Collection and Evaluation of Genetic Variation of *Perilla* Accessions in the Jeju Island

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ABSTRACT In order to understand the genetic variation of the cultivated and weedy types of *Perilla* crop in Jeju Island of Korea, this study has conducted a field expedition for collecting *Perilla* germplasm in 2011 and 2012, respectively. Cultivated *Perilla* crop was almost not cultivated throughout the island, whereas weedy types of both varieties (var.) of *frutescens* and *crispa* were often found in roadsides, around a creek, in wastelands, and in areas around a farmer's fields. The total number of collection was 94 accessions. The seed colors of cultivated var. *frutescens* were white and brown, while the weedy var. *frutescens* were gray, brown, and dark brown. The weedy var. *crispa* exhibited gray and dark brown seed colors. The most accessions of cultivated var. *frutescens* and weedy types of var. *frutescens* and var. *crispa* revealed hard seeds, except one accession of cultivated var. *frutescens* which had soft seeds. A total of 17 simple sequence repeat loci showed polymorphism, producing a total of 149 alleles among the 85 *Perilla* accessions collected from Jeju Island. The average gene diversity for accessions of cultivated var. *frutescens*, weedy var. *frutescens*, and weedy var. *crispa* respectively showed 0.346, 0.649, and 0.463. The accessions of weedy types of var. *frutescens* and var. *crispa* comparatively exhibited higher genetic diversity than those of cultivated var. *frutescens*. The accessions collected would be useful for preserving the genetic diversity of this crop for further breeding programs of the *Perilla* crop in Korea.

Keywords *Perilla frutescens*, Cultivated and weedy types, Field survey, Genetic variation, Germplasm

INTRODUCTION

Perilla frutescens Britt. (Labiatae) is a self-fertilizing annual species, which is widely cultivated and distributed in the Himalayan hills, Southeast Asia, and East Asia. The species includes two varieties that are characterized on the basis of their morphology and utilization. One is *P. frutescens* var. *frutescens*, an oil crop, which is known as Dlggae in Korea. Another is *P. frutescens* var. *crispa*, a Chinese medicine or vegetable crop, and called as Cha-jo-ki in Korea (Lee and Ohnishi 2001). In addition to those two cultigens, weedy plants of both var. *frutescens* and var. *crispa* were reported by Nitta and Ohnishi (1999) and Lee and Ohnishi (2001, 2003). They are commonly found in such habitats as roadsides, wastelands, abandoned fields, and the marginal lands around the farmers' fields in East Asia (Lee and Ohnishi 2001; Nitta *et al.* 2003).

In Korea, cultivated var. *frutescens* has been one of the most important oil crops since ancient times. According to a report by Choi (1984), *Perilla* seed oil has been used not only for edible oil but also for industrial oil. It has been used for lamp oil, waterproofing papers for umbrella and other rain-gear, and lacquer for wooden furniture, wooden vessels, and living households. Also, its seeds, like sesame seeds for the seasoning of flavoring agents, are used as folk medicine for illnesses as inveterate gastritis, cough, and restorative (Lee 2001). Moreover, in Korea, var. *frutescens* is used not only as an oil crop for its seeds but also as a vegetable crop for its leaves to become one of the most important crops. However, germplasm collections and classifications have not been carried out systematically and the breeding of this crop is far behind other major crops in Korea.

The success of breeding or the genetic conservation

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programs is dependent on the understanding of the amount and distribution of the genetic variation present in genepool. In order to clarify the cultivation, utilization, and distribution of *Perilla* species in Korea, this study conducted a field survey throughout the Jeju Island in 2011 and 2012. In Korea, Jeju Island is located in the southernmost portion of South Korea and is also the largest island in the land, coming into existence 700 to 1,200 thousand years ago when lava spewed from a sub-sea volcano and surfaced above the waters, which led to a mild oceanic climate throughout the year with the smallest annual temperature range.

Recently, molecular techniques of polymerase chain reaction (PCR)-based DNA markers such as Random Amplified Polymorphic DNA, Amplified Fragment Length Polymorphisms (AFLPs), and simple sequence repeats (SSRs) have provided useful information regarding genetic diversity and relationships in many crops (Ajmone-Marsan *et al.* 1998; Senior *et al.* 1998; Nitta and Ohnishi 1999; Prasad *et al.* 2000; Lee and Ohnishi 2003; Xia *et al.* 2005; Sa *et al.* 2010). Among the many kinds of DNA-based molecular markers, particularly SSRs or SSRs are very abundant in eukaryotic genomes and hypervariable in the repeat units between plants in a given plant population, and the preferred choice for genetic studies because they are highly reproducible, polymorphic, codominant, and abundant in plant genomes (Powell *et al.* 1996; Park *et al.* 2009). In the previous study, *Perilla* SSRs were developed by Kwon *et al.* (2005) and Park *et al.* (2008), respectively, and also used to analyze the genetic diversity and genetic relationships in *Perilla* germplasm from Korea, East Asian and other countries (Lee *et al.* 2007; Lee and Kim 2007; Park *et al.* 2008; Sa *et al.* 2015).

Moreover, understanding genetic diversity and genetic relationships in *Perilla* crop and their weedy types is essential for the long-term success of breeding programs and maximizes the use of the *Perilla* germplasm resources in Korea. Therefore, in this study, the result of the field survey and the analysis of genetic variation using SSR and morphological markers for understanding the genetic diversity and conservation condition of *Perilla* crop and their weedy types in Jeju Island of Korea are reported.

MATERIALS AND METHODS

Collection for *Perilla* crop and their weedy types

A field survey for the cultivation of *Perilla* crop and their weedy types in Jeju Island of Korea was respectively conducted from October 24 to October 26 in 2011 and from October 31 to November 3 in 2012 (Fig. 1, Table 1). More than 30 villages were visited and numerous farmers were interviewed about their cultivation and utilization of *Perilla* crop. The natural distribution of weedy types of *Perilla* species in farmers' fields, roadsides, wastelands, abandoned fields, and the areas around the farmhouses was also searched. In this study, the *Perilla* samples were tentatively classified into cultivated or weedy type according to the morphological characters and the cultivation conditions where they were collected (cultivated or not by farmers) as based on the previous report by Lee and Ohnishi (2001) and Lee *et al.* (2002).

Morphological characteristics analysis

To assess the morphological variations in *Perilla* crop and their weedy types, five individuals of each accession were grown in a field at the Agriculture and Life Sciences, Kangwon National University, Chuncheon, Gangwon Province. Approximately 10 seeds of each accession were sown in a nursery bed in early May and maintained in a glass house for a month. Five seedlings of each accession

Fig. 1. Routes of the field survey and collection sites for *Perilla* crop and their weedy types in Jeju Island.
○: accession of cultivated type of var. *frutescens*;
●: accession of weedy type of var. *frutescens*;
★: accession of weedy type of var. *crispa*.

Table 1. Collection sites of *Perilla* samples in Jeju-do, Republic of Korea.

Accession no.	Collection date	Source of material (village, town, or city)	Type	Code no. ²⁾
PF11-106	2011/10/24	Seongeup-ri, Pyoseon-myeon, Seogwipo	Weedy var. <i>frutescens</i>	7
PF11-107	2011/10/24	Doryeon-1 dong, Jeju	Weedy var. <i>frutescens</i>	8
PF11-108	2011/10/24	Doryeon-1 dong, Jeju	Weedy var. <i>frutescens</i>	9
PF11-109	2011/10/25	Bongseong-ri, Aewol-eup, Jeju	Weedy var. <i>frutescens</i>	10
PF11-110	2011/10/25	Bongseong-ri, Aewol-eup, Jeju	Weedy var. <i>frutescens</i>	11
PF11-111	2011/10/25	Bongseong-ri, Aewol-eup, Jeju	Weedy var. <i>frutescens</i>	12
PF11-112	2011/10/26	Seonhul-ri, Jocheon-eup, Jeju	Weedy var. <i>frutescens</i>	13
PF11-113	2011/10/26	Seonhul-ri, Jocheon-eup, Jeju	Weedy var. <i>frutescens</i>	
PF11-114	2011/10/26	Bongseong-ri, Aewol-eup, Jeju	Weedy var. <i>crispa</i>	82
PF11-115	2011/10/26	Bongseong-ri, Aewol-eup, Jeju	Cultivated var. <i>frutescens</i>	1
PF12-019	2012/10/31	Samyang-dong, Jeju	Weedy var. <i>frutescens</i>	14
PF12-020	2012/10/31	Samyang-dong, Jeju	Weedy var. <i>frutescens</i>	
PF12-021	2012/10/31	Samyang-dong, Jeju	Weedy var. <i>frutescens</i>	15
PF12-022	2012/10/31	Jochen-ri, Jochen-eup, Jeju	Weedy var. <i>frutescens</i>	16
PF12-023	2012/10/31	Jochen-ri, Jochen-eup, Jeju	Weedy var. <i>frutescens</i>	17
PF12-024	2012/10/31	Jochen-ri, Jochen-eup, Jeju	Weedy var. <i>frutescens</i>	18
PF12-025	2012/10/31	Woljeong-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	19
PF12-026	2012/10/31	Sehwa-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	20
PF12-027	2012/10/31	Sehwa-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	21
PF12-028	2012/10/31	Sehwa-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	22
PF12-029	2012/10/31	Sehwa-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	23
PF12-030	2012/10/31	Sehwa-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	24
PF12-031	2012/10/31	Sehwa-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	25
PF12-032	2012/10/31	Sangdo-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	26
PF12-033	2012/10/31	Sangdo-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	27
PF12-034	2012/10/31	Sangdo-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	28
PF12-035	2012/10/31	Sangdo-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	
PF12-036	2012/10/31	Sangdo-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	29
PF12-037	2012/10/31	Sangdo-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	30
PF12-038	2012/10/31	Sangdo-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	31
PF12-039	2012/10/31	Sangdo-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	32
PF12-040	2012/10/31	Songdang-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	
PF12-041	2012/10/31	Songdang-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	33
PF12-042	2012/10/31	Songdang-ri, Gujwa-eup, Jeju	Weedy var. <i>frutescens</i>	34
PF12-043	2012/10/31	Susan-ri, Seongsan-eup, Seogwipo	Weedy var. <i>frutescens</i>	35
PF12-044	2012/11/01	Siheung-ri, Seongsan-eup, Seogwipo	Weedy var. <i>frutescens</i>	
PF12-045	2012/11/01	Siheung-ri, Seongsan-eup, Seogwipo	Weedy var. <i>frutescens</i>	36
PF12-046	2012/11/01	Siheung-ri, Seongsan-eup, Seogwipo	Weedy var. <i>frutescens</i>	37
PF12-047	2012/11/01	Siheung-ri, Seongsan-eup, Seogwipo	Weedy var. <i>frutescens</i>	38
PF12-048	2012/11/01	Sinyang-ri, Seongsan-eup, Seogwipo	Weedy var. <i>crispa</i>	83
PF12-049	2012/11/01	Nansan-ri, Seongsan-eup, Seogwipo	Weedy var. <i>frutescens</i>	39
PF12-050	2012/11/01	Nansan-ri, Seongsan-eup, Seogwipo	Weedy var. <i>frutescens</i>	40
PF12-051	2012/11/01	Nansan-ri, Seongsan-eup, Seogwipo	Weedy var. <i>frutescens</i>	
PF12-052	2012/11/01	Samdal-ri, Seongsan-eup, Seogwipo	Weedy var. <i>frutescens</i>	41
PF12-053	2012/11/01	Samdal-ri, Seongsan-eup, Seogwipo	Weedy var. <i>frutescens</i>	42
PF12-054	2012/11/01	Seongeup-ri, Pyoseon-myeon, Seogwipo	Weedy var. <i>frutescens</i>	
PF12-055	2012/11/01	Hacheon-ri, Pyoseon-myeon, Seogwipo	Weedy var. <i>frutescens</i>	43

Table 1. Continued.

Accession no.	Collection date	Source of material (Village, town, or city)	Type	Code no. ²⁾
PF12-056	2012/11/01	Hacheon-ri, Pyoseon-myeon, Seogwipo	Weedy var. <i>frutescens</i>	44
PF12-057	2012/11/01	Hacheon-ri, Pyoseon-myeon, Seogwipo	Weedy var. <i>frutescens</i>	45
PF12-058	2012/11/01	Pyoseon-ri, Pyoseon-myeon, Seogwipo	Weedy var. <i>frutescens</i>	46
PF12-059	2012/11/01	Sehwa-ri, Pyoseon-myeon, Seogwipo	Weedy var. <i>frutescens</i>	47
PF12-060	2012/11/01	Singeung-ri, Namwon-eup, Seogwipo	Weedy var. <i>frutescens</i>	
PF12-061	2012/11/01	Singeung-ri, Namwon-eup, Seogwipo	Weedy var. <i>frutescens</i>	48
PF12-062	2012/11/01	Singeung-ri, Namwon-eup, Seogwipo	Weedy var. <i>frutescens</i>	49
PF12-063	2012/11/01	Hannam-ri, Namwon-eup, Seogwipo	Weedy var. <i>frutescens</i>	50
PF12-064	2012/11/01	Wimi-ri, Namwon-eup, Seogwipo	Weedy var. <i>frutescens</i>	51
PF12-065	2012/11/01	Wimi-ri, Namwon-eup, Seogwipo	Weedy var. <i>frutescens</i>	52
PF12-066	2012/11/01	Harye-ri, Namwon-eup, Seogwipo	Weedy var. <i>frutescens</i>	53
PF12-067	2012/11/01	Harye-ri, Namwon-eup, Seogwipo	Weedy var. <i>frutescens</i>	
PF12-068	2012/11/01	Harye-ri, Namwon-eup, Seogwipo	Weedy var. <i>frutescens</i>	54
PF12-069	2012/11/01	Hwasun-ri, Andeok-myeon, Seogwipo	Weedy var. <i>frutescens</i>	55
PF12-070	2012/11/01	Sangye-dong, Seogwipo	Cultivated var. <i>frutescens</i>	2
PF12-071	2012/11/02	Seohong-dong, Seogwipo	Cultivated var. <i>frutescens</i>	3
PF12-072	2012/11/02	Seohong-dong, Seogwipo	Weedy var. <i>frutescens</i>	56
PF12-073	2012/11/02	Seohong-dong, Seogwipo	Weedy var. <i>frutescens</i>	57
PF12-074	2012/11/02	Seohong-dong, Seogwipo	Weedy var. <i>frutescens</i>	58
PF12-075	2012/11/02	Seohong-dong, Seogwipo	Weedy var. <i>frutescens</i>	59
PF12-076	2012/11/02	Deoksu-ri, Andeok-myeon, Seogwipo	Weedy var. <i>frutescens</i>	60
PF12-077	2012/11/02	Deoksu-ri, Andeok-myeon, Seogwipo	Weedy var. <i>frutescens</i>	61
PF12-078	2012/11/02	Deoksu-ri, Andeok-myeon, Seogwipo	Weedy var. <i>frutescens</i>	62
PF12-079	2012/11/02	Yeongrak-ri, Daejeong-eup, Seogwipo	Weedy var. <i>frutescens</i>	63
PF12-080	2012/11/02	Gosan-ri, Hangeong-myeon, Jesu	Cultivated var. <i>frutescens</i>	4
PF12-081	2012/11/02	Dumo-ri, Hangeong-myeon, Jesu	Weedy var. <i>frutescens</i>	64
PF12-082	2012/11/02	Wollyeong-ri, Hanllim-eup, Jesu	Weedy var. <i>frutescens</i>	65
PF12-083	2012/11/02	Geumneung-ri, Hanllim-eup, Jesu	Weedy var. <i>frutescens</i>	66
PF12-084	2012/11/02	Gwideok-ri, Hanllim-eup, Jesu	Weedy var. <i>frutescens</i>	67
PF12-085	2012/11/02	Gwideok-ri, Hanllim-eup, Jesu	Weedy var. <i>frutescens</i>	68
PF12-086	2012/11/02	Bongseong-ri, Aewol-eup, Jesu	Weedy var. <i>frutescens</i>	69
PF12-087	2012/11/02	Gwakji-ri, Aewol-eup, Jesu	Weedy var. <i>frutescens</i>	70
PF12-088	2012/11/02	Sangga-ri, Aewol-eup, Jesu	Weedy var. <i>frutescens</i>	71
PF12-089	2012/11/02	Haga-ri, Aewol-eup, Jesu	Weedy var. <i>frutescens</i>	72
PF12-090	2012/11/02	Susan-ri, Aewol-eup, Jesu	Weedy var. <i>frutescens</i>	73
PF12-091	2012/11/02	Eomjang-ro, Aewol-eup, Jesu	Cultivated var. <i>frutescens</i>	5
PF12-092	2012/11/02	Jangjeon-ri, Aewol-eup, Jesu	Weedy var. <i>frutescens</i>	74
PF12-093	2012/11/02	Jangjeon-ri, Aewol-eup, Jesu	Weedy var. <i>frutescens</i>	75
PF12-094	2012/11/02	Jangjeon-ri, Aewol-eup, Jesu	Weedy var. <i>frutescens</i>	76
PF12-095	2012/11/02	Jangjeon-ri, Aewol-eup, Jesu	Weedy var. <i>frutescens</i>	77
PF12-096	2012/11/02	Jangjeon-ri, Aewol-eup, Jesu	Weedy var. <i>frutescens</i>	78
PF12-097	2012/11/02	Hagwi-ri, Aewol-eup, Jesu	Cultivated var. <i>frutescens</i>	6
PF12-098	2012/11/02	Hagwi-ri, Aewol-eup, Jesu	Weedy var. <i>frutescens</i>	79
PF12-099	2012/11/03	Gyora-ri, Jocheon-eup, Jesu	Weedy var. <i>crispa</i>	84
PF12-100	2012/11/03	Gyora-ri, Jocheon-eup, Jesu	Weedy var. <i>crispa</i>	85
PF12-101	2012/11/03	Gyora-ri, Jocheon-eup, Jesu	Weedy var. <i>frutescens</i>	80
PF12-102	2012/11/03	Waheul-ri, Jocheon-eup, Jesu	Weedy var. <i>frutescens</i>	81

²⁾This mark indicated the accessions used for morphological and simple sequence repeat analysis.

were then transplanted into the field in early June. In this study, four quantitative and nine qualitative characters were examined. Such were reportedly useful for the discrimination between *Perilla* crop and their weedy types (Lee and Ohnishi 2001). The plants were evaluated at the appropriate growth stages for each accession as described in detail in Table 2.

SSR analysis and silver-staining

SSR amplifications were conducted in a total volume of 20 µl consisting of 20 ng genomic DNA, 1× PCR buffer, 0.5 µM of forward and reverse primers, 0.2 mM dNTPs, and 1 unit of Taq polymerase (Biotools, Madrid, Spain). The PCR profile consisted of an initial denaturation at 95°C for 3 minutes, followed by 36 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute 30 seconds, with a final extension step of 5 minutes at 72°C. After PCR, 5 µl of the final products were mixed with 10 µl of electrophoresis loading buffer (98% formamide, 0.02% bromophenol blue, 0.02% Xylene C, and 5 mM NaOH). After denaturing and immediate cooling, 2 µl from each sample was loaded onto 6% denaturing (7.5 M urea) acrylamide- bisacrylamide gel (19:1) in 1× Tris-borate-EDTA (TBE) buffer and then electrophoresed at 1,800 V and 60 W for 130 minutes. The separated fragments were then visualized using a silver staining kit (Promega, Madison, WI, USA).

Data analysis

Fragments amplified by SSR primers were scored as present (1) or absent (0), and the genetic diversity was then calculated for each group of accessions according to the formula developed by Nei (1973): Genetic diversity = $1 - \sum P_i^2$, where P_i is the frequency of the i^{th} SSR allele present in the group of accessions. Anderson *et al.* (1993) measured genetic diversity in terms of polymorphic information content (PIC). The genetic similarities (GS) were calculated for each pair of accessions using the Dice similarity index (Dice 1945). The similarity matrix was used to construct an un-weighted pair group methods using arithmetic averages algorithm (UPGMA) dendrogram with the help of SAHN-Clustering by NTSYS-pc.V.2.1 (Rohlf 2000). In addition, a principal coordinate (PCO) analysis based on the GS matrices was carried out to estimate relationships among accessions of *Perilla* crop and their weedy types using the principal component analysis programs of the NTSYS-pc software package (Rohlf 2000).

RESULTS

Collection for cultivated and weedy types of *Perilla* crop in Jeju Island

Table 1 summarizes the result of the field survey in Jeju Island of Korea. The villages or towns visited are plotted on

Table 2. Characters used in the morphological analysis of *Perilla* crop.

Abbreviation ²⁾	Character	When/How measured	Category
QN1	Days from seeding to flowering	At flowering stage	Day
QN2	Plant height	At flowering stage	cm
QN3	Number of internodes	At flowering stage	Number
QN4	Length of the largest inflorescence	After harvest	cm
QL1	Seed color	After harvest	White-1, gray-2, brown-3, dark brown-4
QL2	Seed hardness	After harvest	Soft-1, hard-2
QL3	Color of leaf surface	At flowering stage	Green-1, green/purple-2, purple-3, deep purple-4
QL4	Color of reverse side of leaf	At flowering stage	Green-1, green/purple-2, purple-3, deep purple-4
QL5	Color of stem	At flowering stage	Green-1, green/purple-2, purple-3
QL6	Degree of pubescence	At flowering stage	Slightly pubescent-1, pubescent-2, heavily pubescent-3
QL7	Color of flower	At flowering stage	White-1, white/purple-2, purple-3
QL8	Shape of leaf	At flowering stage	Wrinkle-1, none wrinkle-2
QL9	Seed size	After harvest	Large (>2 mm)-1, small (<2 mm)-2

²⁾QN: quantitative, QL: qualitative.

Table 3. Morphological characterization of cultivated and weedy types of *Perilla* crop in Jeju Island of Korea.

Morphological character ²⁾	Var. <i>frutescens</i>		Var. <i>crispa</i>
	Cultivated type	Weedy type	Weedy type
QN1 (days from seeding to flowering)	137.8±6.7 (126.0-143.0)	134.6±2.7 (129.0-143.0)	137.8±3.3 (133.0-140.0)
QN2 (plant height)	151.7±14.2 (136.0-177.0)	163.1±15.6 (119.0-195.5)	159.3±8.3 (148.0-168.0)
QN3 (number of internodes)	15.8±1.3 (14.0-18.0)	17.1±1.3 (13.0-20.0)	16.3±1.0 (15.0-17.0)
QN4 (length of the largest inflorescence)	5.7±0.5 (5.0-6.0)	9.3±2.1 (5.0-15.5)	7.3±1.3 (6.0-9.0)
QL1 (seed color)	White (2), brown (4)	Gray (1), brown (1), dark brown (73)	Gray (2), dark brown (2)
QL2 (seed hardness)	Soft (1), hard (5)	Hard (75)	Hard (4)
QL3 (color of surface leaf)	Green (6)	Green (73), pale purple (2)	Green (2), purple (1), deep purple (1)
QL4 (color of reverse leaf)	Green (6)	Green (69), pale purple (6)	Purple (1), deep purple (3)
QL5 (color of stem)	Green (6)	Green (60), pale purple (15),	Pale purple (2), purple (2)
QL6 (degree of pubescence)	Pubescent (6)	Slightly pubescent (16), pubescent (55), heavily pubescent (4)	Slightly pubescent (4)
QL7 (flower color)	White (6)	White (68), purple (7)	Purple (4)
QL8 (leaf shape)	None wrinkle (6)	None wrinkle (75)	None wrinkle (3), wrinkle (1)
QL9 (seed size)	Large (6)	Small (75)	Small (4)

Values are presented as mean±standard deviation (range) or number only.

²⁾QN: quantitative, QL: qualitative.

a map in Fig. 1 which showed that the weedy type of var. *frutescens* was widely distributed in Jeju Island. The accessions of weedy type of var. *frutescens* were commonly found in such habitats as roadsides, wastelands, and the lands around farmer's fields and farmhouses. Meanwhile, the cultivated type of var. *frutescens* was almost not cultivated in there. Some farmers had grown them only a little. In addition, the cultivation of var. *crispa* was not seen anywhere the researchers visited, and even its weedy type was infrequently found as a relict form at roadsides, around a creek, at wastelands, and at the lands around farmhouses. On the interview with the farmers, the farmers said that in Jeju Island, sesame crop was cultivated more than *Perilla* crop in order to produce oil, and the seeds of var. *frutescens* were mainly used for the seasoning of flavoring agents like sesame seeds. The weedy type of var. *crispa* was often used for cooking as the leaves of var. *crispa* for a spice in a pepper pot soup. The total number of collections was 94 accessions which constituted of 6 cultivated var. *frutescens*, 84 weedy types of var. *frutescens*, and 4 weedy types of var. *crispa*, respectively (Table 1). A subset of

Fig. 2. Scatter diagram of 85 *Perilla* crop in Jeju Island based on principal component I (PC1) and II (PC2). ■: cultivated type of var. *frutescens*; ●: weedy type of var. *frutescens*; ▲: weedy type of var. *crispa*.

each collection was deposited in the National Agrobiodiversity Center, Rural Development and Administration, Jeonju, Jeollabuk-do, Republic of Korea, for permanent seed preservation.

Morphological variation for accessions of cultivated and weedy types of *Perilla* crop in Jeju Island

Table 3 shows the morphological features of these collections. The seed colors of the cultivated var. *frutescens* were white and brown, while the weedy type of var. *frutescens* showed gray, brown, and dark brown seed colors. The weedy type of var. *crispa* showed gray and dark brown seed colors. The most accessions of cultivated var. *frutescens* and weedy types of var. *frutescens* and var. *crispa* showed hard seeds, except one accession of

cultivated var. *frutescens* which had soft seeds. The degree of leaf pubescence was variable in each type; dense in the cultivated var. *frutescens*, slight or dense in weedy type of var. *frutescens*, and slight in weedy type of var. *crispa*. In addition, the color of stem and leaf was variable in each type; green in the cultivated var. *frutescens*, green or pale purple in weedy type of var. *frutescens*, purple or deep purple in weedy type of var. *crispa*. The days from seeding to flowering did not show any difference in the cultivated var. *frutescens* and weedy types of var. *frutescens* and var. *crispa*. In case of plant height and plant fragrance, most

Table 4. Characteristics of the 17 simple sequence repeat loci including repeat motif, allele size range, allele numbers and gene diversity among 85 accessions of cultivated and weedy types of *Perilla* crop in Jeju Island.

SSR loci	Primer sequence	Repeat motif	Allele size range (bp)	No. of alleles	MAF ²⁾	GD	PIC
KWPE-5	F - ATCTCCAAGCTTATGAATGC R - CTGGTAGTGAGCCTGTTCAT	[(ATG) ₆ (GA) ₅]	222-250	11	0.306	0.781	0.750
KWPE-19	F - CAACCCTTCACGATCACTAT R - AAATAACGGCCGATTCTAC	(ACG) ₇	226-249	12	0.612	0.597	0.575
KWPE-25	F - ACATTTAAGAGAGAGAGCAAG R - ACGAACGGGCTTCAATCTT	[(GT) ₈ (GA) ₁₄]	212-240	9	0.306	0.803	0.777
KWPE-26	F - GAGGCAATGCTGGTACTTC R - GAACGGGCTTCAATCTTC	[(AG) ₆ (AG) ₇ (GA) ₁₃]	240-280	7	0.318	0.766	0.728
KWPE-29	F - AAGACAAGGAGGAAGATGC R - ATAGGTGTTTCGCTCTCCTGTG	(GAA) ₅	217-236	10	0.224	0.839	0.819
KWPE-32	F - AGAACAACATTGTAGCTCGG R - ACGACCAACCAGTAGATGAT	(CCT) ₄	220-228	4	0.729	0.442	0.414
KWPE-39	F - AGAACAACATTGTAGCTCGG R - GACGAACCAGCAAACGAC	(CCT) ₄	198-230	5	0.765	0.396	0.374
KWPE-48	F - CACCCCATCTTTTGGAT R - AGCAGGATGGTGGTGGTC	(GA) ₉	214-220	5	0.471	0.659	0.600
KWPE-53	F - ACTCACCAGAAGAGAAGAAGA R - GCCACTGACCTGTTAATATCTG	(CT) ₁₆	187-215	14	0.200	0.891	0.880
KWPE-56	F - AAGCAGTGGACTGATTGTTT R - ACAAATCCAATTACTTTCTGC	(TG) ₉	92-98	5	0.694	0.479	0.441
KWPE-57	F - ATCACATCTCTCTTTCTGGA R - CCAGTCACTCCATCATCTCTA	(CT) ₁₆	150-210	17	0.176	0.890	0.880
KWPE-58	F - AGAGAGTTACCTGCGATTTTC R - CTTCAATATTCGGCCATCTT	(TG) ₉ -(GA) ₁₃	161-190	10	0.271	0.833	0.813
GBPFM-70	F - CCCTCCAAATCAATATTCCA R - TAGCTGCCATACGAACATGA	(ATTTG) ₃ , (AC) ₅	230-240	3	0.647	0.465	0.367
GBPFM-75	F - CATAGTTCATGGCTTCCACC R - CCTGAGCACAGAAACAGATCA	(CT) ₁₂	148-260	7	0.435	0.709	0.666
GBPFM-91	F - CCACTCAAATCCGCTTCTAA R - AATGTTGGTTGCGTTTCATT	(AG) ₉	223-235	4	0.765	0.375	0.327
GBPFM-111	F - ATCATGGATGAATCGCACTT R - CATTCTCCAAATGTTACTCTATT	(ACACA) ₈	160-195	5	0.729	0.444	0.418
GBPFM-203	F - GTTTTGTGTCAGCTCGATTT R - TGGGTTTGGAAAGTATTGATG	[(GA) ₅ TAA(AG) ₂₆]	147-225	21	0.388	0.817	0.806
Average				8.8	0.473	0.658	0.626

²⁾MAF: major allele frequency, GD: genetic diversity, PIC: polymorphic information content.

Fig. 3. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on the simple sequence repeat marker. ○ : accession of cultivated var. *frutescens*, ● : accession of weedy var. *frutescens*, ■ : accession of weedy var. *crispa*.

accessions of cultivated var. *frutescens* were 151.7 cm (136-177 cm) in average plant height, and a specific fragrance to the var. *frutescens*. Most accessions of weedy var. *frutescens* was 163.1 cm (119-195.5 cm) in average plant height and most accessions of weedy var. *crispa* was 159.3 cm (148-168 cm) in average plant height, and had weak to strong specific fragrance compared to the cultivated var. *frutescens*.

In the analysis of PCO, the results of PCO analysis indicated that the first and second principal components accounted for 25.7% and 21.1% of the total variation (Fig. 2), respectively. The scatterplot of the first two principal components is presented in Fig. 2.

SSR variation and genetic relationship for accessions of cultivated weedy types of *Perilla* crop in Jeju Island

The genetic variation at each SSR locus was measured in terms of the number of observed alleles, major allele frequency, gene diversity (GD), and PIC (Table 4). A total of 17 SSR loci showed polymorphism, producing a total of 149 alleles in 85 *Perilla* accessions selected among 94 accessions collected from Jeju Island of Korea. The loci varied in the number of observed alleles from 3 at GBPFM70 to 21 at GBPFM203, with an average of 8.8 alleles per locus. The frequency of major alleles per locus varied from 0.176 (KWPE57) to 0.765 (KWPE39, GBPFM91), with an average of 0.473. The GD of each locus ranged from 0.375 at GBPFM91 to 0.891 at KWPE53, with an average of 0.658. In addition, PIC values ranged from 0.327 (GBPFM91) to 0.880 (KWPE53, KWPE57), with an average of 0.626 (Table 4).

The phylogenetic tree constructed using UPGMA revealed that the 85 accessions cluster into five major groups with 29% GS (Fig. 3). Group I contained 11 accessions that consisted of 6 of the cultivated types of var. *frutescens*, 2 of the weedy types of var. *frutescens*, and three of the weedy types of var. *crispa*. Group II contained 62 accessions, 61 of the weedy types of var. *frutescens* and one accession of the weedy type of var. *crispa*. Groups III and IV contained 2 accessions of the weedy type of var. *frutescens*, respectively. Group V contained eight accessions of the weedy type of var. *frutescens*. The clustering patterns observed in the present study indicated a not clear

distinction between cultivated var. *frutescens* and the weedy types of var. *frutescens* and var. *crispa*. Although all accessions of cultivated var. *frutescens* were clearly separated in the three, most accessions of weedy types of var. *frutescens* and var. *crispa* did not form distinct groups.

DISCUSSION

Today, with the increase of meat consumption and the development of various cooking methods for fresh leaves and seeds, var. *frutescens* has become one of the most important vegetable and oil crops in Korea (Lee and Ohnishi 2001; Lee *et al.* 2002). However, according to the field survey in Jeju Island, most farmers did not cultivated *Perilla* crop in there, and only some farmers had grown a little. The interesting thing, however, was many accessions of the weedy type of var. *frutescens* in the field survey in Jeju Island were found. In there, the weedy var. *frutescens* was widely distributed, which was commonly found in such habitats as roadsides, creeks, wastelands, and lands around a farmer's fields and farmhouses. In most cases, plants of the weedy var. *frutescens* were recognized as an escaped form of var. *frutescens* by farmers. In general, weedy types have been reported in several crops; e.g., rice, barley, oat, and sorghum (Hancock 1992; Ladizinsky 1998). In these crops, the weedy types have been considered as either a wild ancestor of the crop or escaped form from cultivated crops. In the previous study by Lee and Ohnishi (2003) and Sa *et al.* (2013), it was suggested that the weedy types of *Perilla* crop in East Asia were the key taxon in understanding the origin of cultivated var. *frutescens* and var. *crispa*. Therefore, the accessions of weedy types of *Perilla* crop collected in Jeju Island were considered to be a highly valuable germplasm for genetic resource conservation and breeding research program in Korea.

In PCO analysis, most accessions of cultivated var. *frutescens* were clearly separated from weedy types of var. *frutescens* and var. *crispa*; that is, all accessions occupied the negative side below. Most accessions of weedy var. *frutescens* were situated on the negative and positive side, and all accessions of weedy var. *crispa* were situated on the

positive side. Additionally, five accessions of weedy var. *frutescens* and two accessions of weedy var. *crispa* were closely positioned to each other. The scattering pattern of accessions of var. *frutescens* in Fig. 2 agreed with the clustering observed on the dendrogram (Fig. 3). In the PCO analysis, several accessions of the weedy types of var. *frutescens* and var. *crispa* could not be clearly discriminated from each other. These results indicate that some accessions of the weedy types of *Perilla* crop might be derived from hybrids between cultivated and weedy types of *P. frutescens*, as in a previous report by Nitta and Ohnishi (1999), Lee *et al.* (2002), Lee and Ohnishi (2003), and Sa *et al.* (2013).

On understanding the genetic diversity between cultivated and weedy types of *Perilla* crop in Jeju Island, the number of observed alleles and GD for 6 accessions of cultivated var. *frutescens*, 75 accessions of weedy var. *frutescens*, and 4 accessions of weedy var. *crispa* were measured (Table 5). As a result, the average number of alleles for accessions of cultivated var. *frutescens*, weedy var. *frutescens*, and weedy var. *crispa* showed 2.1, 8.5, and

2.4 alleles, respectively. The average of GD for accessions of cultivated var. *frutescens*, weedy var. *frutescens*, and weedy var. *crispa* respectively showed 0.346, 0.649, and 0.463 (Table 5). This result indicated that the accessions of weedy types of var. *frutescens* and var. *crispa* comparatively exhibited higher genetic diversity than those of cultivated var. *frutescens*. Although the value of genetic diversity for *Perilla* germplasm collected in Jeju Island was slightly lowered compared to that of Korean and Japanese *Perilla* germplasm (Sa *et al.* 2013) and East Asian and other countries' *Perilla* germplasm (Sa *et al.* 2015), the value of the genetic diversity of accessions of weedy var. *frutescens* collected in Jeju Island showed similarly to those of Korean and Japanese *perilla* germplasm (Sa *et al.* 2013) and East Asian and other countries' *Perilla* germplasm (Sa *et al.* 2015). This result indicated that although cultivated *Perilla* crop almost could not be cultivated in there, the weedy type of var. *frutescens* was considered to be well-made in the genetic conservation in Jeju Island.

In a previous report by Lee and Ohnishi (2001, 2003),

Table 5. Estimates of genetic diversity and allele number of 17 SSR loci among cultivated and weedy types of *Perilla* crop in Jeju Island.

SSR ²⁾ locus	Number of alleles/Genetic diversity		
	Cultivated var. <i>frutescens</i> (n=6)	Weedy var. <i>frutescens</i> (n=75)	Weedy var. <i>crispa</i> (n=4)
KWPE5	3/0.500	10/0.762	3/0.625
KWPE19	3/0.611	11/0.609	1/0.000
KWPE25	1/0.000	9/0.821	3/0.625
KWPE26	3/0.500	6/0.767	3/0.625
KWPE29	2/0.444	10/0.820	2/0.375
KWPE32	2/0.444	4/0.325	3/0.625
KWPE39	1/0.000	5/0.438	1/0.000
KWPE48	2/0.278	5/0.674	2/0.375
KWPE53	4/0.722	14/0.879	3/0.625
KWPE56	2/0.278	5/0.497	2/0.375
KWPE57	2/0.444	17/0.898	3/0.625
KWPE58	2/0.444	10/0.843	3/0.625
GBPFM70	1/0.000	3/0.410	2/0.375
GBPFM75	3/0.611	7/0.686	3/0.625
GBPFM91	1/0.000	4/0.391	2/0.375
GBPFM111	1/0.000	5/0.420	3/0.625
GBPFM203	4/0.611	20/0.790	2/0.375
Average	2.1/0.346	8.5/0.649	2.4/0.463

²⁾SSR: simple sequence repeat.

the discrimination between the cultivated var. *frutescens* and cultivated var. *crispa* was possible by the morphological characters and by the AFLP markers. However, in this study, as shown in Fig. 3, six accessions of cultivated var. *frutescens* and two accessions of weedy var. *crispa* could not be separated by SSR markers. This result indicated that the weedy type of var. *crispa* might be derived from hybrids between cultivated and weedy types of *P. frutescens*, as previously proposed by Nitta and Ohnishi (1999), Lee *et al.* (2002), Lee and Ohnishi (2003), and Sa *et al.* (2013). In addition, the geographic locations of the samples collected from Jeju Island have no relation with their collected position in the phylogenetic tree. This implies that the diffusion of cultivated and weedy types of *Perilla* crop might happen through multiple routes in Jeju Island. A similar result was obtained with AFLP analyses for Korean *Perilla* accessions (Lee *et al.* 2002). The collection of germplasm resources is important in preventing the genetic erosion of the cultivated and weedy types of *Perilla* crop. In this study, the field survey and genetic variation for accessions of *Perilla* crop and their weedy types will be helpful to future studies of the breeding program and genetic resources conservation of *Perilla* crop in Jeju Island of Korea.

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REFERENCES

- Ajmone-Marsan P, Castiglioni P, Fusari F, Kuiper M, Motto M. 1998. Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. *Theor. Appl. Genet.* 96: 219-227.
- Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME. 1993. Optimizing parental selection for genetic linkage maps. *Genome* 36: 181-186.
- Choi YJ. 1984. Deulkkae (*Perilla frutescens* var. *frutescens*): story of folk custom in Korea. *New Hortic.* 26: 52-53.
- Dice LR. 1945. Measures of the amount of ecologic association between species. *Ecology* 26: 297-302.
- Hancock JF. 1992. Plant evolution and the origin of crop species. Prentice Hall, Englewood Cliffs, NJ.
- Kwon SJ, Lee JK, Kim NS, Yu JW, Dixit A, Cho EG, *et al.* 2005. Isolation and characterization of microsatellite markers in *Perilla frutescens* Britt. *Mol. Ecol. Notes* 5: 455-457.
- Ladizinsky G. 1998. Plant evolution under domestication. Chapman & Hall Published, Amsterdam.
- Lee JK. 2001. Genetic differentiation of *Perilla* crops and their weedy types in East Asia revealed by morphological and AFLP analyses [PhD thesis]. Kyoto, Kyoto University.
- Lee JK, Ohnishi O. 2001. Geographic differentiation of morphological characters among *Perilla* crops and their weedy types in East Asia. *Breed. Sci.* 51: 247-255.
- Lee JK, Nitta M, Kim NS, Park CH, Yoon KM, Shin YB, *et al.* 2002. Genetic diversity of *Perilla* and related weedy types in Korea determined by AFLP analyses. *Crop Sci.* 42: 2161-2166.
- Lee JK, Ohnishi O. 2003. Genetic relationships among cultivated types of *Perilla frutescens* and their weedy types in East Asia revealed by AFLP markers. *Genet. Resour. Crop Evol.* 50: 65-74.
- Lee JK, Kim NS. 2007. Genetic diversity and relationships of cultivated and weedy types of *Perilla frutescens* collected from East Asia revealed by microsatellite markers. *Korean J. Breed. Sci.* 39: 491-499.
- Lee JK, Kwon SJ, Park BJ, Kim MJ, Park YJ, Ma KH, *et al.* 2007. Analysis of genetic diversity and relationships of cultivated and weedy types of *Perilla frutescens* collected from Korea by using microsatellite markers. *Korean J. Genet.* 29: 81-89.
- Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. U.S.A.* 70: 3321-3323.
- Nitta M, Ohnishi O. 1999. Genetic relationships among two *Perilla* crops, shiso and egoma, and the weedy type revealed by RAPD markers. *Genes Genet. Syst.* 74: 43-48.
- Nitta M, Lee JK, Ohnishi O. 2003. Asian *Perilla* crops and

- their weedy forms: their cultivation, utilization and genetic relationships. *Econ. Bot.* 57: 245-253.
- Park YJ, Dixit A, Ma KH, Lee JK, Lee MH, Chung CS, *et al.* 2008. Evaluation of genetic diversity and relationships within an on-farm collection of *Perilla frutescens* (L.) Britt. using microsatellite markers. *Genet. Resour. Crop Evol.* 55: 523-535.
- Park YJ, Lee JK, Kim NS. 2009. Simple sequence repeat polymorphisms (SSRPs) for evaluation of molecular diversity and germplasm classification of minor crops. *Molecules* 14: 4546-4569.
- Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, *et al.* 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed.* 2: 225-238.
- Prasad M, Varshney RK, Roy JK, Balyan HS, Gupta PK. 2000. The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. *Theor. Appl. Genet.* 100: 584-592.
- Rohlf FJ. 2000. NTSYS- pc: Numerical taxonomy and multivariate analysis system. Version: 2.1. Exeter Software. New York, NY.
- Sa KJ, Park JY, Park KJ, Lee JK. 2010. Analysis of genetic diversity and relationships among waxy maize inbred lines in Korea using SSR markers. *Genes Genom.* 32: 375-384.
- Sa KJ, Choi SH, Ueno M, Park KC, Park YJ, Ma KH, *et al.* 2013. Identification of genetic variations of cultivated and weedy types of *Perilla* species in Korea and Japan using morphological and SSR markers. *Genes Genom.* 35: 649-659.
- Sa KJ, Choi SH, Ueno M, Lee JK. 2015. Genetic diversity and population structure in cultivated and weedy types of *Perilla* in East Asia and other countries as revealed by SSR markers. *Hortic. Environ. Biotechnol.* 56: 524-534.
- Senior ML, Murphy JP, Goodman MM, Stuber C. 1998. Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. *Crop Sci.* 38: 1088-1098.
- Xia XC, Reif JC, Melchinger AE, Frisch M, Hoisington DA, Beck D, *et al.* 2005. Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers: II. Subtropical, tropical midaltitude, and highland maize inbred lines and their relationships with elite U.S. and European maize. *Crop Sci.* 45: 2573-2582.