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Original Research Article

Intraplant Variations of Sesquiterpene Lactone Content in Lettuce Genetic Resources Grown in Two Cultivation Seasons

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Abstract - Inflorescence, stem, and leaf samples of lettuce grown in a greenhouse in spring and autumn seasons were assayed for sesquiterpene lactones (SLs) content by high performance liquid chromatography. The concentrations of SLs were significantly higher in the inflorescences followed by upper leaf and stem compared to the other plant parts in most of the samples. SLs content (sum of lactucin and lactucopicrin) in various tissues of lettuce cultivated in spring season varied from 5.7 to 22.5 fold ranging from 27.4 ㎍/g dry weight (DW) in the upper stem (cultivar "PI 176588") as the lowest to as high as 2,292.0 ㎍/g DW in the inflorescence (cultivar "709849-1"). During autumn cultivation, the concentration of SLs varied from 2.0 to 14.4 fold ranging from as low of 32.4 ㎍/g DW in the lower stem (cultivar "PI176588") to as high of 838.0 ㎍/g DW in the upper leaf (cultivar "Dambaesangchu"). Higher lactucin (1.2 to 5.6 fold) and lactucopicrin (1.1 to 3.9 fold) concentration was observed during spring compared to autumn cultivation in most of the samples. SLs content in various organs of lettuce increases from the basal plant part going upwards. As lactucin and lactucopicrin are the major SLs which affects the sensory property of lettuce, their quantitative variation in the lettuce cultivars is useful for breeding new varieties with better consumer acceptance.

Key words – Inflorescence, *Lactuca sativa*, *Lactuca serriola*, Lactucin, Lactucopicrin, Leaf, Stem

Introduction

Lettuce is the highest ranked vegetable in production and economic value, and only second to potato in per capita consumption worldwide (Kim *et al*., 2018). Lettuce grows annually in backyards, containers, shade net, greenhouse, and also using hydroponics. Lettuce is grown as leaf vegetable as well as for its stem and seeds. It is one of the most important ready-to-use products and has been principally consumed as salad, a "wrap-up vegetable", as cooked vegetable, and in sandwiches (Martínez-Sánchez *C* 2012; Seo *et al*., 2009; Cho *et al*., 2016). Lettuce varieties differ in: head shape (crisphead, butter head, cos (romaine), and leafy); leaf color (green, red/green, red, purple, and green/purple); and leaf shape (could be circular, broad, elliptic, and transverse). Among the various pharmacologically significant phytoconstituents of lettuce, sesquiterpene lactones (SLs) are one of the important secondary metabolites significant to plants and human (Rees & Harborne, 1985; Chadwick *et al*., 2013; Tamaki *et al*., 1995). Other sources of SLs include, beverages such as chicory root tea, spices such as star anise, and herbs (Chadwick *et al*., 2013). SLs are C-15 terpenoids that naturally occur in the form of hydrocarbons, alcohols, ketones, aldehydes, acids or lactones (Graziani *et al*., 2015). Lactucin and lactucopicrin, the major SLs reported in lettuce, could contribute significantly to the bitterness nature of lettuce cultivars (Price *et al*., 1990; Seo *et al*., 2009). The content of SLs was found to exhibit a significant variability based on the variety in chicory and endive (Ferioli *et al*., 2015). Growing seasons have been reported to influence the concentration of SLs in forage chicory cultivars, keeping in mind the specific response depended on the type of cultivar (Foster *et al*., 2011). Sensory properties including, bitterness, appearance, flavor, texture, and overall acceptability and total phenolic properties of lettuce are also dependent on the growing season (Bunning *et al*., 2010). Genetic improvement of crops partly depends on the existence of variation

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in useful phytochemical traits within germplasm and SLs are one of the important traits for developing a new variety of lettuce (Sung *et al*., 2016, Jang *et al*., 2013). Sweeter taste and more crispy texture of lettuce are the favorable sensory attributes for consumers (Pollard *et al*., 2002). The SLs found in leaves and flowering heads of plants are sometimes available in a range of cell types, produced in high levels constantly, or in some species they may be held in storage organs (Chadwick *et al*., 2013). Knowledge of the profiles of sesquiterpene lactones in different growing seasons and in individual organs of the plant is important. Seo *et al*. (2009) had reported the content of SLs in basal leaves, mid-stalk leaves, and flower stalk leaves, from a single cultivar. Yet, a comprehensive understanding of variation in the content of SLs between different tissues of lettuce and growing seasons is still lacking.

This study was aimed to investigate the variation of two major bitter SLs (lactucin and lactucopicrin) content in different tissues (inflorescences, upper leaf, middle leaf, lower leaf, upper stem, middle stem, and lower stem) of the reproductive stage of lettuce harvested in spring and autumn seasons. As SLs accumulation partly represents the net effect of sensory properties of lettuce, accurate knowledge of the accumulation profile in different organs and seasons provides a baseline data for breeding and also helps in explaining the role of these compounds in interactions with herbivores and pathogens.

Materials and Methods

Reagents and standards

All chemicals and solvents used in extraction and analysis were of analytical grade and purchased from Fisher Scientific Korea Ltd. (Seoul, South Korea) and Sigma-Aldrich (St. Louis, MO, USA). Standards (lactucin and lactucopicrin) were HPLC grade (> 95% purity) and purchased from Extrasynthese (Lyon, France).

Plant materials

Lettuce was grown at the research farm of the National Agrobiodiversity Center (NAC), Jeonju (35°49′18″ N 127°08′ 56″ E), Republic of Korea. Seeds of nine lettuce accessions were sown in plug trays during the autumn (2016) and spring (2017) seasons, and seedlings were grown in a greenhouse. Four-week-old seedlings were transplanted to the field using hydroponics in a plastic house on 20th of September (2016) and 19th of March

Table 1. Morphological characteristics of lettuce varieties

S/No	Accession No	Scientific name	Cultivar Name	Country of Origin	Classification	Shape of cotyledon	leaf attitude	Plant: growth type	outer leaf colour	Leaf blade: degree of undulation of margin	Leaf blade: density of incisions on margin at apical part	Leaf unevenness	Leaf shape
1	IT215805	*L. serriola*	5500	Honduras		Narrow elliptic	Prostrate	Leafy	Green	Weak	Sparse	Low	Narrow elliptic
2	IT235353	*L. sativa*	Choseonsangchu	South Korea	Landrace	Broad elliptic	Semi-erect	Leafy	Greenish purple	Medium	Sparse	Medium	Medium elliptic
3	IT247401	*L. sativa*	Taiguo xiang wo wei	China	Variety	Broad elliptic	Prostrate	Stem	Green	Weak	Sparse	High	Narrow elliptic
4	IT259238	*L. serriola*	1572	Denmark		Medium elliptic	Prostrate	Leafy	Green	Medium	Sparse	Medium	Triangular
5	IT260859	*L. sativa*	Cheongsangchu	South Korea	Landrace	Medium elliptic	Semi-erect	Leafy	Deep green	Weak	Sparse	Low	Medium elliptic
6	IT260861	*L. sativa*	Dambaesangchu	South Korea	Landrace	Broad elliptic	Prostrate	Leafy	Deep green	Weak	Sparse	Medium	Medium elliptic
7	IT271096	*L. sativa*	709849-1	South Korea	Landrace	Medium elliptic	Semi-erect	Leafy	Grey green	Weak	Sparse	Medium	Medium elliptic
8	IT271121	*L. sativa*	PI 141680			Medium elliptic	Semi-erect	Leafy	Deep green	Weak	Sparse	Low	Medium elliptic
9	IT271129	*L. sativa*	PI 176588	Turkey		Medium elliptic	Semi-erect	Leafy	Deep green	Weak	Sparse	High	Medium elliptic

(2017). Planting density was 20 x 20 ㎝. Rural Development Administration (RDA)'s recommendation for the cultural management practices of lettuce were followed in the field. Each accession was consisted of 24 plants. Plant growth was maintained using nutrient solution throughout the growing season. Qualitative morphological characteristics were recorded on the basis of the standard lettuce descriptors for Protection of New Varieties of Plants (UPOV, 1981). The description of some selected qualitative characters of lettuce is given in Table 1. The qualitative characters were recorded based on plant observation on field when plants of each variety completed flowering.

Extraction, separation, and analysis of sesquiterpene lactones (SLs)

Each of the nine lettuce varieties consisting of 24 individuals were harvested and dissected into pedicel, upper leaf, middle leaf, lower leaf, upper stem, middle stem, and lower stem, placed in vinyl freezer bags and held at -80°C. The frozen samples were subsequently lyophilized for 48 hr using LP100 vacuum freeze-drier (Ilshibiobase, Rijssen, Netherlands). The freeze-dried samples were then ground to a fine powder using a mortar and pestle, and held at -80ºC until analysis. Samples were extracted based on the method described by Price *et al.* (1990) with some modification. Briefly, powdered lyophilized lettuce (0.25 g) was extracted with 100 ㎖ of methanol by boiling under reflux at 65°C for 1 hr and 20 min and filtered through Whatman #2 filter paper. The methanol was evaporated under reduced pressure using High Capacity Centrifugal Evaporator (Genevac, HT-4X, 5 mm Hg, 30 -35°C). The crude extract was then partitioned two times between water/chloroform (20 ㎖; 1:1 mixture by volume) with the chloroform separated, dried over anhydrous magnesium sulfate, and evaporated using a High Capacity Centrifugal Evaporator (Genevac, HT-4X, 5 ㎜ Hg, 30~35°C). The residue was dissolved in 0.4 ㎖ methanol/chloroform (1:2, v/v) and the SLs separated using high-performance liquid chromatography (HPLC).

The sesquiterpene lactones were analysed using Agilent 1260 infinity high-performance liquid chromatography (HPLC) system equipped with UV visible Diode Array Detector (SPD-M10A). A Phenomenex Luna C18 (250 x 4.6 mm, 5 ㎛) column connected to a Phenomenex Security Guard (ODS Octadecyl, 4 x 3.0 ㎜) was used for separation of SLs. The column thermostat was maintained at 30°C. The solvent system was comprised of water (mobile phase A) and acetonitrile (mobile phase B). The elution program was as follows: 0-3 mins, 10% B; 5-15 mins, 35% B; 15-25 mins, 35-100% B, and 25-30 mins, 100% B. The SLs were monitored at a detection wavelength of 256 ㎚. The flow rate and injection volume were kept at 0.8 ㎖/min and 20 ㎕, respectively. Quantification was done using calibration equations (lactucin, Y= 3938.6 X - 4.7184 R^2= 1; lactucopicrin, Y= 2606.8X + 1.2828, R^2= 1) derived from the calibration curves of the corresponding standards.

Statistical analyses

Results were expressed as means ± standard deviation (SD) of nine measurements three from biological replicates and three tests, on a dry weight basis. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using SPSS V.17.0 statistical program (SPSS Inc., Chicago, USA). Significant level was set at $p < 0.05$.

Results and Discussions

This study was designed to investigate the patterns of variation in SLs concentration in nine lettuce cultivars with respect to plant part and cultivation season. A representative sample was prepared from each cultivar which had 24 individual lettuce plants. Samples were taken from the major organs at the reproductive stage, when the elongated stem has produced inflorescences and flowers are present. Each plant was segregated into inflorescence, upper leaf, middle leaf, lower leaf, upper stem, middle stem, and lower stem as shown in Fig. 1.

The mean SLs concentrations of different organs of lettuce plant cultivated in spring and autumn seasons, expressed in microgram per gram of dry weight (㎍/g DW), are given in Table 2. A representative HPLC chromatogram showing authentic standards (lactucin and lactucopicrin) and a randomly selected lettuce sample is presented in Fig. 2. Analysis of variance (ANOVA) indicated that individual SLs as well as total SLs concentration showed a significant inter-cultivar and intra-plant variations. The concentration of total SLs in various tissues of lettuce cultivated in spring season varied from 5.7 to 22.5 fold ranging from as low as 27.4 ㎍/g DW in the upper stem (cultivar "PI 176588") to as high as 2,292.0 ㎍/g DW in the inflorescence

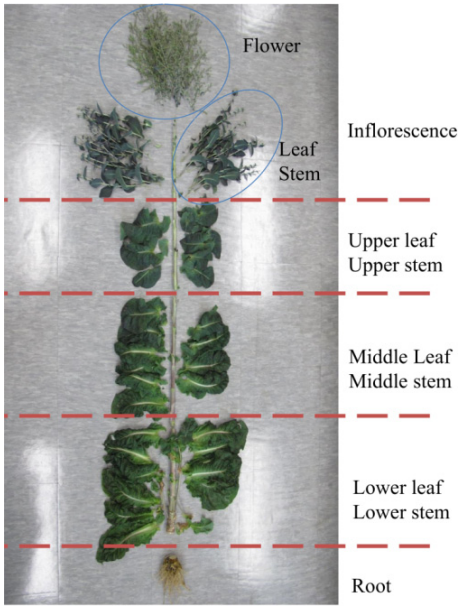

Fig. 1. A representative photo of lettuce at reproductive stage showing its organs.

(cultivar "709849-1"). During autumn cultivation, the concentration of SLs varied from 2.0 to 14.4 fold ranging from as low as 32.4 ㎍/g DW in the lower stem (cultivar "PI176588") to as high as 838.0 ㎍/g DW in the upper leaf (cultivar "Dambaesangchu"). In almost all the samples considered in this study, the concentration of lactucin and lactucopicrin were significantly higher in the inflorescences compared to other parts. The total SLs in in the inflorescences ranged from 457.7 to 2,292.0 ㎍/g DW and 141.1 to 768.6 ㎍/g DW in spring and autumn seasons, respectively. Intermediate levels were observed in upper leaves (205.8 to 1,222.8 ㎍/g DW, spring cultivation; 37.1 to 838.0 ㎍/g DW, autumn cultivation) and upper stem tissues (27.4 to 396.9 ㎍/g DW, spring cultivation; 43.5 to 546.1 ㎍/g DW, autumn cultivation), and lesser amounts in lower stem and leaves.

To better visualize the SLs accumulation in various organs of lettuce, two-dimensional pie charts for selected representative lettuce varieties are presented in Fig. 3, where the area of the

Table 2. The concentration of SLs in various tissues of lettuce cultivars and two cultivation seasons

S/No	Cultivar name	Plant part	Lactucin (㎍/g DW)		Lactucopicrin (㎍/g DW)		Total SLs (㎍/g DW)	
			Spring	Autumn	Spring	Autumn	Spring	Autumn
1	5500	Inflorescence	121.8±9.6b	230.2±10.1a	335.9±20.4a	538.4±25.2b	457.7±30.0a	768.6±35.3a
		upper leaf	44.8±1.8c	15.9±0.5de	184.8±10.0c	712.9±8.2a	229.6±11.4c	728.8±8.4a
		middle leaf	20.5±1d	23.2±2.9d	71.1±9.6e	163.3±5.3d	91.6±8.7e	186.5±5.5d
		lower leaf	16.9±0.7d	37.1±2.8c	35.9±1.0f	338.5±35.0c	52.8±1.4f	375.6±37.9c
		upper stem	144.2±1.0a	88.1±3.0b	252.8±3.6b	362.5±22.3c	397±3.3b	450.6±25.1b
		middle stem	43.1±3.7c	26.0±12.1d	121.0±7.4d	157.0±69.8d	164.1±11.1d	183.0±81.9d
		lower stem	22.0±1.5d	11.6±0.9f	55.5±3.0e	92.4±3.4f	77.5±4.4e	104.0±4.4e
2	Choseon-sangchu	Inflorescence	40.6±3.0a	18.3±2.4a	607.6±22.6a	620.8±80.8a	648.2±24.0a	639.1±83.2a
		upper leaf	19.5±1.0b	44.4±4.2b	186.3±11.6b	203.6±15.8b	205.8±11.6b	248.0±18.8b
		middle leaf	NA	ND	NA	99.1±4.7c	NA	99.1±4.6c
		lower leaf	7.2±0.8d	ND	59.6±2.8e	108.7±5.8c	66.8±3.6e	108.7±5.8c
		upper stem	16.3±1.8c	ND	91.0±8.4d	81.5±4.0c	107.3±10.0d	81.5±4.0c
		middle stem	NA	ND	NA	75.1±5.9c	NA	75.1±5.9c
		lower stem	5.6±0.5d	4.6±0.3c	130.4±5.9c	85.7±7.7c	136.0±6.3c	90.3±8.0c
3	Taiguo xiang wo wei	Inflorescence	23.9±1.1a	NA	496.2±7.0a	NA	520.1±6.3a	NA
		upper leaf	12.9±1.5bc	NA	270.7±32.4b	NA	283.6±33.8b	NA
		middle leaf	7.6±0.4d	NA	85.0±1.0d	NA	92.6±1.4d	NA
		lower leaf	5.3±0.1e	NA	47.6±1.4e	NA	52.9±1.3e	NA
		upper stem	11.7±0.2c	NA	135.0±7.1c	NA	146.7±6.9c	NA
		middle stem	14.1±1.6b	NA	95.5±7.8d	NA	109.6±7.8d	NA
		lower stem	14.2±1.3b	NA	46.3±5.2e	NA	60.5±6.2e	NA

Table 2. Continued

S/No	Cultivar name	Plant part	Lactucin (μg/g DW)		Lactucopicrin (μg/g DW)		Total SLs (μg/g DW)	
			Spring	Autumn	Spring	Autumn	Spring	Autumn
4	1572	Inflorescence	138.4±5.8a	NA	352.7±17.7a	NA	491.1±23.4a	NA
		upper leaf	64.3±5.0b	8.7±0.7d	232.7±8.8b	28.4±1.9c	296.9±7.2b	37.1±2.6c
		middle leaf	25.8±3.4d	NA	123.1±9.1c	NA	148.9±12.3d	NA
		lower leaf	67.2±1.8b	13.1±0.5b	111.3±3.2c	49.0±4.6ab	178.5±2.6c	62.1±5.0b
		upper stem	53.9±10.0c	23.1±0.6a	248.7±15.8b	53.5±3.6a	302.6±24.4b	76.6±4.0a
		middle stem	15.2±0.6e	NA	51.6±4.9e	NA	66.8±5.3f	NA
		lower stem	15.4±0.4e	11.9±0.3c	91.1±5.4.0d	45.7±1.2b	106.5±5.6e	57.6±1.5b
5	Cheong-sangchu	Inflorescence	58.0±4.2a	37.5±0.9a	745±50.7a	645.3±21.1a	803±54.9a	682.8±21.9a
		upper leaf	33.4±2.2b	35.8±1.3a	689.4±16.4b	345±19.9b	722.8±18.4b	380.8±21.2b
		middle leaf	NA	NA	NA	NA	NA	NA
		lower leaf	9.9±0.5d	14.4±1.1c	93.1±8.1d	140.9±12.3d	103.0±8.5d	155.3±13.3d
		upper stem	21.2±1.8c	19.5±1.6b	170±17.9c	230.7±22.1c	191.2±19.6c	250.2±23.3c
		middle stem	NA	NA	NA	NA	NA	NA
		lower stem	8.1±0.4d	3.1±0.3d	110.2±8.3d	44.3±2.8e	118.3±8.5d	47.4±2.6e
6	Dambae-sangchu	Inflorescence	70.2±7.6a	18.7±0.8cd	953.2±51.5b	590.2±31.6b	1023.4±58.1b	608.9±32.4b
		upper leaf	41.0±4.4b	26.6±0.9b	1181.9±28.9a	811.4±36.6a	1222.9±32.5a	838.0±37.5a
		middle leaf	9.1±1.0cd	17.3±0.3d	302.6±33.4c	312.3±10.4d	311.7±34.3d	329.6±10.2f
		lower leaf	5.3±0.5d	10.6±1.2f	142.5±5.2d	193.7±10.0e	147.8±5.4e	204.3±9.2e
		upper stem	46.0±2.8b	30.6±2.5a	332.7±30.8c	515.5±50.0c	378.7±33.4c	546.1±52.5c
		middle stem	12.8±1.6c	20.4±1.4c	301.5±27.0c	478.4±35.3c	314.3±28.6d	498.8±36.7c
		lower stem	6.4±0.4cd	13.0±1.1e	122.3±1.5d	280.0±4.2d	128.7±1.9e	293.0±4.9d
7	709849-1	Inflorescence	193.7±3.7a	41.4±3.8a	2098.3±39.8a	642.5±39.8a	2292.0±43.4a	683.9±43.6a
		upper leaf	28.4±1.3c	10.9±1.1c	413.0±15.3b	138.0±12.0b	441.4±16.6b	148.9±13.0b
		middle leaf	13.7±0.2d	NA	116.6±3.3e	NA	130.3±3.5e	NA
		lower leaf	9.0±0.7e	29.3±3.9b	92.7±10.8e	628.1±50.0a	101.7±10.7e	657.4±53.8a
		upper stem	48.2±1.8b	8.5±0.5c	232.6±13.4c	70.7±7.4c	280.8±15.1c	79.2±7.7c
		middle stem	11.7±0.8de	NA	185.5±17.7d	NA	197.2±18.2d	NA
		lower stem	13.2±0.4d	4.7±0.4d	241.4±4.0c	61.2±5.9c	254.6±4.2c	65.9±5.8c
8	PI 141680	Inflorescence	78.3±3.3a	18.1±1.1a	662.6±20.5a	273.9±12.7a	740.9±20.3a	292±13.8a
		upper leaf	15.6±1.5de	10.5±0.2d	229.7±12.9cd	121.1±8.5b	245.3±14cd	131.6±8.3b
		middle leaf	16.8±1.4d	16.4±0.7ab	194±20.8d	115.7±10.2b	210.8±22.2d	132.1±10.4b
		lower leaf	12.1±0.5e	7.1±0.5e	117.3±6.7e	50.7±0.7c	129.4±7.2e	57.8±1.2c
		upper stem	NA	13.0±1.8c	NA	122.3±15.4b	NA	135.3±17.1b
		middle stem	42.6±0.6b	14.8±1.6bc	292.7±8.1b	107.8±10.7b	335.3±8.7b	122.6±12.0b
		lower stem	31.4±3.1c	7.8±0.6e	242.2±41.6c	108.2±9.2b	273.6±44c	116.0±9.8b
9	PI 176588	Inflorescence	95.8±0.9a	10±1.0a	365.5±15.5a	131.2±8.5a	461.3±16.4a	141.2±9.5a
		upper leaf	36.4±2.4b	6.7±0.7b	236.6±24.5b	34.0±0.8cd	273.0±26.9b	40.7±1.0cd
		middle leaf	NA	5.9±0.8bc	NA	28.1±0.7d	NA	34.0±1.1de
		lower leaf	10.6±1.0d	4.7±0.5cd	44.8±3.0c	31.1±1.5cd	55.4±4.0c	35.8±1.5de
		upper stem	8.5±0.3d	5.7±0.2bc	19±1.4d	37.7±2.7c	27.5±1.6d	43.4±2.7c
		middle stem	NA	4.8±0.2cd	NA	50.7±2.0b	NA	55.5±2.2b
		lower stem	14.6±0.6c	3.9±0.5d	63.3±1.4c	28.5±2.2d	77.9±2.0c	32.4±2.5e

ND = not detected; NA= Not analyzed, BSLs = Bitter sesquiterpene lactones. Different letters between rows within each sample indicate statistically significant differences between plant parts at $p < 0.05$.

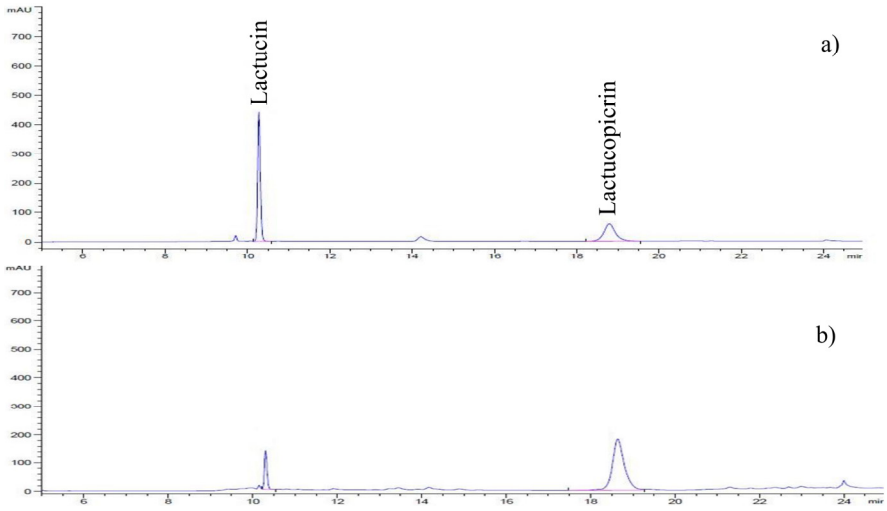

Fig. 2. Typical HPLC chromatogram of SLs standards (a) and randomly selected sample (b).

sectors are proportional to the concentration of SLs. The concentrations of the SLs (lactucin + lactucopicrin) in roots and different parts of inflorescence (flower, leaf, and stem) of cultivar "709849-1", which had the highest SLs in its inflorescence, were further evaluated. The results were 1416.7 ± 55.1, 533.2 ± 39.4, 342.1 ± 34.0, 441.4 ± 16.6, 280.8 ± 15.1, 130.3 ± 197.2, 101.7 ± 10.7, 254.6 ± 4.2, and 164.6 ± 5.2 ㎍/g DW in flower, leaf in inflorescence, stem in inflorescence, upper leaf, upper stem, middle leaf, middle stem, lower leaf, lower stem, and root parts, respectively.

To assess the effect of cultivar and season of cultivation on SLs concentration, a combinative consideration of results were taken into account, calculating average values of SLs in various tissue samples within each cultivar. Higher lactucin (1.2 to 5.6 fold) and lactucopicrin (1.1 to 3.9 fold) concentration was observed in spring compared to autumn cultivation in all samples except in cultivars "5500" and "709849-1", which showed 1.9- and 1.2-fold higher concentration of total SLs, respectively. The concentrations of lactucin and lactucopicrin cultivated in spring season varied 4.6 and 3.3 fold ranging from 12.8 (cultivar "Taiguo xiang wo wei") to 59.0 (cultivar "5500") ㎍/g DW and 145.8 (cultivar "PI 176588") to 482.9 (cultivar "709849-1") ㎍/g DW, respectively. The concentration of lactucin and lactucopicrin in autumn season cultivation ranged from 6.0 (cultivar "PI 176588") to 61.7 (cultivar "5500") ㎍/g DW and 44.1 (cultivar "1572") to 454.5 ㎍/g DW (cultivar "Dambaesangchu"), respectively.

Plants accumulate phytochemicals, including SLs, in all their vegetative and reproductive parts. However, the content of SLs in individual lettuce organs varies significantly. Generally, the concentration of SLs increases from lower part to the upper of the plant. These patterns are in accordance with a previous report (Seo *et al*., 2009). SLs could function in defense against herbivores and pathogens because of the bitter taste repelling chewing insects and birds (Chadwick *et al*., 2013; Mithöfer & Boland, 2012). The pattern of differences of SLs among organs in lettuce is consistent with predictions of optimal defense theory which states that defense should be preferentially allocated to more valuable parts with high probability of attack (Zangerl & Bazzaz, 1993). Inflorescences which contain the reproductive organ, such as flowers, contribute most to plant fitness and hence expected to contain the highest content of defense compounds. The concentration of SLs in lettuce tissues also depends on stages of development, where younger plant organs (small leaves & stems in inflorescences and upper leaves & stems) relatively have higher concentration of SLs compared to older plant organs (middle/lower leaves and stems).

Like the position of the various organs of lettuce, cultivar and harvesting date influenced the concentration of individual as well as total SLs. Our results were in accordance with the report of Foster *et al*. (2011), where SLs concentration significantly varied in forage chicory cultivars. These authors have also reported that concentration of SLs was lower during autumn than in late spring and early summer. Bitterness of lettuce

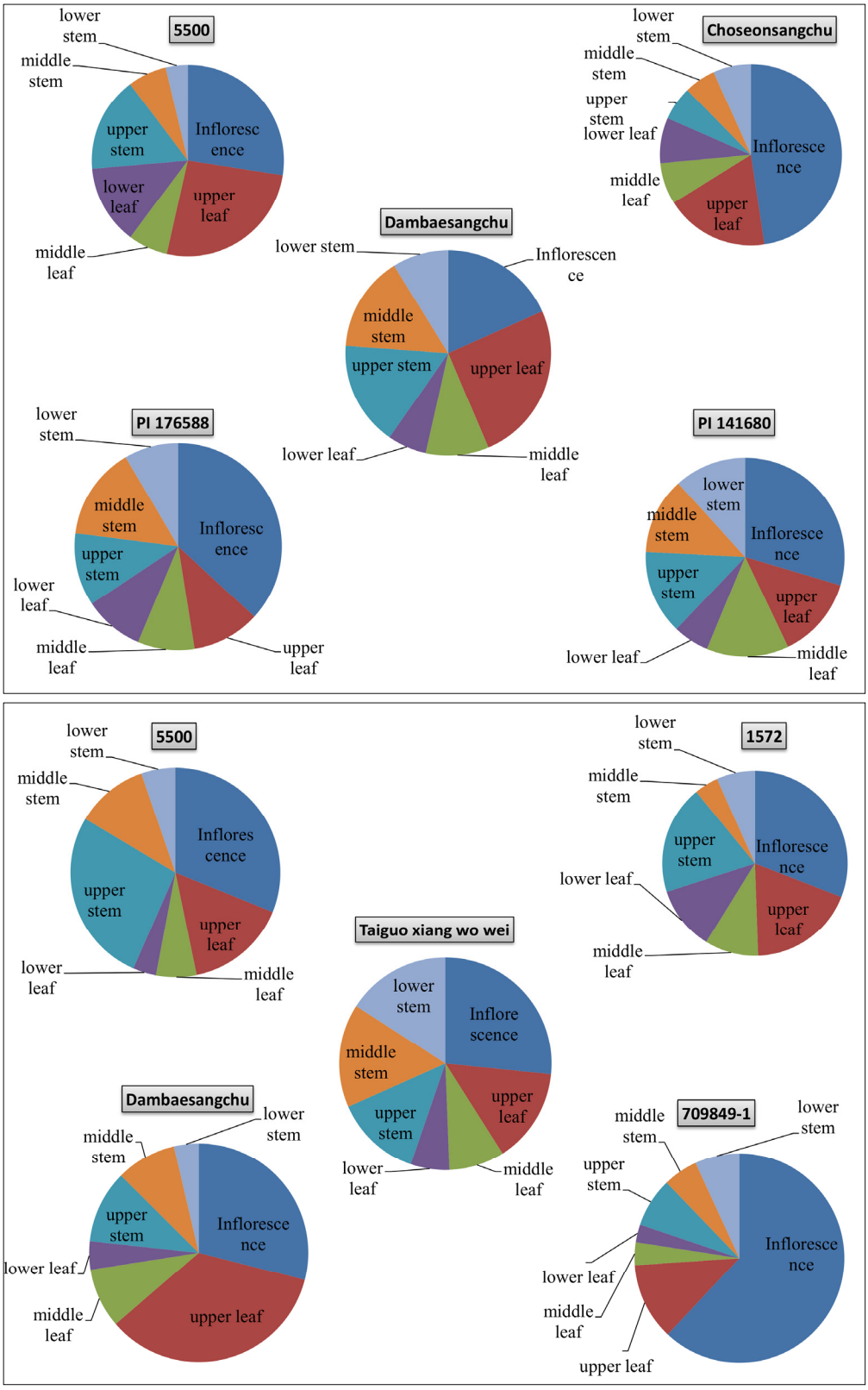

Fig. 3. Representative pie charts showing the percentage contribution of the parts of lettuce to the overall concentration of SLs of different cultivars cultivated in spring (top) and autumn (bottom).

cultivars, which has been partly linked to the presence of sesquiterpene lactones (notably lactucin and lactucopicrin) (Price *et al*., 1990), was found to increase with temperature (Bunning *et al*., 2010).

In conclusion, we have investigated the pattern of variability of SLs content in various tissues of lettuce. Generally, the concentration of SLs in various organs of lettuce increases as one goes upward from the base. A more detailed physiological study to determine the effect of synthesis, transport, and degradation of SLs to its variability in plant distribution is needed. Lettuce cultivars harvested in spring had relatively accumulated higher SLs compared to autumn harvest. Moreover, the study showed a wide variation in SLs contents among lettuce varieties. This study focused mainly on SLs quantification of nine lettuce varieties. Aside from this, molecular characterization is recommended for further understanding of their genetic variation. As lactucin and lactucopicrin are the major SLs which affects the sensory property of lettuce, their quantitative variation in the lettuce cultivars is useful for breeding new varieties with better consumer acceptance.

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References

Bunning, M.L., P.A. Kendall, M.B. Stone, F.H. Stonaker and C. Stushnoff. 2010. Effects of seasonal variation on sensory properties and total phenolic content of 5 lettuce cultivars. J. Food Sci. 75(3):S156-S161.

Cho, G.T., Y.Y. Lee, S. Choi, J.Y. Yi, G.A. Lee, H.C. Ko, Y.W. Na, H.J. Park, K.H. Ma and J.G. Gwag. 2016 Variation of sesquiterpene lactones content in lettuce (*Lactuca sativa* L. germplasm. J. Korean Soc. Int. Agric. 28:220-226 (in Korean).

Chadwick, M., H. Trewin, F. Gawthrop and C. Wagstaff. 2013. Sesquiterpenoids lactones: Benefits to plants and people. Int. J. Mol. Sci. 14:12780-12805.

Ferioli, F., M.A. Manco and L.F.D. Antuono. 2015. Variation of sesquiterpene lactones and phenolics in chicory and endive germplasm. J. Food Compost. Anal. 39:77-86.

Foster, J.G., K.A. Cassida and M.A. Sanderson. 2011. Seasonal variation in sesquiterpene lactone concentration and composition of forage chicory (*Cichorium intybus* L.) cultivars. Grass Forage Sci. 66(3):424-433.

Graziani, G., R. Ferracane, P. Sambo, S. Santagata, C. Nicoletto and V. Fogliano. 2015. Profiling chicory sesquiterpene lactones by high resolution mass spectrometry. Food Res. Int. 67: 193-198.

Jang, M., J.B. Seo, M.S. Jo, K.C. Ma, J.W. Lee, B.K. Yoon and Y.S. Yoon. 2013. Functional Contents in Varieties of indigenious Lettuce (*Lactuca sativa* L.). Korean J. Plant Res. 10:109-109 (in Korean).

Kim, D.E., X. Shang, A.D.Assefa, Y.S. Keum and R.K. Saini. 2018. Metabolite profiling of green, green/red, and red lettuce cultivars: Variation in health beneficial compounds and antioxidant potential. Food Res. Int. 105:361-370.

Martínez-Sánchez, A., M.C. Luna, M.V. Selma, J.A. Tudela, J. Abad and M.I. Gil. 2012. Baby-leaf and multi-leaf of green and red lettuces are suitable raw materials for the fresh-cut industry. Postharvest Biol. Tech. 63(1):1-10.

Mithöfer, A. and W. Boland. 2012. Plant defense against herbivores: Chemical aspects. Annu. Rev. Plant Biol. 63(1): 431-450.

Pollard, J., S.F.L. Kirk and J.E. Cade. 2002. Factors affecting food choice in relation to fruit and vegetable intake: a review. Nutr. Res. Rev. 15(2):373-387.

Price, K.R., M.S. Dupont, R. Shepherd, H.W.S. Chan and G.R. Fenwick. 1990. Relationship between the chemical and sensory properties of exotic salad crops-coloured lettuce (*Lactuca sativa*) and chicory (*Cichorium intybus*). J. Sci. of Food Agr. 53(2):185-192.

Rees, S.B. and B.J. Harborne. 1985. The role of sesquiterpene lactones and phenolics in the chemical defence of the chicory plant. Phytochemistry 24(10):2225-2231.

Seo, M.W., D.S. Yang, S.J. Kays, G.P. Lee and K.W. Park. 2009. Sesquiterpene lactones and bitterness in korean leaf lettuce cultivars. HortScience 44(2):246-249.

Sung, J.S., O.S. Hur, K.Y. Ryu, H.J. Baek, S. Choi, S.G. Kim, P.L. Binod, H.C. Ko, J.G. Gwak and J.H. Rhee. 2016. Variation in phenotypic characteristics and contents of sesquiterpene lactones in lettuce (*Lactuca sativa* L.) germplasm. Korean J. Plant Res. 10:115-115.

Tamaki, H., R.W. Robinson, J.L. Anderson and G.S. Stoewsand.

1995. Sesquiterpene lactones in virus-resistant lettuce. J. Agr. Food Chem. 43(1):6-8.

UPOV, 1981. Guidelines for the conduct of tests for distinctness, homogeneity and stability, lettuce (*Lactuca sativa* L.). Document No. TG/13/4 (26.10.1981).

Zangerl, A. and F. Bazzaz. 1993. Theory and pattern in plant defense allocation: *In* Fritz, R.S. and E.L. Simms (eds.), Plant Resistance to Herbivores and Pathogens, University of Chicago Press, Chicago, IL (USA). pp. 363-391.

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