	-		0.05	-	0.055	-
Diisopropylsilyl isopropyl ether	21.931	-	-	-	0.05	-	0.02	-	0.06
Tetramethylheptane	22.22	-	-	-	0.02	-	-	-	0.02
1-Propene (34)	22.225	0.1	-	-	0.19	0.09	0.15	0.052	0.09
1,4-Dithiane (35)	23.702	0.27	-	0.1	-	0.17	-	0.186	-
4-Thiapentanoic acid	27.379	-	-	-	0.04	-	0.01	-	0.01
3-propanoic acid (36)	27.7	0.09	-	-	-	0.05	-	0.041	-
Total	99.16	95.88	72	97.32	92.17	88.81	99.09	95.84	

²Ovendry weight, 1g, ^yNot detected ^xThe numbers represents compounds used to PDA analysis

Table 3. Number and area of alk(en)yl group on bulb and stem tissues from different habitat in Ulleung Island.

Alkyl	Jungmaehwa valley		Nari	basin	Taehw	va pass	Sungin peak		
group	Bulb	Stem	Bulb	Stem	Bulb	Stem	Bulb	Stem	
Propyl	0 ^z	0	1	0	1	0	1	0	
	(0) ^y	(0)	(0.05)	(0)	(0.02)	(0)	(0.06)	(0)	
A 11-1	2	5	4	4	4	5	5	4	
Allyl	(58.33)	(18.32)	(10.69)	(2.54)	(15.99)	(12.06)	(19.99)	(16.70)	
Methyl	8	6	8	8	9	8	8	8	
	(28.89)	(13.63)	(17.45)	(19.78)	(21.98)	(14.62)	(29.31)	(19.33)	
Other	18	18	13	11	16	17	18	19	
	(20.66)	(74.21)	(80.88)	(62.68)	(59.66)	(71.49)	(53.36)	(68.07)	

^zThe value represents number of alkyl group in different population

^yThe value represents area (%) of alkyl group in different population

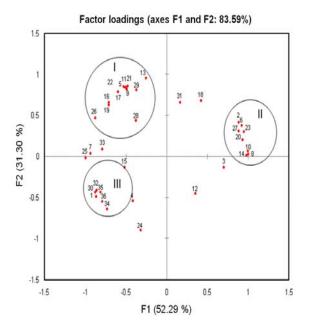


Fig. 3. Distribution of stem volatile components on the correlation circle defined by the three first principal components (or factors). Numbers represent compounds according to Table 2.

Among the volatile constituents, sulfur containing compounds were higher in garlic herb. The large differences in volatile chemical composition can be explained on the basis of previous reports. Bernhard (1980) has analyzed the composition of many Allium species and concluded that the volatile constituents of Chinese chive mainly consisted of allyl methyl disulfide and dimethyl disulfides. Iida et al., (1983) and Pino et al., (2001) separated many sulfur compounds from the steam distillate of Chinese chive and identified 29 of them, the most important being dimethyl disulfide (32%), dimethyl trisulfide (16%), allyl methyl trisulfide (10%), and allyl methyl disulfide (8%). In this study the major volatile constituents of A. victorialis differed from other Allium sp. The composition of propyl related compounds is comparatively low especially in A. victorialis.

Volatile constituents of the studied material differ. Diallytrisulphide, a well-known constituent of garlic with a typical odour, is predominant in the oil, for instance diallyltetrasulphide, diallyldisulfide and methylallyl trisulfide are the minor constituents (Jirovetz et al., 1992). Among these compounds the garlic bulb contained: diallyltetrasulphide (29.7%), diallyltetrasulphide (4.4%), and diallyldisulfide (3.2%); whereas methylallylsulphide, dimethyl trisulfide, diallyl tetrasulphide, allyl disulfide and diallyl trisulfide are found in both bulb and stem of our specimens. Diallyl trisulfide, allyl disulfide, methylsulfide, diallyl trisulfide are also the components of garlic bulb and stems. Differences between Korean wild garlic and other Allium species may be species specific and due to the influence of environmental factors at growing sites.

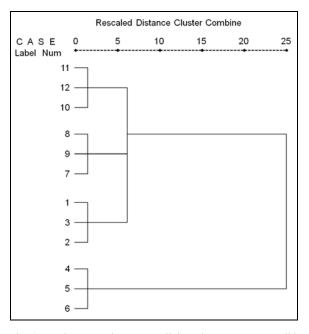


Fig. 4. Dendrogram using average linkage between Korean wild garlic population groups.

Nari basin (4, 5, and 6), Sungin peak (1, 2, and 3), Taehwa pass (7, 8, and 9), and Jungmaehwa valley (10, 11, and 12).

PCA involving all 36 detected volatile constituents analyzed in this study gives some visual evidence of the ability to differ between factor groups. Differences of these volatile constituents were not reported in the Korean wild garlic. This phenomenon was considered to be due to environmental influences on the alk(en)yl constituents.

Volatile constituents differed in the material studied, both in stem and bulbs. MacKenzie & Ferns (1977) found a large variation in the volatile compositions in different parts of *Allium tuberosum* plant. In *Allium* sp, the leaf contains 2-3% of carbohydrate and ascorbic acid whereas the bulbs possesses organosulfuric compounds (Lawson *et al.*, 1991). These organosulfuric compounds exist in the form of S-alkenyl- or S-alky-L-cysteine sulfoxide but these hydrophilic compounds are converted to disulfides with garlic smell when allinase is activated by tissue injury.

Volatile constituent profile of our bulb extract was dependant on the collection site characteristics in Ulleung Island. Among volatile constituents, alk(en)yl group also varied depending upon the habitat. Volatile constituents in Nari basin were different than three other habitats in spite of indifferent ecological characteristics of Nari basin (Table 2). The forest within Nari basin was composed of pine, beech, alder and deciduous-mixed stand, and the species composition was greatly different among the four stands (Chung *et al.*, 2010). The high chemical variability among habitats could be mainly explained by genetic rather than ecological factors (Chograni *et al.*, 2010). Variation of volatile constituents between the four habitats may be attributed to the result of selective factors such as rainfall, temperature, population size and soil quality. These parameters affected the species volatile composition (Zaouali *et al.*, 2005). However, Saghir *et al.*, (1965) reported that habitat had no effect on the proportions of sulfide radicals.

In this study, the PCA analysis used to assess the variation of compounds among the habitats showed that several major constituents (dimethyl tridisulfide, allyl disulfide, diallyl disulfide and dimethl pentasulfide) can be distinguished related to the habitats. Thus, habitats exhibiting high amounts of these compounds would constitute an effective strategy to preserve a high level of chemical diversity in the species. Our study needs to be supported by organization of isozyme and molecular polymorphism analyses to understand the relationship between environmental traits (drought, phenological parameters, etc.), variations in volatile constituents and genetic diversity in Korean wild garlic populations.

These results distinguish 3 volatile groups in *A. victorialis* mainly characterized by compositional analysis, and contribute towards their cultivation in the forest and nursery as well as for the assessment of their genetic and chemical characteristics. In the long term the outcome of the study may allow the propagation of interesting superior genotypes.

Acknowledgements

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