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Assessment of Genetic Diversity and Fatty acid Composition of Perilla (*Perilla frutescens* var. *frutescens*) Germplasm

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Abstract - The objective of this study was to analyze the genetic diversity using SSR marker and investigate the fatty acid composition of perilla (*P. frutescens* var. *frutescens*) germplasm. Genetic diversity among 95 accessions, which consisted of 29 weedy types and 66 landrace accessions, was evaluated based on 12 SSR markers carrying 91 alleles. The mean values of observed (H_O) and expected heterozygosities (H_E) were 0.574 and 0.640, respectively, indicating a considerable amount of polymorphism within this collection. A genetic distance-based phylogeny grouped into two distinct groups, which were the landrace, moderate and weedy type, genetic distance (GD) value was 0.609. The physicochemical traits about crude oil contents and fatty acid compositions were analyzed using GC. Among tested germplasm, the total average oil contents (%) showed a range from 28.57 to 49.67 %. Five fatty acids and their contents in the crude oils are as follows: α-linolenic acid (41.12%-51.81%), linoleic acid (15.38%-16.43%), oleic acid (18.93%-27.28%), stearic acid (2.56%-4.01%), and palmitic acid (7.38%-10.77%). The average oil content of wild types was lower than landrace, and the oil content of middle genotype accessions was higher than other germplasm, but no significant variation between landrace and wild types was shown. Nevertheless, IT117174, landrace of Korea, was highest in crude oil content (47.11%) and linolenic acid composition (64.58%) among the used germplasm. These traits of the selected accessions will be helped for new functional plant breeding in perilla crop.

Key words - Microsatellite, Genetic diversity, Genetic conservation, *Perilla frutescens*, Fatty acid composition

Introduction

Perilla crop (*Perilla* L.) comprises self-fertilizing annual species as an annual herbaceous plant of the *Labiatae* family, which is widely distributed in East Asian regions such as China, Korea and Japan (Li, 1974; Lee *et al*., 2002). Nitta *et al*. (2005) reported that the genus *Perilla* was composed of one cultivated species, *P. frutescens* (L.) Britt., and three wild species, *P. citriodora* (Makino) Nakai, *P. hirtella* Nakai and *P. setoyensis* G. Honda, and the cultivated species are classified two main types, *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa*, basis on their fragrance of leaves and the hardness of seeds (Nitta *et al*., 2003). *P. frutescens* var. *frutescens* is known as Dlggae in South Korea, Egoma in Japan, and Renin in China. The applicable parts of *P. frutescens* var. *frutescens* are the leaves for vegetation and seeds to oil in Korea.

P. frutescens var. *frutescens* seeds are good source of polyunsaturated fatty acids (PUFAs). The seeds of perilla contained approximately 35-45% oil and perilla seed oil consistently contains the one of the highest proportion of omega-3 (ALA) fatty acids, at 54-64% in comparison to other plant oils. In addition, the omega-6 (linoleic acid) component is usually around 14% and omega-9 (Oleic acid) is also present in perilla oil (Asif, 2011). Omega fatty acids are the essential for our health, so the omega-3 and 6 must be obtained through our diet or by supplementation (Kopecky *et al*., 2009; Russo, 2009). Perilla oil has been studied in humans and is converted into DHA and EPA by the body. Due to its anti-inflammatory effects, it has been shown to be beneficial in patients with asthma, resulting in decreased inflammatory markers, and improved ventilatory parameters (Okamoto *et al*., 2000).

In Korea, landraces of *P. frutescens* var. *frutescens* were

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cultivated in farmer's fields in several areas of South Korea (Park *et al*., 2008) and the weedy types are grown naturally (Lee *et al*., 2003), even though some varieties have been developed for mainly seed or leaf production. Landraces have been continuously maintained by farmers within their local biological, cultural and socio-economic context. As environments, as well as the people who live within them, are different in different agricultural areas, many landraces of a crop were developed within a certain region after domestication or introduction. In addition, farmers tended to suit the intra-farm multiple eco-agricultural conditions by growing a diverse range of landraces per crop (Christensen *et al*., 2011). Landraces and weedy types have distinct identity and lack formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems (Villa *et al*., 2006). Studies of genetic diversity using molecular markers are necessary to understand the genetic structure of a population and to orientate effective strategies of germplasm conservation (Ganapathy *et al*., 2011).

However, the lack of molecular characterizations and evaluations limits the utility of this conserved *P. frutescens* var. *frutescens* germplasm in breeding programs. Because the ability to identify genetic variation is indispensable to the effective management and use of any plant genetic resources (Rao, 2004), knowledge of the genetic diversity and genetic relationships among conserved and on-farm perilla landrace and weedy type accessions is critical importance, and may ensure the long-term success of perilla breeding programs. Recently, the variability of some perilla genotypes has been described in terms of their morphology, cultivation, use, and diversification (Lee and Ohnishi, 2001; Nitta *et al*., 2003 and 2005), as well as their genetic diversity and relationships with weedy forms using random amplified polymorphic DNA (RAPD) (Nitta and Ohnishi, 1999), amplified fragment length polymorphism (AFLP; Lee *et al*., 2002; Lee and Ohnishi, 2003), and SSR markers(Park *et al*., 2008; Verma *et al*., 2010). However, limited information is available on population structure and fatty composition of Korea landraces and weedy types in *P. frutescens* var. *frutescens* germplasm.

The objectives of this study were (i) to evaluate genetic polymorphism between landraces and weedy type collections, (ii) to investigate the mean of GD estimates between the subgroups, and (iii) to estimate the oil content and fatty acid composition to subgroups.

Materials and Methods

Plant materials

The collection contained 94 accessions of *Perilla frutescens* var. *frutescens* consisted including 56 Korean landraces and 29 Korean weedy types from eight provinces of Korea, and 6 Japanese landraces and 3 North Korean landraces from National Institute of Crop Sciences (NICS). The accession numbers and localities of the experimental accessions are presented in Table 1. Samples were sown in a nursery in May 2011, and one month later 30 seedlings were transplanted into the experimental field. Seeds of all accessions were taken during leaf yellowing stage after the flowering.

Table 1. List of 94 perilla accessions collected by the RDA

Sample number	IT/Temp. IT	Origin	Variety type	Collection region
6	104291	KOR	Landrace	Gyeongju, Gyeongsanbuk-do
7	104849	KOR	Landrace	Gunsan(Okku), Jeollabuk-do
8	105168	KOR	Landrace	Mungyeong, Gyeongsanbuk-do
11	105282	KOR	Landrace	Chuncheon, Gangwon-do
12	105409	KOR	Landrace	Yecheon, Gyeongsanbuk-do
16	105556	KOR	Landrace	Gimcheon, Gyeongsanbuk-do
17	105584	KOR	Landrace	Boseong, Jeollanam-do
18	105803	KOR	Landrace	Andong, Gyeongsanbuk-do
26	108768	KOR	Landrace	Gimcheon, Gyeongsanbuk-do
34	109181	KOR	Landrace	Uljin, Gyeongsanbuk-do
35	109199	KOR	Landrace	Uiseong, Gyeongsanbuk-do
36	110938	KOR	Landrace	Gochang, Jeollabuk-do

Table 1. Continued

Sample number	IT/Temp. IT	Origin	Variety type	Collection region
37	113045	KOR	Landrace	Seongju, Gyeongsanbuk-do
44	113329	KOR	Landrace	Gochang, Jeollabuk-do
49	117006	KOR	Landrace	Suwon, Gyeonggi-do
51	117028	KOR	Landrace	Chilgok, Gyeongsanbuk-do
57	117048	KOR	Landrace	Pohang, Gyeongsanbuk-do
59	117067	KOR	Landrace	Ulsan, Gyeongsangnam-do
67	117088	KOR	Landrace	Yeongyang, Gyeongsanbuk-do
69	117094	KOR	Landrace	Paju, Gyeonggi-do
82	117119	KOR	Landrace	Dangjin, Chungcheongnam-do
83	117124	KOR	Landrace	Cheongju, Chungcheongbuk-do
84	117132	KOR	Landrace	Nonsan, Chungcheongnam-do
97	117167	KOR	Landrace	Hwaseong, Gyeonggi-do
99	117174	KOR	Landrace	Cheongju, Chungcheongbuk-do
120	157401	KOR	Landrace	Chuncheon, Gangwon-do
130	157434	KOR	Landrace	Yangyang, Gangwon-do
132	157438	KOR	Landrace	Yangyang, Gangwon-do
136	157447	KOR	Landrace	Danyang, Chungcheongbuk-do
137	157448	KOR	Landrace	Danyang-gun, Chungcheongbuk-do
138	157450	KOR	Landrace	Jincheon, Chungcheongbuk-do
139	157455	KOR	Landrace	Hwaseong, Gyeonggi-do
142	157472	KOR	Landrace	Pyeongtaek, Gyeonggi-do
143	157478	KOR	Landrace	Pocheon, Gyeonggi-do
144	157481	KOR	Landrace	Yangpyeong, Gyeonggi-do
163	157504	KOR	Landrace	Bonghwa, Gyeongsanbuk-do
171	157514	KOR	Landrace	Yeongdeok, Gyeongsanbuk-do
173	157534	KOR	Landrace	Naju, Jeollanam-do
181	157567	KOR	Landrace	Boryeong, Chungcheongnam-do
184	157570	KOR	Landrace	Buyeo, Chungcheongnam-do
185	157576	KOR	Landrace	Seocheon, Chungcheongnam-do
186	157590	KOR	Landrace	Iksan, Jeollabuk-do
187	157596	KOR	Landrace	Gimje, Jeollabuk-do
188	157598	KOR	Landrace	Gimje, Jeollabuk-do
205	201758	JPN	Landrace	
208	201760	JPN	Landrace	
210	201765	JPN	Landrace	
211	201769	JPN	Landrace	
212	201770	JPN	Landrace	
213	201773	JPN	Landrace	
216	207970	KOR	Landrace	Suncheon, Jeollanam-do
217	209211	KOR	Landrace	Ganghwa, Incheon
230	214468	KOR	Landrace	Sinan, Jeollanam-do
232	214498	KOR	Landrace	Goseong, Gyeongsangnam-do
251	712180	KOR	Landrace	Seogwipo, Jeju-do
254	805105	KOR	Landrace	Ongjin, Gyeonggi-do
258	K001444	KOR	Landrace	Haenam, Jeollanam-do
259	K001517	KOR	Landrace	Goseong, Gyeongsangnam-do
264	K011623	KOR	Landrace	Cheorwon, Gangwon-do
268	K015829	KOR	Landrace	Pyeongchang, Gangwon-do
282	K131002	KOR	Weedy	Hwacheon, Gangwon-do
283	K131004	KOR	Weedy	Hwacheon, Gangwon-do
284	K131010	KOR	Weedy	Yanggu, Gangwon-do

Table 1. Continued

Sample number	IT/Temp. IT	Origin	Variety type	Collection region
288	K131018	KOR	Weedy	Inje, Gangwon-do
289	K131027	KOR	Weedy	Hongcheon, Gangwon-do
331	K141364	KOR	Landrace	Jeju, Jeju-do
332	K151656	KOR	Landrace	Ganghwa, Incheon
341	K153700	PRK	Landrace	
342	K153701	PRK	Landrace	
344	K153703	PRK	Landrace	
352	K131026	KOR	Weedy	Hongcheon, Gangwon-do
356	K131034	KOR	Weedy	Jeongseon, Gangwon-do
357	K131035	KOR	Weedy	Bonghwa, Gyeongsanbuk-do
358	K131036	KOR	Weedy	Ulleung, Gyeongsanbuk-do
359	K131037	KOR	Weedy	Bonghwa, Gyeongsanbuk-do
360	K131040	KOR	Weedy	Bonghwa, Gyeongsanbuk-do
361	K131041	KOR	Weedy	Bonghwa, Gyeongsanbuk-do
364	K131044	KOR	Weedy	Yeongyang, Gyeongsanbuk-do
365	K131045	KOR	Weedy	Yeongyang, Gyeongsanbuk-do
367	K131047	KOR	Weedy	Cheongsong, Gyeongsanbuk-do
368	K131048	KOR	Weedy	Andong, Gyeongsangbuk-do
369	K131051	KOR	Weedy	Changnyeong, Gyeongsangnam-do
370	K131052	KOR	Weedy	Changnyeong, Gyeongsangnam-do
372	K131055	KOR	Weedy	Haman, Gyeongsangnam-do
373	K131056	KOR	Weedy	Haman, Gyeongsangnam-do
374	K131062	KOR	Weedy	Uiryeong, Gyeongsangnam-do
375	K131064	KOR	Weedy	Uiryeong, Gyeongsangnam-do
376	K131065	KOR	Weedy	Hapcheon, Gyeongsangnam-do
377	K131067	KOR	Weedy	Haenam, Jeollanam-do
379	K131083	KOR	Weedy	Sacheon, Gyeongsangnam-do
380	K131090	KOR	Weedy	Wando, Jeollanam-do
381	K131094	KOR	Weedy	Jindo, Jeollanam-do
392	K130953	KOR	Weedy	Chungju, Chungcheongbuk-do
393	K131016	KOR	Weedy	Inje, Gangwon-do

†KOR, Korea; JPN, Japan; PRK, North Korea.

DNA extraction

Fresh leaves of 15-day-old seedlings were used for the genomic DNA isolation. Homogenization was accomplished by freezing fresh leaf tissue in liquid nitrogen and then grinding with pestle. DNA was extracted using QIAGEN DNA extraction kit (QIAGEN Co. Germany). The relative purity and concentration of extracting DNA was estimated with the help of NanoDrop ND-1000 (Dupont Agricultural Genomics Lab.). The final concentration of each DNA sample was adjusted to 20 ng/ul in TE buffer before conducting PCR.

Amplification of SSR loci and fragment separation

The sequences of seven primer pairs of SSR markers were obtained Park *et al*. (2008) and additional five polymorphic SSR markers were isolated from the microsatellite enriched library prepared using the protocol described by Dixit *et al*. (2005) in this study. A final set of 12 SSR primer pairs was used for the analyses of 94 genotypes (Table 2).

The polymerase chain reactions (PCRs) were performed in PTC-220 thermocyclers (MJ Research, Waltham, MA, USA) under the following amplification conditions : initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 52-56°C for 45 s, then 15 cycles at 94°C for 30 s, 53°C for 45 s, and extension at 72°C for 45 s followed by a final extension at 72°C for 10 min and 4°C as the holding step. The 20μL reaction mix consisted of 0.1 μM of each primer, 2.5 U of *Taq* polymerase (Takara, Tokyo,

Table 2. Simple sequence repeat (SSR) marker primer pairs sequence and the number of alleles obtained from each SSR locus in 94 genotypes of perilla

Marker	Genebank Acc. No.	Forward	Reverse	Repeated motif	Tm (℃)	Size range (bp)‡	NA	He	Ho	PIC
GB-PF-075[1]	GBPFM75	CATAGTTCATGGCTTCCACC	CCTGAGCACAGAAACAGATCA	$(CT)_{12}$	52	150-220	11	0.643	1.000	0.583
GB-PF-091[1]	GBPFM91	CCACTCAAATCCGCTTCTAA	AATGTTGGTTGCGTTTCATT	$(AG)_9$	54	282-294	4	0.692	1.000	0.644
GB-PF-111[1]	GBPFM111	ATCATGGATGAATCGCACTT	CCATTCTCCAAATGTTACTCTATTT	$(ACACA)_8$	56	190-199	4	0.631	0.444	0.607
GB-PF-134[2]	JQ287711	CCTCCACTTCTTCTTCTCCC	TTTGCATCCTGTCTCTCACA	$(CT)_5$	52	163-181	5	0.441	0.656	0.344
GB-PF-135[2]	JQ287712	CTTCTGAGGCCAACATTGAG	AGGGCTCGGTTGAATCTTAC	$(CT)_{20}$	52	168-206	10	0.481	0.000	0.462
GB-PF-155[1]	GBPFM155	TTTGTGACAATACGCACCAC	CCAATTGCTCAATGCTCTCT	$(GAA)_{10}$	56	172-200	3	0.886	0.047	0.875
GB-PF-157[1]	GBPFM157	AAAGAGCTGATGGACGTGAG	AGGTGCTACTGTGTCAAGGC	$(CAA)_7$	56	180-238	18	0.686	0.043	0.657
GB-PF-172[2]	JQ287713	ATCGGTCTTTGAAATCACCA	TGAAATTTCTTGCCGTTACC	$(GA)_{11}$	52	158-172	6	0.646	0.066	0.574
GB-PF-179[1]	GBPFM203	TGAATCATCCCAAACGAGAT	TCGCTTCTCTCTCATGGATT	$(TGA)_5$	52	236-272	7	0.504	0.659	0.449
GB-PF-198[2]	JQ287714	GTATCGTTTCGCAGCAATTT	CCACACTTCCTTACCCCTCT	$(GA)_7(G)(GA)_4$	52	175-189	2	0.621	0.989	0.550
GB-PF-201[2]	JQ287715	AGACTCGTTTCACAATTTCTCC	CATTCCCACCTCATGTTACG	$(GA)_6(GT)(GA)^5$	52	210-264	8	0.617	1.000	0.542
GB-PF-203[1]	GBPFM203	GTTTTGTTGCAGCTCGATTT	TGGGTTTGGAAAGTATTGATG	$(AG)^{26}$	54	277-319	13	0.834	0.978	0.816
Mean							7.6	0.640	0.574	0.592

[1]Derived from Park et al. (2008). [2]Newly developed in this study. N_A = total number of alleles per locus; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content.

Japan), 0.16 mM of each dNTP, 1x PCR Buffer (150 mM Tris−HCl, pH 8.0, and 500 mM KCl), 2.5 mM $MgCl_2$, and 1 μL template DNA. The PCR products were fractionated on an ABI PRISM 3130XL Genetic Analyzer according to the manufacturer's instructions (Applied Biosystems, USA). Allele sizes were scored to ensure proper scoring of band size using GeneMapper v4.0 software (Applied Biosystems, USA).

Diversity statistics

PowerMarker version 3.25 (Liu and Muse, 2005) was utilized to create a genetic distance matrix between all pairwise combinations and summarize information on unique alleles, frequency of alleles, distribution of alleles. With this software, the MAF, the observed (H_O) and expected (H_E) heterozygosities were determined, and the degree of polymorphism was estimated from polymorphic information content (PIC) values.

Population structure analysis

The software package Structure 2.0 (Pritchard *et al*., 2000) was utilized to infer population structure and assign individuals to populations based on the SSR genotypes. Structure uses model-based clustering in which a Bayesian approach is used to identify clusters based on Hardy−Weinberg equilibrium and linkage equilibrium. In our study, the membership of each genotype was tested in the range of genetic clusters from K = 1 to 10 with the admixture model, without prior information on their origin. The burn-in time and replication number was set to 100,000 for each run and each run was replicated five times as suggested by Pritchard and Wen (2004), assuming that allele frequencies are uncorrelated across clusters. Furthermore, principal co-ordinate analysis (PCoA) in GenAlEx 6.0 (Peakall and Smouse, 2006) was employed to examine further the genetic relationships among detected populations on the basis of the SSR data. PCoA analysis was performed for all markers as well as separately for the markers representing the each group, respectively.

Oil content and fatty acids composition

About three grams of seeds were ground to a particle size of 3 mm or less with pestle using liquid nitrogen, and 1 g of sample was used for analysis. Crude oil extraction was carried out in Foss soxhlet apparatus (Foss, USA), using hexane as solvents and calculated the amount of recovered

lipid. The fatty acid methyl esters (FAMEs) of the total lipid extract were prepared by the BF3 method (Morrison and Smith, 1964). The boron trifluoride-methanol solution (Sigma, St. Louis, MO) was added to a final concentration of 14%, and the solution was heated on a water bath at 70°C, shaken every 30 min, and cooled to room temperature. The FAMEs were separated by capillary gas chromatography (GC) to the retention times of standard fatty acids. GC analysis was performed on a HP 5890 system (Hewlett Packard, USA) by a flame ionization detector (FID). GC separation was performed on a capillary column (0.25 mm X 30 m, film 0.5 mm) of HP-INNOWAX (Hewlett Packard, USA). Each extraction (1 ul) was injected using the splitless injection method. The column temperature was initially 150℃, then raised the temperature to 200℃ at a rate of 4℃/min, and finally held at 250℃ for 5 min. The injection port was set at 250℃ and detector was 300℃. Nitrogen gas was used as the carrier at a flow rate of 0.6 ml/min. Fatty acids were identified by comparing the gas chromatograph retention time of each peak with standard mixtures that were prepared by methylation similar to the sample preparation and the results calculated using the response area under each peak. Standard chemicals were obtained from Sigma-Aldrich (St. Louis, Mo, USA)

Results

Genetic Diversity Statistics

The average number of alleles, the frequency of the major allele and gene diversity were given in Table 2. All the 12 SSR primers were used for inter-population diversity analysis and detected 91 alleles among 94 perilla accessions. The average alleles per primer pair was 7.6, ranging from 2 for GB-PF-198 to 18 for GB-PF-157. In general, higher values both of Ho and He revealed a higher genetic variability among the germplasm accessions. The values of H_O and H_E were ranged from 0.00 to 1.00 (mean 0.57) and from 0.44 to 0.89 (mean 0.64), respectively, and the highest H_O and H_E were revealed by GB-PF-075 (1.000), GB-PF-091 (1.000), GB-PF-201 (1.000) and GB-PF-155 (0.886). The PIC values, a reflection of allele diversity and frequency among the used germplasm, also varied from one locus to another. The PIC values were ranged from 0.344 to 0.875, with an average of 0.592. In comparing of repeat motif, SSR primers containing AG repeat motif showed the larger variability than the other used repeat motif. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus (DeWoody *et al*., 1995). The mean PIC value observed in this study was higher than the PIC value of 0.5, but the genetic diversity of each SSR locus not appeared to be associated with the number of alleles detected per locus.

Population Structure

In the joint PCoA of the molecular data of 94 *Perilla frutescens* var. *frutescens* accessions, 56 Korean landraces, 29 Korean weedy types, 6 Japanese landraces and 3 North Korean landraces, The first principal coordinate (PC1) accounted for 44.1% of the total variation and the second (PC2) accounted for 18.1% of the total molecular variation of all 94 populations (Fig. 2). The first principal coordinate

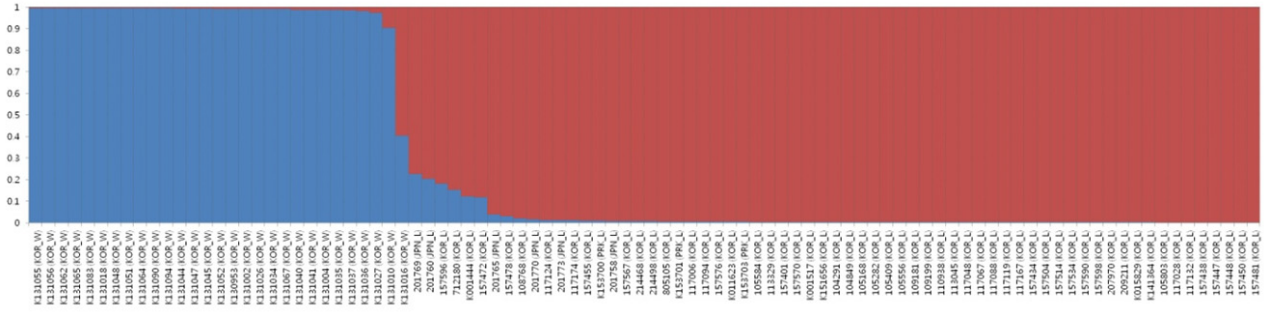

Fig. 1. Membership probability of assigning genotypes of the entire germplasm set to two subgroups. The height of blue and red bars represents the probability of two different subgroups. Each vertical bar represents one perilla accessions; KOR_L, Korean_landraces; KOR_W, Korean_weedy type; JPN_L, Japanese_landraces; PRK_L, North Korean_landraces.

clearly differentiated between the weedy type accessions and landrace germplasm. Even though the weedy types were clearly separated from all other landrace accessions in PC1, weedy types were more separated than landraces in PC2 analysis. Most landrace accessions clustered in quadrants III and IV with a substantial overlap by the second principal coordinate (PC2). Six landraces of Japan were mainly assigned to quadrant III, whereas the Korean landraces was distributed among quadrants III and IV. As in the cluster analysis, accessions of cultivated perilla from Korea showed more genetical variations than those from Japan, although the number of Japanese accessions used in this study was deficiency. The Bayesian model-based STRUCTURE software (Pritchard et al., 2000) was used to determine population structure. The LnP(D), log likelihood, revealed by STRUCTURE increased gradually from K = 1 to K = 10 and showed no clear optimum values. Therefore, the *ad hoc* measure ΔK was applied to estimate the number of subgroups. For the entire germplasm set, the maximum ΔK value was observed for K = 2 (data not shown). The results of the population structure analysis indicate that the 94 diverse genotypes analyzed belongs to two subpopulations (Fig. 1) that represent landrace and weedy type, while 7 genotypes were categorized as admixed form of genotype that was consisted with PCoA results.

According to the results obtained in the previous studies, by Ito *et al*. (1998) using RFLP markers, by Nitta *et al*. (2003) using RAPD markers and by Lee *et al*. (2007) using SSR markers, *P. frutescens* var. *frutescens* were not distinctively separated between landrace and weedy type and hence considered to belong to the same gene pool. Lee *et al*. (2007) suggested that natural hybrids and the outcrossing rate in perilla species have not been reported by this time, but these results due to the possibility that cultivated type might be originated from its weedy type, and some accessions of weedy types might be derived from escaped forms from cultivation. Compared with the above published literature, our results showed some differences. The clustering patterns based on the used SSR markers in this paper permitted clear distinctions landraces and weedy types, and it indicated enough polymorphism to differentiate the intra- and inter-population diversity. These 12 SSR marker set, that consist of

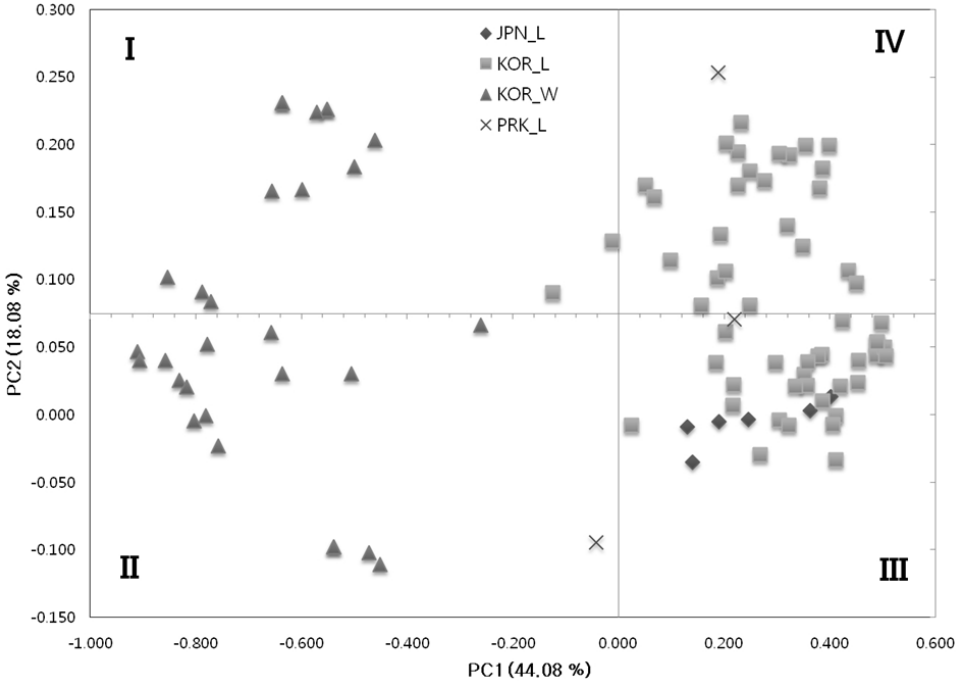

Fig. 2. Scatter plot of the first two principle coordinate scales for 94 accessions. The principle coordinate analysis (PCoA) performed with the matrix of genetic similarity (GS) estimates from SSR markers. PC1 and PC2 are the first and second principle coordinates, respectively.

newly developed and derived from Park *et al*. (2008), will be useful tools for assessing genetic diversity and population structure in *P. frutescens*.

Crude Oil Content and Fatty acid Composition

The crude oil content and constituents of fatty acid are presented in Table 3 and 4. Yield of crude oil from perilla accessions was ranged from 31.39 to 47.11%. Five different kinds of fatty acids, representing over than 99% of identified compound, were identified as α-linolenic acid (55.57%-64.58%), linoleic acid (11.81%-17.94%), oleic acid (9.95%-16.08%), stearic acid (2.31%-4.48%), and palmitic acid (6.55%-8.99%). Two essential fatty acids, oleic and linolenic acids, were the significant fatty acids in terms of quality and quantity of perilla seed oils. Most predominant fatty acid was linolenic acid as 55-64% of total fatty acid and oleic acid was about 13%. These results were similar to those reported in previous studies on perilla seed where linolenic acid content of the seed was about 58 to 60 % (Asif, 2011; Gunstones *et al*., 1994).

It can be clearly seen that the oil content of weedy types was lower than landraces, but the ratio of fatty acid composition did not differ significantly between weedy types and landraces. Furthermore, the oil content was not positively correlated with the contents of oleic acid and linolenic acid. In this study, we selected an accession IT117174 as one of the elite genetic resources, showing higher contents of oil (47.11%) and linolenic acid (64.58%) than the cultivated varieties in Korea (Table 5).

Ding *et al*. (2012) reported that *Perilla frutescens* var. *frutescens* had seven fatty acids, including myristic acid (0.37%), cis-11-eicosenoic acid palmitic acid (0.16%), but these kinds of fatty acid were not found in this study. The ratio of the saturated, monounsaturated and polyunsaturated fatty acids was 1:1.15:6.65 on average (Table 3). This result was comparable with those obtained by Kim and Choi (2004) in Korea (1:1.50:7.50), Siriamornpun *et al*. (2006) in Thailand (1:1.19:7.76) and Ding *et al*. (2012) in China (1: 2.00: 6.02). It suggested that environmental factors affect on fatty acid compositions.

Table 3. Saturated and unsaturated fatty acids in seed oils

Country	Oil content (%)			Saturated FA	Monounsaturated FA	Polyunsaturated FA	Total unsaturated FA	Saturated : monounsaturated : polyunsaturated
	Max	Min	Mean					
KOR_L	47.11	37.85	43.59±2.28	10.46	12.62	74.5	87.12	1:1.21:7.21
KOR_W	39.67	31.39	36.51±2.04	11.44	12.71	73.54	86.25	1:1.11:6.43
PRK_L	42.7	37.58	40.34±2.58	11.6	12.93	73.16	86.09	1:1.15:6.31
JPN_L	44.7	42.32	43.45±1.05	10.88	12.98	74.13	87.11	1:1.19:6.81
Mean	43.55±3.15	37.28±4.49	40.97±3.33	11.10±0.52	12.81±0.17	73.83±0.60	86.64±0.55	1:1.15:6.65

KOR_L, Korean_landraces; KOR_W, Korean_weedy type; JPN_L, Japanese_landraces; PRK_L, North Korean_landraces.
Saturated FA, Myristic, Palmitic, Stearic, Arachidic and Behenic; Monounsaturated FA, Oleic; Polyunsaturated FA, Linoleic and inolenic.

Table 4. Difference in fatty acid composition in 94 perilla accessions collected from different countries

Country	Fatty acid composition														
	Palmitic (%)			Stearic (%)			Oleic (%)			Linoleic (%)			Linolenic (%)		
	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
KOR_L	8.59	6.55	7.61±0.54	3.6	2.31	2.85±0.31	15.37	9.95	12.62±1.11	17.74	11.81	14.06±1.12	64.58	56.26	60.44±1.63
KOR_W	8.99	6.67	7.87±0.60	4.48	2.92	3.57±0.37	16.08	10.42	12.71±1.24	17.94	11.66	13.88±1.2	62.97	55.91	59.66±1.77
PRK_L	8.99	7.92	8.35±0.57	4.19	2.71	3.25±0.82	14.66	11.81	12.93±1.52	15.05	13.56	14.31±0.74	59.79	57.44	58.85±1.24
JPN_L	8.35	7.55	7.99±0.31	3.15	2.66	2.89±0.20	15.6	10	12.98±2.02	17.87	13.65	16.14±1.68	62.5	55.57	57.99±2.76
Mean	7.95±0.31			3.14±0.34			12.81±0.18			14.60±1.04			59.24±1.05		

KOR_L, Korean_landraces; KOR_W, Korean_weedy type; JPN_L, Japanese_landraces; PRK_L, North Korean_landraces.

Table. 5 List of 10 accessions containing highest oil content (%) among 94 perilla accessions

Acc.No.	Country	Oil content (%)	Fatty acid composition (%)				
			Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
IT117174	KOR-L	47.114	7.254	2.569	11.046	12.784	64.583
IT157576	KOR-L	46.771	7.584	2.776	12.307	13.448	62.079
IT157472	KOR-L	46.753	7.795	2.827	11.901	14.731	60.608
IT117094	KOR-L	46.459	7.668	3.397	15.374	11.814	59.288
IT105584	KOR-L	46.452	6.658	2.326	12.784	13.760	62.572
IT805105	KOR-L	46.272	8.221	3.024	12.816	13.909	59.237
IT117048	KOR-L	46.139	7.314	2.320	9.950	14.519	64.085
IT157598	KOR-L	45.932	8.043	3.021	13.533	12.891	60.639
IT157567	KOR-L	45.924	7.620	2.850	13.751	13.549	60.475
IT157596	KOR-L	45.582	7.808	2.840	12.934	12.910	61.721

Discussion

P. frutescens (L.) Britt. is widely cultivated in East Asia and used as a spicy vegetable or an oil crop. Leaves of perilla plants have specific scent derived from their variety of essential oil components (Ito et al., 1999 and 2002). However the leaves have poor source of oil, containing only 0.2%. In addition, only the seed oil contains the omega 3 fatty acid and alpha-linolenic acid, in perilla (Asif, 2011). The content of the ω-3 linolenic acid was the highest one among soybean, maize, sunflower, sesame, earthnut, walnut, olive and oil-tea, and even 10% higher than that of the flaxseed oil, and it has also been introduced to Europe, Russia and USA as a commercial oilseed crop (Ding *et al*., 2012). Some of varieties have been focused on mainly seed or leaf production, but many landraces are cultivated in Korea as a household vegetable and oil crop. Next to breeding stocks and wild relatives, landraces are one of the important sources for genetic improvement of cultivars in oil and linolenic acid content of perilla. The detailed assessment of oil content and fatty acid composition is important to use the landraces as genetic resources for breeding.

In this study, we determined genetic diversity of perilla germplasm based on SSR markers. Cluster analyses performed with landrace accessions and weedy type showed clearly distribution pattern. The genetic variation among the accessions revealed by SSRs reflected a high level of polymorphism at the DNA level. Model-based structure analysis was divided into two groups, weedy types and landrace accessions, which were basically consistent with the clustering results PCoA based on genetic distance.

The results suggest that these SSR markers of perilla would be a valuable marker resource for the genetic diversity analysis of perilla germplasm and distinguish the weedy types and landraces. Furthermore, the selected perilla accession with the high oil content and linolenic composition would be one of good genetic resources for perilla breeding to improve the oil yield and quality. The information obtained here would be useful to evaluate perilla genetic resources and for the utilization of these plants for the breeding of new valuable cultivars.

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