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Original Research Article

Variation in Phenotypic Characteristics and Contents of Sesquiterpene Lactones in Lettuce (*Lactuca sativa* L.) Germplasm

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Abstract - Lettuce is an important annual leafy vegetable and bitterness is its potent flavor character. Lettuce germplasm differ their phenotypic characters and sesquiterpene lactones (SLs) contents which are important for consumer's acceptance. This study was carried out to evaluate the phenotypic characters and SLs contents in one hundred lettuce germplasm in Jeonju, Korea. Twenty-three agro-morphological (16 qualitative and 7 quantitative) traits and two SLs (lactucin and lactucopicrin) contents were studied in these germplasm. Germplasm exhibited the variation in qualitative and quantitative characters. Average plant weight was 423.9 g with a range from 116.0 to 905.0 g. Lactucin content was varied from 19.7 (IT 294226) to 194.4 ㎍/g (IT 294298) with an average concentration of 84.7 ㎍/g. Lactucopicrin ranged from 82.5 (IT 300134) to 2228.6 ㎍/g (IT 294210) with an average concentration of 586.3 ㎍/g. Total SLs content was ranged from 120.1 (IT 300134) to 2286.6 (IT 294210) ㎍/g with the average concentration of 671.0 ㎍/g. Significant ($p \leq 0.05$) differences were found between crisp head and butter head germplasm for lactucin, lactucopicrin and total SLs content. Crisp head germplasm revealed the highest average lactucin (112.9 ㎍/g), lactucopicrin (734.8 ㎍/g) and total SLs content (847.7 ㎍/g). Crisp head and leafy type germplasm exhibited more total SLs content (847.7 and 744.7 ㎍/g, respectively) than cos (524.9 ㎍/g) and butter head type (519.4 ㎍/g). Principal component analyses of the quantitative traits indicated that the first principal component axis accounted more than 91% of the total variation. This study revealed the ample genetic variation in the agro-morphological traits and SLs contents to support the selection for improved lettuce varieties.

Key words - Bolting, germplasm, *Lactuca sativa*, Principal component, Sesquiterpene lactone

Introduction

Lettuce belongs to the family Asteraceae, the most important leafy vegetable and it is exclusively used as fresh vegetable as salads but some forms of lettuce are used as cooked vegetables (Lebeda *et al*., 2007). It is an important commercial crop in Asia, North and Central America, and Europe. China, United States, Spain, Italy, India and Japan are the largest lettuce producers in the World (Lebeda *et al*., 2007). In Korea, lettuce is commonly consumed as 'wrap-up vegetable' with perilla, kale, and pak-choi (Park and Lee, 2006). Year-round production in hydrophonic culture system under greenhouses established the lettuce as an important commercial crop in Korea. Lettuce having romaine, green and red leaf are better sources of vitamin A, niacin, riboflavin, thiamine, calcium, iron, potassium, manganese, selenium, and beta-carotene (USDA-ARS, 2008).

Generally, morphological characters are important for lettuce breeding (Kristokva *et al*., 2008). Genetic improvement of crop depends on the existence of useful genetic variation within germplasm (Ojuederie *et al*., 2014). Presence of genetic diversity among breeding population provides long-term genetic gain (Messmer *et al*., 1993). Likewise, Smith and Smith (1989) highlighted the importance of morphological characterization for the description and classification of germplasm. In lettuce, deep red leaf, late bolting and higher leaf yield and lower SLs are considered as important agro-morphological and biochemical traits for developing new variety (Jang *et al*., 2010). Additionally, sweeter taste and more crispy texture of lettuce are the favorable sensory

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attributes for consumers (Pollard *et al*., 2002).

SLs are a group of over 500 compounds, characteristics of the Asteraceae (Ferioli and D'Antuono, 2012). They show anti-tumour, anti-leukaemic, cytotoxic, antimicrobial activities and allergenic properties (Malarz *et al*., 2002; Sayyah *et al*. 2004). Differences in SLs concentration in lettuce cultivars were also reported by previous researchers (Cho *et al*., 2016; Beharav *et al*., 2015; Arakawa *et al*. 2009; Seo *et al*., 2009). Bitterness is important flavor characteristics of lettuce and degree of bitterness varied among lettuce cultivars (Simmone *et al*., 2002). Differences of bitter components quantities including the SLs: lactucin and lactucopicrin might be attributed the degree of bitterness in lettuce cultivars (Price *et al*., 1990). In Korea, bitterness is the most important factor affecting the consumers to purchase the lettuce (Park and Lee, 2006). Differences in SLs in lettuce can strongly influence taste and consumer acceptability and genotypic information with regard to SLs is of great significance for the breeder.

National Agrobiodiversity Center of Korea has been collecting numerous lettuce germplasm for few years and characterization of these germplasm is essential for breeding new varieties. In addition, these germplasm require characterization to remove duplicate accessions, and to obtain a genetically distinct core sample for effective breeding. Germplasm characterization can be done at morphological, molecular and biochemical levels. Morphological traits are useful for preliminary evaluation and diversity assessment (Asare *et al*., 2011). Morphological evaluation also provides useful information on degree of genetic variation. Previous studies were done on antioxidant potential of lettuce cultivars (Chon, 2005) and its efficient regeneration system through *in vitro* culture (Kim and Kwon, 1999). However, studies on morphological and biochemical (sesquiterpene lactones) characterization among the lettuce germplasm are not well documented yet in Korea. Therefore, this study was done to assess the variation in phenotypic characters and SLs content in lettuce germplasm collected in the genebank of Korea.

Materials and Methods

Plant materials

This experiment was conducted at the Research Farm of National Agrobiodiversity Center, Jeonju (35°49′18″ N 127°08′56″ E), Republic of Korea. The list of lettuce germplasm used in this study is presented in Table 1. Seeds of 100 lettuce accessions were obtained from NAC, RDA and sown in plug trays in the spring and autumn for SLs analysis and phenotypic characterization, respectively, and seedlings were grown in a greenhouse, 2015. Four-week-old seedlings were transplanted at the field of plastic house on 19th of March, and 20th of Sept., respectively, 2015. Planting density was 20 × 20 ㎝. Plant cultural practices were followed by the recommendation of RDA in the field. Each accession consisted of 24 plants and planted at un-replicated method. Plant growth was maintained using nutrient solution throughout the growing season.

Morphological characterization

Twenty-three agronomic and morphological characteristics were recorded on the basis of the standard lettuce descriptors for Protection of New Varieties of Plants (UPOV, 1981). The qualitative and quantitative characters, stage and method of their measurement are given in Table 2. The qualitative characters were recorded based on plant observation on field and bolting and flowering time was measured when 50% plants at each accession were initiated to bolt and flower. Quantitative measurement for each variable was taken on three plants and was averaged it.

Isolation of sesquiterpene lactones

The plant leaf samples were harvested and placed in vinyl freezer bags and held at -80ºC. The frozen samples were subsequently lyophilized for 48 h using vacuum freeze-drier samples. The freeze-dried samples were then ground to a fine powder using a mortar and pestle and held at -80 ºC until analysis. Samples were analyzed using the method of Price *et al*. (1990) in which powdered lyophilized aliquots (0.25 g) were extracted with 100 ㎖ of methanol by boiling under reflux at 65ºC for 1 h 20 min and filtered through Whatman # 2 filter paper. The methanol was evaporated under reduced pressure in a High Capacity Centrifugal Evaporator (Genevac, HT-4X, 5 ㎜ Hg, 30~35°C). It was analyzed using standard lactucin ($C_{15}H_{16}O_5$, 276.3) lactucopicrin ($C_{23}H_{22}O_7$, 410). The extract was then partitioned two times between water/chloroform (20 ㎖; 1:1 mixture by volume) with the

Table 1. List of lettuce germplasm used for the study of phenotypic characters and SLs evaluation in Jeonju, Korea

S. N.	Germplasm	Cultivar	Source[z]	S. N.	Germplasm	Cultivar	Source[z]
1	IT 300576	Crival	FRA	51	IT 300165	Bronowicka	-
2	IT 294200	Tania	GBR	52	IT 300166	Rakowicka	-
3	IT 300136	Trianon	HUN	53	IT 300167	Hohlblattriger Butter	-
4	IT 300148	DL 1644	ISR	54	IT 300168	Kasseler	-
5	IT 300144	DL 1633	KOR	55	IT 300171	Prefere	-
6	IT 300146	DL 1633-2	KOR	56	IT 300172	Prinz V. Lowenstein	-
7	IT 300145	DL 1633-1	KOR	57	IT 300175	Nochowska	-
8	IT 300142	Choseonheukchima	KOR	58	IT 300176	Bronowicka	-
9	IT 300158	Dambaesangchu	KOR	59	IT 300177	Trocadero S.N.	-
10	IT 300157	Dambaesangchu	KOR	60	IT 300178	Budai Hajtato	-
11	IT 300096	Gosina 32	KOR	61	IT 300207	Marula Zelena	-
12	IT 300095	Mihongjeokchukmyeon	KOR	62	IT 300222	Bunte Forellen	-
13	IT 100525	Cheongchima	KOR	63	IT 300224	Furchtenichts	-
14	IT 217003	Highballgyeolgu	KOR	64	IT 300225	Hilmar	-
15	IT 300189	PI 342447	NLD	65	IT 300226	Hoffmanns Aurora	-
16	IT 300190	PI 342454	NLD	66	IT 300234	Black-Seeded Simpson	-
17	IT 300193	PI 342495	NLD	67	IT 300250	Imperial D	-
18	IT 300196	PI 342522	NLD	68	IT 300275	Bologna	-
19	IT 300197	PI 342545	NLD	69	IT 300279	Sweetie	-
20	IT 300198	PI 342549	NLD	70	IT 300282	Feville Dechene	-
21	IT 300571	Romaine Verte	NLD	71	IT 300299	01-778M	-
22	IT 300572	Prego	NLD	72	IT 300307	Balady Aswan	-
23	IT 300569	Cavallona	NLD	73	IT 300308	Balady Cairo	-
24	IT 299997	CAMARA	NLD	74	IT 300313	Da Ye Wo Sun	-
25	IT 300099	Great lakes	PER	75	IT 300210	Bucistinska	-
26	IT 300577	Alface Embola	PRT	76	IT 300289	Floricos	-
27	IT 300134	PI 278073	TUR	77	IT 300300	01-781M	-
28	IT 300579	Zolotoj Shar	UKR	78	IT 300301	01-789M	-
29	IT 294170	Marvel	USA	79	IT 300972	Ramcos	-
30	IT 294194	Domingos 41	USA	80	IT 300977	Dena	-
31	IT 294207	Sumi	USA	81	IT 300970	Wiener Maidivi	-
32	IT 294210	Red Sails	USA	82	IT 300974	Mona	-
33	IT 294226	Redprize	USA	83	IT 300976	Stenhoved	-
34	IT 294298	Ice Cube	USA	84	IT 300574	Forellenschluss Dez	-
35	IT 300270	Excell	USA	85	IT 300570	Kaiser Selbstschlus	-
36	IT 300271	Genesys Green	USA	86	IT 300980	Romaine Ballon	-
37	IT 300272	Honcho 11	USA	87	IT 300973	Cherna Gyumyurdzinska	-
38	IT 300273	Champ	USA	88	IT 300966	Trocadero	-
39	IT 300274	Tiara	USA	89	IT 300971	Spafaulschie Pender Greber	-
40	IT 300156	Jeokchimasangchu	USA	90	IT 32824	Imperial No 44	-
41	IT 209988	VNM-AWS-1998-44	VNM	91	IT 32817	Calmar	-
42	IT 300140	Lakalna	YUG	92	IT 32814	Vanguard	-
43	IT 294111	Great Lakes 659 LMC	-	93	IT 251833	WIR1830	-
44	IT 294131	Imperial 152	-	94	IT 216900	Costamari	-
45	IT 294133	Imperial 615	-	95	IT 227858	Saladbaul(cheong)	-
46	IT 294145	Drumhead White Cabbage	-	96	IT 32798	Great Lakes 66	-
47	IT 294152	Valrio	-	97	IT 218327	Italienischer Gruner	DEU
48	IT 294161	Primaverde	-	98	IT 100526	Un-known	KOR
49	IT 294165	Mesa 659	-	99	IT 100516	Cheongchima	KOR
50	IT 294175	PI 561053	-	100	IT 219889	HAROMFAI	HUN

[z]YUG = Yugoslavia, VNM = Vietnam, USA = United States of America, UKR = Ukraine, TUR = Turkey, PRT = Portugal, PER = Peru, NLD = Netherland, KOR = Korea, ISR = Israel, GBR = Great Britain, FRA = France, DEU = Deutschland, and - = unknown.

Table 2. Plant morphological characters used in phenotypic evaluation of lettuce germplasm at Jeonju, Korea

Characters	When to measure	How to measure/scale
Qualitative characters		
Shape of cotyledon	Seedling stage	3 = narrow elliptic, 5 = medium elliptic, 7 = broad elliptic
Size of cotyledon	Seedling stage	3 = small, 5 = medium , 7 = large
Plant growth type	Vegetative stage	1 = leaf, 2 = crisp head, 3 = cos (romaine), 4= butter head, 5 = stem, 6 = oil seed
Leaf attitude	Vegetative stage	3 = erect, 5 = semi-erect, 7 = prostrate
Green color of outer leaves	Vegetative stage	1 = light green, 2 = green, 3 = deep green, 4 = grey green, 5 = purple red
Intensity of leaf color at outer leaves	Vegetative stage	1 = very light, 2 = light, 3 = medium, 4 = dark, 5 = very dark
Leaf anthocyanin color	Vegetative stage	0 = absent, 1 = present
Leaf shape	Vegetative stage	1 = narrow elliptic, 2 = elliptic, 3 = broad elliptic, 4 = circular, 5 = elliptic, 6 = obovate, 7 = transverse broad, 8 = triangular
Leaf blade undulation at margin	Vegetative stage	3 = weak, 5 = medium, 7 = strong
Leaf blade incision density	Vegetative stage	1 = sparse, 3 = medium, 5 = dense 7 = very dense
Head formation	Vegetative stage	1 = none heading, 2 = semi- heading, 3 = closed heading (overlapping)
Head size	Vegetative stage	0 = no head, 1 = very small, 3 = small, 5 = medium, 7 = large, 9 = very large
Head density	Vegetative stage	0 = very loose, 3 = loose, 5 = medium, 7 = dense, 9 = very dense
Head shape at longitudinal section	Vegetative stage	0 = no shape,1 = narrow elliptic, 2 = circular, 3 = broad elliptic, 4 = transverse broad elliptic
Flower color	Flowering stage	1 = white, 2 = light yellow, 3 = yellow, 4 = deep yellow
Seed color	Harvesting stage	1 = grey, 2 = brown , 3 = mixed (grey + brown)
Quantitative characters		
Outer leaves per plant	Vegetative stage	no.
Leaf length	Vegetative stage	cm
Leaf width	Vegetative stage	cm
Leaf length/width	Vegetative stage	-
Bolting time	Vegetative stage	days
Flowering time	Flowering stage	days
Plant weight	Harvesting stage	g

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 by volume) and the SLs separated using high-performance liquid chromatography (HPLC) and monitored at 256 ㎚.

Analysis of sesquiterpene lactones

The sesquiterpene lactones were separated using high-performance liquid chromatography (HPLC) system (Agilent1260/LC) equipped with UV visible Diode Detector (SPD-M10A). A Luna C18 column (250 × 4.6 ㎜ i. d. 5 ㎛ particles; Phenomenex Security Guard (ODS Octadecyl, 4 × 3.0 ㎜) was used with water/acetonitrile mobile phase at flow rate of 0.8 ㎖/min. The solution gradient started at 90:10 (by volume) and was altered in a linear manner to a final ratio of 65:35 over 30 min. The SLs (lactucin and lactucopicrin) were monitored at 256 ㎚ (Fig. 1).

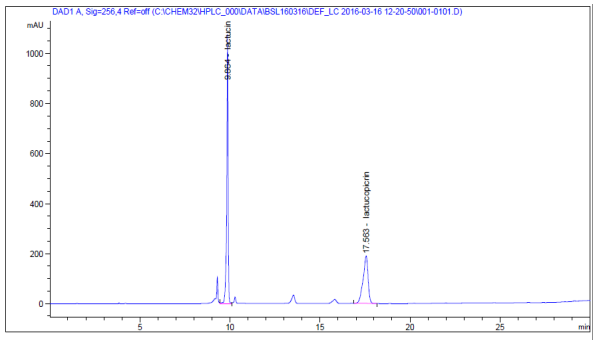

Fig. 1. High-performance liquid chromatography of lettuce extracts containing lactucin and lactucopicrin.

Statistical analyses

The qualitative and quantitative data were subjected to descriptive statistics using SPSS Statistics 17.0 (SPSS Inc., Chicago, IL, USA). Lettuce accessions were further categorized based on plant growth habit i.e. leafy type, crisp head, cos (romaine), butter head, stem and oil seed and, agronomic and SLs contents data were further analyzed using one-way analysis of variance followed by Student-Newman-Keuls test for multiple comparisons of means. Principal component analysis (PCA) was performed on qualitative and quantitative characters using Microsoft Excel (version 10.0, Microsoft, Redmond, WA, USA), Multibase program (http://www.numerical dynamics.com).

Results and Discussion

Phenotypic variation

Sixteen qualitative traits exhibited variation among accessions (Table 3). Plant growth type varied from leaf, crisp, cos butter head to stem type. Erect, semi-erect and prostrate leaf attitude were observed. Outer leaves had green, deep green, grey green and purple red colored. Leaf shape was varied from narrow elliptic, elliptic, broad elliptic, circular, prostrate elliptic obovate and transverse broad. Likewise, head size and density was varied among the accessions. Head shape at longitudinal section had no head, narrow elliptic, circular, and transverse broad elliptic. Flower and seed color were varied among the accessions.

Quantitative characters also exhibited wide variation in the germplasm (Table 4). Number of outer leaves per plant was varied from 20.7 (IT 300099) to 120.0 (IT 300308) with average of 43.0. Average leaf length was 30.4 ㎝ with varied from 10.7 (IT 300190) to 47.0 ㎝ (IT 300307). Leaf width was varied from 9.4 (IT 300134) to 32.2 ㎝ (IT 32817) with an average of 20.8 ㎝. Leaf length and width ratio was ranged from 0.8 (IT 300190) to 4.8 (IT 300134) with an average of 1.6. Bolting time was varied from 38.0 (IT 300134) to 102.0 (IT 294298) days with average of 65.0 days whereas flowering days was varied from 68.0 (IT 300308) to 118.0 (IT 300270) with average of 92.0 days. Plant weight was ranged from 116.0 (IT 300190) to 905.0 g (IT 32814) with an average of 423.9 g.

Lactucin content was varied from 19.7 (IT 294226) to 194.4 ㎍/g (IT 294298) with an average concentration of 84.7 ㎍/g. Likewise, lactucopicrin was ranged from 82.5 (IT 300134) to 2228.6 ㎍/g (IT 294210) with the average

Table 3. Frequency distribution of qualitative traits in lettuce germplasm evaluated at Jeonju, Korea

Characters	Frequency[z]	Characters	Frequency[z]
Shape of cotyledon	narrow elliptic (21), medium elliptic (61), broad elliptic (18)	Leaf blade undulation at margin	Weak (59), medium (30), strong (11)
Size of cotyledon	small (10), medium (48), large (42)	Leaf blade incision density	Sparse (15), medium (51), dense (30), very dense (4)
Plant growth type	leaf (28), crisp (25), cos (21), butter head (25), stem (1)	Head formation	no head (38), semi head (15), closed head (47)
Leaf attitude	erect (6), semi-erect (43), prostrate (51)	Head size	No head (29), small (8), medium (45), large (15), very large (3)
Color of outer leaves	green (54), deep green (19), grey green (13), purple red (14)	Head density	very loose (34), loose (20), medium (30), dense (14), very dense (2)
Intensity of leaf color at outer leaves	very light (1), light (9), medium (47), dark (43)	Head shape at longitudinal section	No head shape (29), narrow elliptic (17), circular (27), broad elliptic (12), transverse broad elliptic (15)
Leaf anthocyanin color	absent (86), present (14)	Flower color	white (75), yellow (21), deep yellow (4)
Leaf shape	narrow elliptic (13), elliptic (29), broad elliptic (14), circular (11), prostrate elliptic (2), obovate (20), transverse broad (11)	Seed color	grey (61), brown (37), mixed (2)

[z]Number in parenthesis indicate germplasm.

Table 4. Variation in quantitative traits and sesquiterpene lactones in lettuce germplasm evaluated at Jeonju, Korea

Traits	Mean	SD[y]	Minimum	Maximum
Quantitative traits				
Outer leaves/plant (no.)	43.1	18.8	20.7 (IT 300099)[z]	120.0 (IT 300308)[z]
Leaf length (㎝)	30.4	6.6	10.7 (IT 300190)	47.0 (IT 300307)
Leaf width (㎝)	20.8	5.3	9.4 (IT 300134)	32.2 (IT 32817)
Leaf length/width	1.6	0.6	0.8 (IT 300190)	4.8 (IT300134)
Bolting time (days)	65.0	13.6	38.0 (IT 300134)	102.0 (IT 294298)
Flowering time (days)	92.0	10.8	68.0 (IT 300308)	118.0 (IT 300270)
Plant weight (g)	423.9	155.0	116.0 (IT 300190)	905.0 (IT 32814)
Sesquiterpene lactones				
Lactucin (㎍/g)	84.7	40.0	19.7 (IT 294226)	194.4 (IT 294298)
Lactucopicrin (㎍/g)	586.3	367.3	82.5 (IT 300134)	2228.6 (IT 294210)
Total SLs content (㎍/g)	671.0	381.0	120.1 (IT 300134)	2286.6 (IT 294210)

[y]SD = Standard deviation. [z]Number in parentheses indicates germplasm.

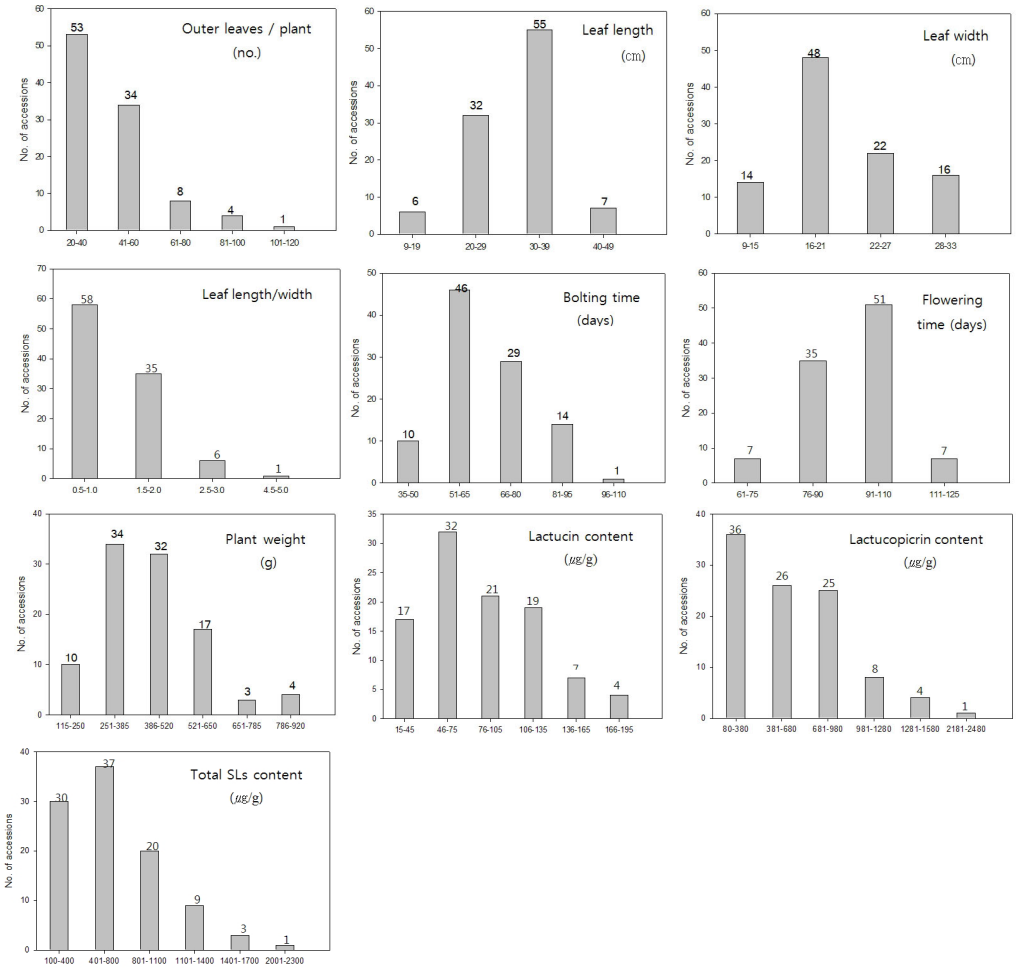

Fig. 2. Frequency distribution of quantitative traits among 100 lettuce germplasm evaluated at Jeonju, Korea.

concentration of 586.3 ㎍/g. Total SLs content was varied from 120.1 (IT 300134) to 2286.0 ㎍/g (IT 294210) with an average of concentration of 671.0 ㎍/g.

Frequency distribution of quantitative traits among lettuce germplasm is given in Fig. 2. Number of outer leaves per plant within 20-40 ㎝ range had the highest (53) accessions whereas

leaf length ranging from 30 to 39 ㎝ had the highest (55) accessions. Bolting time between 51 and 65 days had the highest number (46) of accessions. The plant weight ranging from 251 to 385 g contained the highest number (34) of accessions. The lactucin content ranged from 46 to 75 ㎍/g contained the highest number (32) of accessions whereas lactucopicrin ranged from 80 to 380 ㎍/g contained 36 accessions. This showed wide variation of agrononic traits and there was a good potential for developing a good variety of lettuce using this variation.

The quantitative characters and SLs contents in different varietal types (leafy type, crisp, cos and butter head type) were statistically analyzed (Table 5). Number of outer leaves per plant was significantly ($p \leq 0.05$) different between leafy and crisp head type, and crisp head and cos head type. Outer leaves per plant produced the highest number (52.8) in cos head type which ranged from 31.3 to 120.0. Leaf length was significantly different between leafy and butter head type but it was non-significant with leafy, crisp and cos head type.

Bolting time in crisp head type was significantly different ($p \leq 0.05$) from leafy type, cos head and butter head type but bolting days between cos and butter head was non-significant. Late bolting (average, 82.6 days) was recorded in crisp head type with a range from 69.0-102.0 days and leafy (average, 57.3 days) and butter head (average, 59.3 days) types were early bolting types. Flowering time of leafy type was significantly different from crisp, cos and butter head types. Plant weight was significantly different between crisp and butter head type. Crisp head germplasm showed the highest plant weight (490.3 g) which ranged from 176.0 - 905.0 g.

Significant differences were found between crisp and cos head, and butter head for lactucin and lactucopicrin ($p \leq 0.05$). Crisp head lettuce accession exhibited the highest (average, 112.6 ㎍/g) lactucin which ranged from 45.4 to 194.4 ㎍/g. Average lactucopicrin content was highest (734.8 ㎍/g) with a range from 313.9 to 1116.3 ㎍/g in crisp head type followed by leaf type (average, 665.0 ㎍/g). Total lactones in leafy and crispy head type were statistically significant from cos head

Table 5. Variation in quantitative traits and sesquiterpene lactones in different varietal types of lettuce germplasm evaluated at Jeonju, Korea

Characters	Statistics	Leafy type (N = 28)[x]	Crisp head (N = 25)[x]	Cos head (N=21)[x]	Butter head (N = 25)[x]	Stem type (N=1)[y]
Quantitative traits						
Outer leaves (no.)	Mean	39.0 ± 11.6 b[z]	29.1 ± 9.6 c	52.8 ± 24.3 ab	52.9 ± 17.6 ab	-
	Range	20.7 - 64.3	20.7 - 66.3	31.3 - 120.0	30.7 - 99.0	-
Leaf length (㎝)	Mean	32.5 ± 5.5 a	30.7 ± 5.0 a	34.4 ± 5.3 a	24.0 ± 5.8 b	-
	Range	15.6 - 42.8	15.0 - 39.7	26.5 - 47.0	10.7 - 36.5	-
Leaf width (㎝)	Mean	20.6 ± 4.7 ab	26.7 ± 4.1 a	16.6 ± 3.6 c	18.8 ± 1.7 bc	-
	Range	11.3 - 31.7	14.9 -32.2	9.4 - 26.2	13.6 - 21.3	-
Leaf length/width	Mean	1.6 ± 0.4 a	1.2 ± 0.1 c	2.2 ± 0.7 a	1.3 ± 0.2 b	-
	Range	1.1 - 2.4	0.9 - 1.5	1.3 - 4.8	0.8 - 1.9	-
Bolting time (days)	Mean	57.3 ± 10.5 c	82.6 ± 8.1 a	62.4 ± 9.0 b	59.3 ± 6.9 bc	-
	Range	38.0 - 75.0	69.0 -102.0	49.0 -77.0	41.0 -71.0	-
Flowering time (days)	Mean	87.6 ± 10.4 c	103.8 ± 7.8 a	88.4 ±8.5 ab	89.2 ± 5.2 b	-
	Range	69.0 - 113.0	91.0 - 118.0	72.0 - 103.0	77.0 - 98.0	-
Plant weight (g)	Mean	386.9 ± 146.8 abc	490.3 ± 173.2 a	481.1 ± 158.7 ab	351.6 ± 96.7 c	-
	Range	176.0 - 836.0	176.0 - 905.0	236.0 - 895.0	116.0 - 548.0	-
Sesquiterpene lactones						
Lactucin (㎍/g)	Mean	79.7 ± 41.0 ab	112.9 ± 39.6 a	79.6 ± 37.3 b	66.1 ± 27.4 bc	-
	Range	19.7 - 169.1	45.4 - 194.4	27.6 - 153.6	26.4 - 136.6	-
Lactucopicrin (㎍/g)	Mean	665.0 ± 526.8 a	734.8 ± 224.2 a	445.4 ± 253.7 bc	453.3 ± 262.2 b	-
	Range	111.9 - 2228.6	313.9 - 1116.3	82.5 - 1094.9	109.9 - 1132.5	-
Total SLs content (㎍/g)	Mean	744.7 ± 524.7 a	847.7 ± 253.4 a	524.9 ± 266.0 b	519.4 ± 280.3 bc	-
	Range	134.7 - 2286.6	381.2 - 1247.3	120.1 - 1157.0	138.1 - 1225.9	-

[x]N = Number of germplasm. [y]Stem type contained one accession and not considered to analyze. [z]Values are presented as mean ± SD (standard deviation). Means in the rows for each character followed by different letter (s) are significantly different at $P \leq 0.05$.

and butter head type. Total lactones was highest (average, 847.7 ㎍/g) in crispy type which was statistically similar with leafy type.

Principal component analyses

PCA of the 16 qualitative traits revealed that the first six axes (PC1-PC6) cumulatively accounted for 85.8% of the total variation observed among the germplasm (Table 6). The first PC axis accounted 41.9% of the variation. The traits that contributed most of the variation were head density, head size, leaf shape and head formation. The second PC also contributed 15.3% of the total variation and associated with leaf shape and leaf attitude. The third PC explained 12.3% of the total variation and associated with size and shape of the cotyledon, and plant growth type. The fourth PC axis contributed 6.4% of the total variation and was associated with leaf blade incision density, head size and color of outer leaves. The fifth and sixth PCs contributed 5.3 and 4.2% of total variation, respectively and were associated with head density, color of outer leaves, leaf shape and size of cotyledon.

PCA using 10 quantitative traits (Table 7) indicated that the first two principal components (PC1 and PC2) contributed

Table 6. Principal component (PC) analysis of 16 qualitative characters showing their contribution to the total variation among 100 lettuce germplasm evaluated at Jeonju, Korea

Characters	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Shape of cotyledon	-0.135	0.122	0.346	-0.132	-0.258	-0.057
Size of cotyledon	-0.094	0.178	0.398	0.058	-0.040	0.344
Plant growth type	0.175	-0.153	0.334	-0.070	-0.024	0.155
Leaf attitude	0.057	0.303	0.282	-0.031	-0.234	-0.135
Color of outer leaves	-0.056	0.047	-0.153	0.339	0.435	0.479
Intensity of leaf color at outer leaves	0.036	-0.090	-0.164	-0.037	0.048	0.012
Leaf anthocyanin color	-0.035	0.044	0.006	0.111	0.096	0.133
Leaf shape	0.336	0.574	-0.292	0.039	-0.478	0.351
Leaf blade undulation at margin	0.131	0.144	-0.499	0.211	0.014	-0.304
Leaf blade incision density	0.087	0.261	0.320	0.683	0.142	-0.450
Head formation	0.313	0.078	0.027	-0.086	0.074	-0.070
Head size	0.419	-0.605	0.051	0.427	-0.431	0.151
Head density	0.690	0.049	0.162	-0.281	0.449	0.002
Head shape at longitudinal section	0.210	0.092	0.032	-0.094	0.041	-0.138
Flower color	-0.053	0.158	0.115	0.233	0.181	0.335
Seed color	-0.006	0.011	0.007	0.008	0.000	-0.108
% Variance	41.98	15.32	12.39	6.49	5.36	4.28
% Cumulative variance	41.98	57.30	69.69	76.18	81.54	85.82

Table 7. Principal component (PC) analysis of 10 quantitative characters showing their contribution to the total variation among 100 lettuce germplasm evaluated at Jeonju, Korea

Characters	PC 1	PC 2
Outer leaves/plant (no.)	0.0054	0.0140
Leaf length (㎝)	0.0042	0.0066
Leaf width (㎝)	-0.0033	0.0417
Leaf length/width	0.0003	0.0196
Bolting time (days)	0.0032	0.0111
Flowering time (days)	-0.0002	0.0003
Plant weight (g)	0.0360	0.9979
Lactucin (㎍/g)	0.0261	0.0163
Lactucopicrin (㎍/g)	0.6932	-0.0341
Total SLs contents (㎍/g)	0.7193	-0.0177
% Variance	91.35	7.75
% Cumulative variance	91.35	99.1

99.1% of the total variation observed among the germplasm. PC1 contributed 91.3% of the total variation and was associated with total lactones and lactucopicrin. The second PC contributed 7.7% of the total variation and was associated the plant weight.

Discussion

Lettuce is an annual plant and contained enormous morphological characteristics, and varieties are mainly categorized based on their shape of leaves, and head formation. This study explained the morphological and SLs variation in one hundred lettuce germplasm. Dolezalova *et al*. (2002) mentioned that the description of morphological features of lettuce accessions increases their potential successful utilization. Plant growth type, leaf colors, shape, head shape and density are important features to identify the lettuce germplasm. The morphological descriptor provides the important tools for species determination within the genus *Lactuca* and for a characterization of *Lactuca* intraspecific morphological variability (Dolezalova *et al*., 2002). The phenotypic characters are useful in separating the genotypes into distinct group based on their sources and variability for the growth traits. Bolting is one of the important traits in lettuce and the bolted lettuce undergoes various undesirable physical and chemical changes which affect consumer acceptability and marketability. Late bolting, deep red leaf and high leaf yield are the important characters to select lettuce variety (Jang *et al*., 2010). This study also characterized the lettuce germplasm for deep red, late bolting and high plant weight type which is important for lettuce breeding.

Lettuce produces SLs compounds which impart the bitter taste and ultimately affects the consumer acceptance and consumption (Chadwick *et al*., 2015). The concentration of lactucin and lactucopicrin ranged from 2.9 to 17.2 and 8.8 to 36.1 ㎍/g dry weight, respectively in 10 lettuce cultivars (Seo *et al*., 2009) but present study revealed that lactucin and lactucopircrin concentration ranged from 19.7 to 194.4 and 82.5 to 2228.6 ㎍/g dry weight, respectively in 100 lettuce germplasm. The SLs content varied widely between populations of the wild lettuce and the differences in SLs contents are genotype dependent (Beharav *et al*., 2015). Lactucopicrin was the primary contributor to bitterness due to its high concentration (Seo *et al*., 2009) and the high concentration of lactucopicrin found in the lettuce germplasm in this study might be contributed to more bitterness but bitterness activity in the studied germplasm is yet to be confirmed. In the study of Price *et al*., (1990), they reported lactucin and lactucopicrin are bitter SLs and their concentrations determine the taste. The genetic differences among the lettuce cultivars contribute to the wide range in bitterness in lettuce (Price *et al*., 1990; Seo *et al*., 2009). Besides, concentrations of SLs in lettuce depend on plant parts (e.g. basal, midstalk, flower stalk) (Seo *et al*., 2009). Our study showed the highest average total SLs content in crisp and leaf head type lettuce germplasm which confirms the findings of Cho *et al*. (2016). But Choi *et al*. (2014) reported the highest SLs content in romaine (cos head) type lettuce cultivars.

PCA of both qualitative and quantitative characters showed that the germplasm were morphologically diverse for traits such as head density, size, leaf shape, head formation, leaf attitude, cotyledon shape and size, plant growth type, leaf color, plant weight, lactucopicrin, and total lactones. For the qualitative traits, leaf shape, leaf color, head size and plant growth type are useful traits that distinguished the genotypes. Leaf shape, head size, and head density also play the role in plant weight and yield. Likewise, lactucopicrin is the quantitative trait and is a major contributor to bitterness in lettuce due to its concentration. Therefore, the lactucopicrin is important trait for the breeders to select the lettuce germplasm for varietal improvement. Lower SLs content is also the main criteria for developing lettuce variety (Jang *et al*., 2010). The presence of phenotypic variation in germplasm provides opportunity for developing improved lettuce varieties particularly on preferred traits.

To summarize, the study showed variation among lettuce germplasm for all phenotypic and SLs contents. We employed sixteen qualitative traits and nine quantitative traits for the study of 100 lettuce germplasm. Qualitative characters including plant growth type, leaf shape and color, head size and density showed the variation among the germplasm and are useful to differentiate the germplasm. Large variation found in quantitative traits such as leaves number, leaf size, bolting time, plant weight, lactucin, lactucopicrin and total SLs

content among the germplasm. Significant differences were found between leaf and crisp head type for the number of outer leaves per plant and bolting time. Lactucin, lactucopicrin ant total SLs content in crisp head type was significantly different from butter head type. Germplasm belonging to crisp head and leafy type found more SLs content and might be bitter than cos and butter head type lettuce. Present study focused mainly on morphological characterization and SLs quantification of the lettuce germplasm. Besides this, molecular characterization of the germplasm is recommended for further understanding of their genetic variation. Lactucin and lactucopicrin are the major bitter SLs compounds in lettuce and their quantitative variation in the lettuce germplasm is useful for selecting the new varieties.

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