Genes & Genomics (2019) 41:267–281
https://doi.org/10.1007/s13258-018-0756-3

Online ISSN 2092-9293
Print ISSN 1976-9571

RESEARCH ARTICLE

Genetic diversity and population structure analysis in *Perilla* crop and their weedy types from northern and southern areas of China based on simple sequence repeat (SSRs)

Shi Jun Ma[1,2] · Kyu Jin Sa[1] · Tak-Ki Hong[1] · Ju Kyong Lee[1]

Received: 9 April 2018 / Accepted: 24 October 2018 / Published online: 14 November 2018

Abstract

Introduction Identification of genetic variation is an essential ability for the long-term success of breeding programs and maximizes the use of germplasm resources. In East Asia, China has a long history of the cultivation of *Perilla* crop, but there has been little research on the genetic diversity and genetic relationships among accessions of *Perilla* crop and their weedy types.

Objectives To better understand the genetic variations of the cultivated and weedy types of *Perilla* crop in China, the 91 accessions were evaluated for genetic diversity by 21 simple sequence repeat (SSR) markers.

Methods SSR amplifications were conducted in a total volume of 20 µL, consisting of 20 ng genomic DNA, 1X PCR buffer, 0.5 µM forward and reverse primers, 0.2 mM dNTPs, and 1 U Taq polymerase. Power Marker version 3.25 was applied to obtain the information on the number of alleles, allele frequency, major allele frequency, gene diversity (GD), and polymorphic information content (PIC). The similarity matrix was used to construct an unweighted pair group method with arithmetic mean dendrogram by the application of SAHN-Clustering from NTSYS-pc.V.2.1.

Results A total of 147 alleles were identified with an average of 7 alleles per locus. The average values of PIC and GD were 0.577 and 0.537, respectively. The genetic diversity level of accessions from Northern China was lower than accessions from Southern China. The genetic diversity level and PIC values for accessions of var. *crispa* were the highest. For accessions of cultivated var. *frutescens*, genetic diversity in Southern China was higher than that in Northern China.

Conclusion Most cultivated *Perilla* accessions were clearly separated from weedy *Perilla* accessions, but there was no clear geographic structure between cultivated *Perilla* crop and weedy types based on their regional distribution. This study demonstrated the utility of SSR analysis for performing genetic and population analysis of cultivated and weedy types of *Perilla* accessions in China.

Keywords *Perilla frutescens* · Genetic similarity · SSR marker · Geographical location · Polymorphic information content · Population structure

Introduction

Perilla frutescens Britt. (Labiatae) is a perennial crop that performs self-pollination and grows intensively in Himalayan mountain areas in Southeast Asia and East Asia. Recently, it has been introduced to Europe and North America due to its economic properties as an oil crop or a garden plant (Nitta et al. 2003). In East Asia, *Perilla* crop is cultivated in a large scale and used widely; therefore, East Asia is considered to be the birth place of *P. frutescens* (Makino 1961; Li 1969; Nitta 2001). China has been assumed to be the primary center of biodiversity for *Perilla* crop (Li 1969; Zeven and de-Wet 1982). Lee and Ohnishi (2001, 2003)

✉ Ju Kyong Lee
jukyonglee@kangwon.ac.kr

[1] Department of Applied Plant Sciences, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon 24341, South Korea

[2] School of Life Sciences, Shandong University, No.72 Binhai Road, Jimo District, Qingdao 266237, P.R. China

suggested that Korea is the secondary center of diversity of cultivated var. *frutescens* due to its large-scale cultivation, various usages, and higher level of morphological diversity.

Perilla crop has been divided into two cultivated types, *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa*, based on their morphology and use. *P. frutescens* var. *frutescens* is a kind of oil crop and called by different names in East Asian countries, such as Ren in China, Dlggae in Korea, and Egoma in Japan. It is also used as a leafy vegetable crop only in Korea, and its seeds were traditionally used in the same way with sesame seeds in China, Korea, and Japan from old times (Lee and Ohnishi 2001, 2003; Nitta et al. 2003). Meanwhile, *P. frutescens* var. *crispa* is a Chinese medicine crop that is called Cha-jo-ki in Korean, Shiso in Japan, and Zisu in China (Lee and Ohnishi 2001, 2003). It is also used as a spicy vegetable or pickle crop in Japan. Thus, these two cultivated types of *Perilla* crop have been important in East Asia from ancient times (Lee and Ohnishi 2003; Nitta et al. 2003).

In China, *Perilla* crop can be found in multiple provinces, such as Heilongjiang, Liaoning, Shanxi, Ningxia, Gansu, Anhui, Hubei, Sichuan, Taiwan, Hunan, Jiangxi, Jiangsu, Anhui, Henan, and Hulunbuir city of Inner Mongolia (Liu et al. 1996; Liu and Zhang 1998; Zhang et al. 2009; Hu et al. 2010). The main producing areas for *P. frutescens* var. *crispa* are concentrated in Guizhou and Sichuan provinces (Zhang et al. 2009). The weedy forms of the two cultivated types of *Perilla* crop are grown and commonly found in such habitats as roadsides, the edge of village and around farmers' fields or farmhouses. In northern areas of China, the cultivated area of *P. frutescens* var. *frutescens* is large in Changchun, Jilin, and Songyuan of Jilin province. Particularly, Yanbian area of Northeast China, inhabited by Chinese and Korean, var. *frutescens* is cultivated in a larger scale. There, the seeds of var. *frutescens* are used for flavoring in traditional foods, *Perilla* seed oil had been used for vegetable oil or industrial purpose, while cultivated and weedy types of var. *crispa* were found all over the provinces in South China. In South China, var. *crispa* is used majorly due to its medicinal function (Tan et al. 2012; Wang and Guo 2012; Wei et al. 2015). For instance, leaves of var. *crispa* exhibit the function of detoxification and have been used in cooking crab and fish for more than 2000 years (Yu et al. 2016). In addition, seeds and leaves of var. *crispa* have been considered effective in the treatment of cough, common cold, asthma, and digestive problem (Yu et al. 2016).

Taxonomic studies have underpinned the management of genetic resources in many aspects. Traditionally, the researches on plant taxonomy are conducted based on the comparative analysis in morphological traits. For example, morphological traits of *Perilla* accessions collected from East Asian areas were investigated by Lee and Ohnishi (2001) and Ma and Lee (2017) for the better understanding of morphological and geographical variation among different types of *Perilla* crop. They found many traits such as leaf size, seed size, plant height, branch number, flower, leaf and stem color, degree of pubescence, and plant fragrance that can be used for the discrimination of var. *frutescence* and var. *crispa*. Among these morphological traits, seed size can be used as the most reliable trait for separating cultivated type of var. *frutescence* from var. *crispa* and weedy var. *frutescence*. However, morphological analysis can be impacted by the environmental factors and provide the dubious results of taxonomy (Rao and Hodgkin 2002). Significant differences were not found between the cultivated and weedy var. *crispa* in morphological characters (Lee and Ohnishi 2001). Some accessions of var. *frutescens* performed similar qualitative traits (color of leaf, flower and stem) with var. *crispa* (Lee and Ohnishi 2001; Ma and Lee 2017), which created difficulties in the classification among *Perilla* accessions. The application of molecular technologies in taxonomic analysis has remedied the shortages of analysis based on morphological traits and has provided new insights into the phylogeny and taxonomy of many plant species. A number of excellent properties for molecular techniques have been demonstrated by Palmer et al. (1988). They reported that molecular techniques can reduce the impact of environment on the final data and analyze more characters than morphological analysis.

Therefore, various molecular markers have provided useful information regarding genetic diversity and genetic relationships in many crops (Senior et al. 1998; Nitta and Ohnishi 1999; Prasad et al. 2000; Lee et al. 2002; Hamza et al. 2004; Xia et al. 2005; Lee and Kim 2007; Ganapathy et al. 2011; Park et al. 2008, 2015, Sa et al. 2013, 2015; Zhang et al. 2016; Ma et al. 2017). Among them, random amplified polymorphic DNA or RAPD (Nitta and Ohnishi 1999), amplified fragment length polymorphism or AFLP (Lee et al. 2002; Lee and Ohnishi 2003), and simple sequence repeats or SSR (Lee and Kim 2007; Park et al. 2008, Sa et al. 2013, 2015; Ma et al. 2017) markers have been applied in the analysis of genetic diversity and relationships among cultivated and weedy types of *Perilla* crop in East Asia and other countries. Particularly, SSR markers are highly reproducible, polymorphic, generally codominant, and abundant in plant genomes (Powell et al. 1996; Park et al. 2009). As a result of these better features, SSRs have been used to establish genetic diversity and genetic relationships in many crop species (Prasad et al. 2000; Da Cunha et al. 2014; Yook et al. 2014; Park et al. 2015).

Identification of genetic variation is an essential ability for the long-term success of breeding programs and maximizes the use of germplasm resources (Xia et al. 2005; Park et al. 2008). In East Asia, China has a long history of the cultivation of *Perilla* crop, but there has been little research on the genetic diversity and genetic relationships among accessions of *Perilla* crop and their weedy types. Therefore,

in this study, we used 21 *Perilla* SSR primers in order to determine the genetic diversity, genetic relationships, and population structure of some collected *Perilla* accessions in the northern and southern areas of China.

Materials and methods

Plant materials

A total of 91 accessions (54 cultivated var. *frutescens*, 27 weedy var. *frutescens*, 4 cultivated var. *crispa*, and 6 weedy var. *crispa*) were collected from northern and southern areas of China and deposited in the National Agrobiodiversity Center, Rural Development and Administration, Jeonju, Republic of Korea, for permanent seed preservation. The number of accessions and the name of the collection place for each *Perilla* accessions have been shown in Table 1 and Fig. 1.

DNA extraction, SSR analysis, and silver staining

Genomic DNA was extracted from the young leaves for each *Perilla* accession at the seedling stage following the Plant DNAzol Reagent protocol (GibcoBRL Inc., Grand Island, NY, USA). The *Perilla* SSR primers used in our experiments were developed and used by Kwon et al. (2005), Park et al. (2008), Song et al. (2012), and Sa et al. (2013, 2015). SSR amplifications were conducted in a total volume of 20 µL, consisting of 20 ng genomic DNA, 1X PCR buffer, 0.5 µM forward and reverse primers, 0.2 mM dNTPs, and 1 U Taq polymerase (Biotools, Spain). The PCR profile consisted of initial denaturation at 95 °C for 3 min, followed by 36 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min 30 s, with a final extension step of 5 min at 72 °C. After PCR, 5 µL of the final products were mixed with 10 µL electrophoresis loading buffer (98% formamide, 0.02% BPH, 0.02% Xylene C, and 5 mM NaOH). After denaturing and quick cooling, 2 µL of each sample was loaded onto 6% denaturing (7.5 M urea) acrylamide-bisacrylamide gel (19:1) in 1X TBE buffer and then electrophoresed at 1800 V and 60 W for 130 min. The separated fragments were then visualized through a silver staining kit (Promega, Madison, WI, USA).

Data analysis

Fragments amplified using the SSR primers were scored as present (1) or absent (0). Power Marker version 3.25 (Liu and Muse 2005) was applied to obtain the information on the number of alleles, allele frequency, major allele frequency (MAF), gene diversity (GD), and polymorphic information content (PIC). Genetic similarities were calculated for each pair of lines using the Dice similarity index (Dice 1945). To illustrate the genetic relationship of samples, the similarity matrix was then used to construct an unweighted pair group method with arithmetic mean (UPGMA) dendrogram by the application of SAHN-Clustering from NTSYS-pc.V.2.1 (Rohlf 1998). The STRUCTURE 2.2 software (Pritchard and Wen 2003) was used for the investigation of a population structure for 91 accessions of *Perilla* crop. To identify the number of clusters (K), the software was run using admixture model with correlated allele frequencies following Hardy–Weinberg equilibrium. Five independent runs were performed for each simulated value of K = 1–10, with a burn-in period of 100,000 runs followed by 100,000 Monte Carlo Markov Chain (MCMC) replications. The real number of population was detected by plotting Ln (PD) derived ΔK, an ad hoc quantity against each K (Evanno et al. 2005). The result was computed and visualized using the Structure Harvester software (http://taylor0.biology.ucla.edu/struct_harvest/).

Results

SSR polymorphism and genetic variation between *Perilla* accessions from northern and southern areas of China

The 21 SSR primer pairs with polymorphism were applied for final detection in this study. The information on primer pairs, allele size range, number of alleles, MAF, and values of GD and PIC was shown in Table 2. A total of 147 alleles were produced among 91 *Perilla* accessions. The average number of alleles revealed by each primer pair was 7, varying from 3 for GBPF201 and KWPE56 to 12 for KWPE57. The MAF for each SSR marker ranged from 0.228 at GBPF135 to 0.957 t GBPF201, with the average value of 0.549. The values of GD ranged from 0.084 revealed by GBPF201 to 0.828 revealed by KWPE57 with the average value of 0.577. Several higher values of genetic diversity were also observed at KWPE53 (0.783), KWPE19 (0.776), GBPF203 (0.741), GBPF135 (0.827), GBPF204 (0.804), and GBPF111 (0.762), respectively. Allelic diversity and frequency among these *Perilla* accessions could be reflected by the PIC values, which ranged from 0.082 at GBPF201 to 0.808 at KWPE57, with an average value of 0.537. 14 markers—GBPF179, GBPF135, KWPE25, KWPE26, GBPF203, KWPE39, KWPE57, KWPE53, KWPE58, KWPE19, KWPE29, GBPF111, GBPF155, and GBPF204—exhibited PIC values higher than 0.5.

To compare the genetic diversity of *Perilla* accessions from Northern China (45 accessions) and Southern China (46 accessions), the number of alleles, values of genetic diversity, and polymorphic information content

Table 1 *Perilla* accessions from different regions of China used for SSR analysis

Code no.	Accession no.	City and province	Country	Type
1	CH3	Harbin, Hei Longjiang	CHN	Cultivated type of var. *frutescens*
2	CH35	Hailin, Hei Longjiang	CHN	Cultivated type of var. *frutescens*
3	CH38	Jiamusi, Hei Longjiang	CHN	Cultivated type of var. *frutescens*
4	CH46	Heihe, Hei Longjiang	CHN	Cultivated type of var. *frutescens*
5	CH62	Helong, Jilin	CHN	Cultivated type of var. *frutescens*
6	CH64	Helong, Jilin	CHN	Cultivated type of var. *frutescens*
7	CH65	Helong, Jilin	CHN	Cultivated type of var. *frutescens*
8	CH66	Helong, Jilin	CHN	Cultivated type of var. *frutescens*
9	CH72	Yanji, Jilin	CHN	Cultivated type of var. *frutescens*
10	CH75	Yanji, Jilin	CHN	Cultivated type of var. *frutescens*
11	CH79	Yanji, Jilin	CHN	Cultivated type of var. *frutescens*
12	CH45	Yanji, Jilin	CHN	Cultivated type of var. *frutescens*
13	CH82	Longjing, Jilin	CHN	Cultivated type of var. *frutescens*
14	CH83	Longjing, Jilin	CHN	Cultivated type of var. *frutescens*
15	CH16	Longjing, Jilin	CHN	Cultivated type of var. *frutescens*
16	CH23	Longjing, Jilin	CHN	Cultivated type of var. *frutescens*
17	CH24	Baishan, Jilin	CHN	Cultivated type of var. *frutescens*
18	CH32	Changbai, Jilin	CHN	Cultivated type of var. *frutescens*
19	CH33	Tonghua, Jilin	CHN	Cultivated type of var. *frutescens*
20	CH14	Siping, Jilin	CHN	Cultivated type of var. *frutescens*
21	CH42	Jilin, Jilin	CHN	Cultivated type of var. *frutescens*
22	CH5	Shenyang, Liaoning	CHN	Cultivated type of var. *frutescens*
23	CH8	Liaoyang, Liaoning	CHN	Cultivated type of var. *frutescens*
24	CH34	Zhengzhou, Henan	CHN	Cultivated type of var. *frutescens*
25	CH37	Tianshui, Gansu	CHN	Cultivated type of var. *frutescens*
26	CH30	Tianshui, Gansu	CHN	Cultivated type of var. *frutescens*
27	CH39	Tianshui, Gansu	CHN	Cultivated type of var. *frutescens*
28	CH29	Longnan, Gansu	CHN	Cultivated type of var. *frutescens*
29	CH51	Qingyang, Gansu	CHN	Cultivated type of var. *frutescens*
30	CH44	Haozhou, Anhui	CHN	Cultivated type of var. *frutescens*
31	CH50	Suqian, Jiangsu	CHN	Cultivated type of var. *frutescens*
32	CH31	Huai'an, Jiangsu	CHN	Cultivated type of var. *frutescens*
33	CH12	Changchun, Jilin	CHN	Cultivated type of var. *frutescens*
34	CH6	Shenyang, Liaoning	CHN	Cultivated type of var. *frutescens*
35	CH10	Anguo, Hebei	CHN	Cultivated type of var. *frutescens*
36	CH48	Tianshui, Gansu	CHN	Cultivated type of var. *frutescens*
37	CH9	Cangzhou, Hebei	CHN	Cultivated type of var. *frutescens*
38	CH25	Weifang, Shandong	CHN	Cultivated type of var. *frutescens*
39	CH26	Jilin, Jilin	CHN	Cultivated type of var. *frutescens*
40	CH36	Zhengzhou, Henan	CHN	Cultivated type of var. *frutescens*
41	CH47	Baoding, Hebei	CHN	Cultivated type of var. *frutescens*
42	CH52	Yantai, Shandong	CHN	Cultivated type of var. *crispa*
43	CH2	Harbin, Hei Longjiang	CHN	Cultivated type of var. *crispa*
44	CH54	Zhaoyuan, Shandong	CHN	Cultivated type of var. *crispa*
45	CH53	Zhaoyuan, Shandong	CHN	Cultivated type of var. *crispa*
46	CSY4	Chuxiong, Cangling	CHN	Cultivated type of var. *frutescens*
47	CSY18	Dali, Yangbi, Huan'an cun	CHN	Cultivated type of var. *frutescens*
48	CSY21	Dali, Eryuan, Fengyuzhen	CHN	Cultivated type of var. *frutescens*
49	CSY22	Dali, Eryuan, Fengyuzhen	CHN	Cultivated type of var. *frutescens*
50	CSY31	Lijiang, Baishazhen	CHN	Cultivated type of var. *frutescens*

Table 1 (continued)

Code no.	Accession no.	City and province	Country	Type
51	CSY33	Lijiang, Baishazhen	CHN	Cultivated type of var. *frutescens*
52	CSY34	Lijiang, Shiguzhen	CHN	Cultivated type of var. *frutescens*
53	CSY35	Lijiang, Jinanxiang	CHN	Cultivated type of var. *frutescens*
54	CSY36	Lijiang, Jinanxiang	CHN	Cultivated type of var. *frutescens*
55	CSY37	Lijiang, Yongshengxian	CHN	Cultivated type of var. *frutescens*
56	CSY38	Lijiang, Yongshengxian	CHN	Cultivated type of var. *frutescens*
57	CSY39	Lijiang, Yongshengxian	CHN	Cultivated type of var. *frutescens*
58	CSY40	Lijiang, Yongshengxian	CHN	Cultivated type of var. *frutescens*
59	CSY1	An'ning-si, Qinglong	CHN	Weedy type of var. *frutescens*
60	CSY6	Dali, Yangbi, Pingpo zhen	CHN	Weedy type of var. *frutescens*
61	CSY9	Dali, Yangbi, Pingpo zhen	CHN	Weedy type of var. *frutescens*
62	CSY10	Dali, Yangbi, Xi Menguan	CHN	Weedy type of var. *frutescens*
63	CSY11	Dali, Yangbi, Xi Menguan	CHN	Weedy type of var. *frutescens*
64	CSY12	Dali, Yangbi, Xi Menguan	CHN	Weedy type of var. *frutescens*
65	CSY13	Dali, Yangbi, He Xixiang	CHN	Weedy type of var. *frutescens*
66	CSY14	Dali, Yangbi, He Xixiang, Machang	CHN	Weedy type of var. *frutescens*
67	CSY15	Dali, Yangbi, He Xixiang, Machang	CHN	Weedy type of var. *frutescens*
68	CSY19	Dali, Yangbi, Jin Xing cun	CHN	Weedy type of var. *frutescens*
69	CSY24	Dali, Eryuan, Niujiexiang	CHN	Weedy type of var. *frutescens*
70	CSY25	Dali, Eryuan, Niujiexiang	CHN	Weedy type of var. *frutescens*
71	CSY28	Dali, Jianchuan, Shaxizhen	CHN	Weedy type of var. *frutescens*
72	CSY32	Lijiang, Baishazhen	CHN	Weedy type of var. *frutescens*
73	CSY41	Lijiang, Yongshengxian, Qinazhen	CHN	Weedy type of var. *frutescens*
74	CSY42	Lijiang, Yongshengxian, Qinazhen	CHN	Weedy type of var. *frutescens*
75	CSY44	Dali, Binchuanxian, Taihecun	CHN	Weedy type of var. *frutescens*
76	CSY45	Dali, Binchuanxian, Daluocheng	CHN	Weedy type of var. *frutescens*
77	CSY46	Guizhou Province, Tongren, Jiangkouxian	CHN	Weedy type of var. *frutescens*
78	CSY3	Chuxiong, Guangtong	CHN	Weedy type of var. *frutescens*
79	CSY26	Dali, Jianchuan, Shaxizhen	CHN	Weedy type of var. *frutescens*
80	CSY27	Dali, Jianchuan, Shaxizhen	CHN	Weedy type of var. *frutescens*
81	CSY29	Dali, Jianchuan, Shaxizhen	CHN	Weedy type of var. *frutescens*
82	CSY30	Dali, Jianchuan, Shaxizhen	CHN	Weedy type of var. *frutescens*
83	CSY2	Chuxiong, Jiuzhuang	CHN	Weedy type of var. *frutescens*
84	CSY5	Dali, Yangbi, Pingpo zhen	CHN	Weedy type of var. *frutescens*
85	CSY20	Dali, WanqiaoZhen, Zhongzhuang cun	CHN	Weedy type of var. *frutescens*
86	CSY7	Dali, Yangbi, Pingpo zhen	CHN	Weedy type of var. *crispa*
87	CSY8	Dali, Yangbi, Pingpo zhen	CHN	Weedy type of var. *crispa*
88	CSY16	Dali, Yangbi, He Hou cun	CHN	Weedy type of var. *crispa*
89	CSY17	Dali, Yangbi, He Hou cun	CHN	Weedy type of var. *crispa*
90	CSY23	Dali, Eryuan, Bihuzhen, Xiaonanshancun	CHN	Weedy type of var. *crispa*
91	CSY48	Jinhua, Zhejiang	CHN	Weedy type of var. *crispa*

CH: Perilla from northern areas of China, 1–45; CSY: Perilla from southern areas of China, 46–91

of *Perilla* accessions from Northern and Southern China were detected respectively and summarized in Table 3. The average numbers of alleles per locus were 5.29 and 5.81 for *Perilla* accessions from northern and southern areas of China, respectively. The average values of GD were 0.462 and 0.566 for *Perilla* accessions from northern and southern areas of China, respectively. The average values for PIC were 0.432 and 0.526 for *Perilla* accessions from northern and southern areas of China, respectively. Obviously, the genetic diversity level of *Perilla*

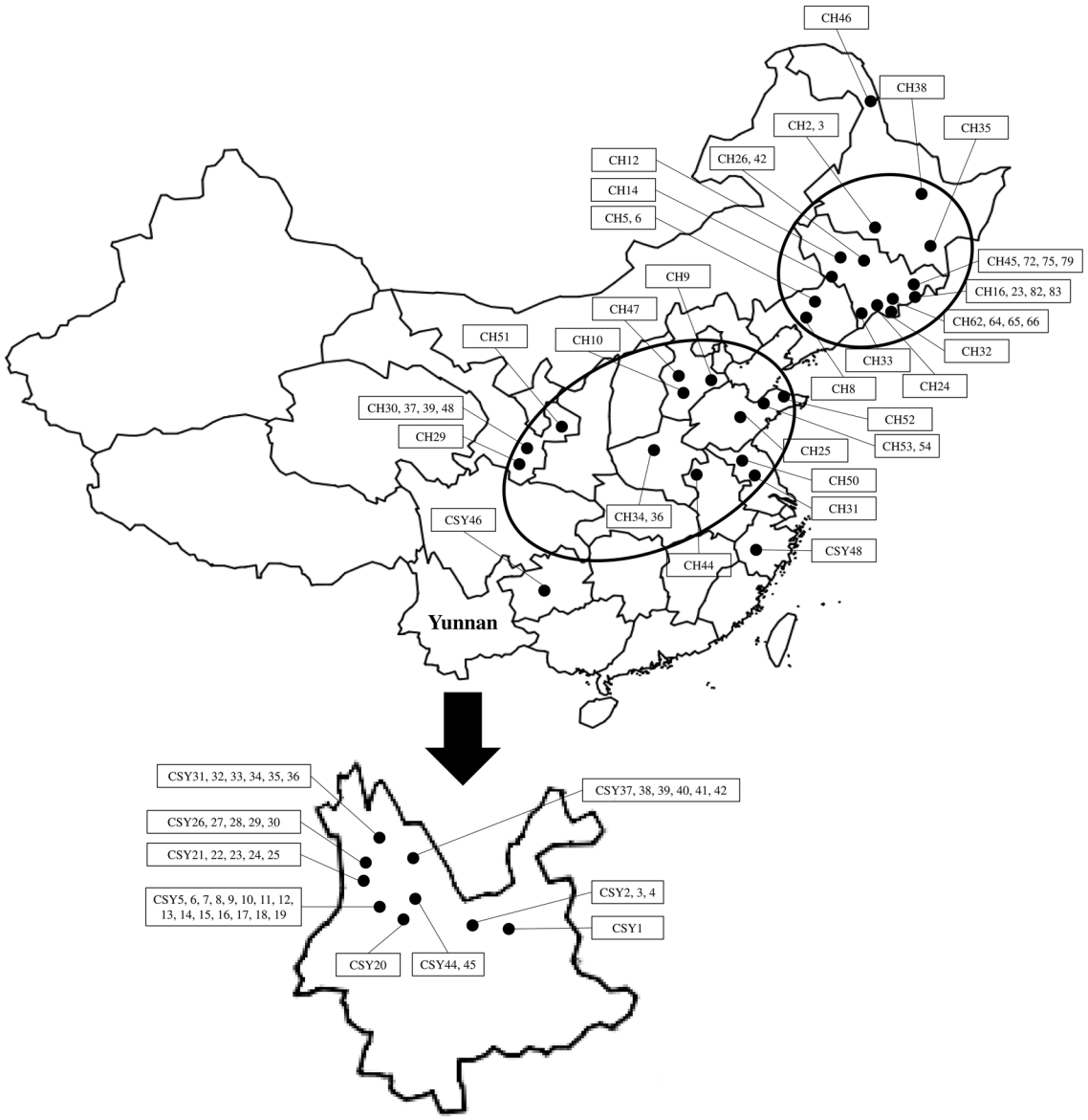


Fig. 1 Collecting sites of 91 *Perilla* accessions collected from Northern and Southern China. The two circles receptively showed the *Perilla* accessions collected in the northern (upper) and southern (lower) regions of China. The small map shown by the arrow is an enlargement of the Yunnan Province area in China

accessions from southern areas of China was higher than those accessions from northern areas of China. As shown in Table 4, average numbers of alleles per loci were 4.81 and 4.10 for accessions of cultivated var. *frutescens* from northern and southern areas of China, respectively. The average values of GD were 0.400 and 0.532 for accessions of cultivated var. *frutescens* from northern and southern areas of China, respectively. The average values for PIC were 0.374 and 0.490 for accessions of cultivated var. *frutescens* from northern and southern areas of China, respectively. This indicated that the genetic diversity for accessions of cultivated var. *frutescens* in Southern China was higher than that in Northern China. In Table 5, the average values for the number of alleles, GD, and PIC for accessions of cultivated var. *frutescens* were 6, 0.467, and 0.439, respectively. The average values for the number of alleles, GD, and PIC for accessions of weedy var. *frutescens* were 4.71, 0.500, and 0.460, respectively. The average values for the number of alleles, GD, and PIC for accessions of var. *crispa* were 4.05, 0.567, and 0.524,

Table 2 Characteristics of the 21 SSR loci including primer sequence, repeat motif, allele size range, no. of alleles, gene diversity, and polymorphic information content among 91 accessions from northern and southern areas of China

SSR primer	Primer sequence	Repeat motif	Allele size (bp)	No. of alleles	MAF	GD	PIC
GBPF201	F-AGACTCGTTTCACAATTTCTCC R-CATTCCCACCTCATGTTACG	$(GA)_6(GT)\ (GA)_5$	153–155	3	0.957	0.084	0.082
GBPF179	F-TGAATCATCCCAAACGAGAT R-TCGCTTCTCTCTCATGGATT	$(TGA)_5$	172–184	5	0.511	0.641	0.586
GBPF172	F-ATCGGTCTTTGAAATCACCA R-TGAAATTTCTTGCCGTTACC	$(GA)_{11}$	280–290	4	0.674	0.453	0.369
GBPF135	F-CTTCTGAGGCCAACATTGAG R-AGGGCTCGGTTGAATCTTAC	$(CT)_{20}$	230–260	9	0.228	0.827	0.804
GBPF75	F-CATAGTTCATGGCTTCCACC R-CCTGAGCACAGAAACAGATCA	$(CT)_{12}$	147–160	7	0.772	0.391	0.375
GBPF70	F-CCCTCCAAATCAATATTCCA R-TAGCTGCCATACGAACATGA	$(ATTTG)_3$, $(AC)_5$	236–250	4	0.707	0.446	0.390
KWPE25	F-ACATTTAAGAGAGAGAGCAAG R-ACGAACGGGCTTCAATCTT	$[(GT)_8(GA)_{14}]$	212–225	6	0.511	0.628	0.567
KWPE26	F-GAGGCAATGCTGGTACTTC R-GAACGGGCTTCAATCTTC	$[(AG)_6(AG)_7(GA)_{13}]$	246–260	6	0.500	0.650	0.596
GBPF203	F-GTTTTGTTGCAGCTCGATTT R-TGGGTTTGGAAAGTATTGATG	[(GA)5TAA(AG)26]	132–181	10	0.380	0.741	0.703
KWPE39	F-AGAACAACATTGTAGCTCGG R-GACGAACCAGCAAACGAC	$(CCT)_4$	242–263	5	0.489	0.586	0.501
KWPE48	F-CACCCCATCTTTTTGGAT R-AGCAGGATGGTGGTGGTC	$(GA)_9$	212–219	4	0.826	0.299	0.275
KWPE57	F-ATCACATCTCTCTCTTTCTGGA R-CCAGTCACTCCATCATCTCTA	$(CT)_{16}$	156–203	12	0.272	0.828	0.808
KWPE56	F-AAGCAGTGGACTGATTGTTT R-ACAAAATCCAATTACTTTCTGC	$(TG)_9$	105–107	3	0.630	0.474	0.371
KWPE53	F-ACTCACCAGAAGAGAAGAAGA R-GCCACTGACCTGTTAATATCTG	$(CT)_{16}$	153–169	10	0.348	0.783	0.754
KWPE58	F-AGAGAGTTACCTGCGATTTTC R-CTTCAATATTCGGCCATCTT	$(TG)_9$-$(AG)_{12}$	165–175	7	0.652	0.546	0.521
KWPE19	F-CAACCCTTCACGATCACTAT R-AAATAACGGCCGATTCTAC	$(ACG)_7$	226–270	10	0.370	0.776	0.747
KWPE29	F-AAGACAAGGAGGAAGATGC R-ATAGGTGTTCGCTCTCCTGTG	$(GAA)_5$	220–247	9	0.489	0.665	0.618
GBPF111	F-ATCATGGATGAATCGCACTT R-CATTCTCCAAATGTTACTCTATTT	$(ACACA)_8$	155–200	10	0.391	0.762	0.732
GBPF155	F-TTTGTGACAATACGCACCAC R-CCAATTGCTCAATGCTCTCT	$(GAA)_{10}$	290–320	8	0.587	0.619	0.593
GBPF204	F-TCGAAAAATTGCAGATCACC R-TTGTCTTTTGCCTCTTTTGC	$(AG)_{17}$	130–156	11	0.293	0.804	0.780
GBPF91	F-CCACTCAAATCCGCTTCTAA R-AATGTTGGTTGCGTTTCATT	$(AG)_9$	224–235	4	0.946	0.104	0.102
Average				7	0.549	0.577	0.537

MAF major allele frequency, *GD* genetic diversity, *PIC* polymorphic information content

respectively. That implied that accessions of var. *crispa* performed the highest level of genetic diversity among different types of *Perilla* accessions in China.

Genetic relationships among accessions of *Perilla* crop from different regions of China

The phylogenetic tree was constructed using UPGMA, and 91 *Perilla* accessions were revealed to have clustered into

Table 3 No. of alleles, genetic diversity, and polymorphic information content (PIC) obtained from each SSR locus in *Perilla* accessions from northern and southern areas of China

Markers	Accessions of Northern China ($n=45$)			Accessions of Southern China ($n=46$)		
	No. of alleles	GD	PIC	No. of alleles	GD	PIC
GBPF201	3	0.124	0.120	3	0.082	0.081
GBPF179	5	0.705	0.654	4	0.553	0.501
GBPF172	3	0.396	0.333	4	0.513	0.420
GBPF135	6	0.745	0.711	9	0.827	0.804
GBPF75	4	0.305	0.288	6	0.474	0.440
GBPF70	4	0.300	0.275	4	0.560	0.487
KWPE25	4	0.305	0.288	6	0.661	0.617
KWPE26	5	0.340	0.324	5	0.693	0.649
GBPF203	4	0.464	0.413	9	0.630	0.603
KWPE39	3	0.373	0.318	5	0.585	0.541
KWPE48	4	0.336	0.315	3	0.289	0.258
KWPE57	9	0.803	0.779	10	0.802	0.776
KWPE56	3	0.502	0.396	3	0.463	0.374
KWPE53	7	0.692	0.661	7	0.694	0.659
KWPE58	6	0.310	0.300	7	0.704	0.667
KWPE19	8	0.715	0.686	8	0.735	0.691
KWPE29	8	0.693	0.662	6	0.368	0.352
GBPF111	7	0.410	0.398	6	0.720	0.678
GBPF155	5	0.202	0.197	6	0.791	0.759
GBPF204	9	0.819	0.797	8	0.663	0.621
GBPFM91	4	0.164	0.159	3	0.082	0.081
Mean	5.29	0.462	0.432	5.81	0.566	0.526

GD genetic diversity, *PIC* polymorphic information content

3 major groups (Group I, Group II, and Group III) with a genetic similarity of 38% (Fig. 2). Group I could be further divided into four subgroups under a genetic similarity of 46%. Group I-1 contained 36 accessions of cultivated var. *frutescens* from Northern China and 2 accessions (CSY21 and CSY22) of cultivated var. *frutescens* from Southern China. Group I-2 included seven cultivated accessions of var. *frutescens* and one accession of weedy var. *frutescens* from Southern China. Group I-3 consisted of two accessions of cultivated var. *frutescens* from Northern China. Group I-4 was comprised of three accessions of cultivated var. *frutescens* from Northern China and one accession of cultivated var. *crispa* (CH53) from Northern China. Group II was further classified into four subgroups under a genetic similarity of 43%. Group II-1 contained 21 accessions of weedy var. *frutescens*, 5 accessions of weedy var. *crispa*, and 1 accession (CSY4) of cultivated var. *frutescens* from Southern China. Group II-2 included 3 accessions of cultivated var. *frutescens* and 2 accessions of weedy var. *frutescens* from Southern China. Group II-3 consisted of 2 accessions (CSY2 and CSY3) of weedy var. *frutescens*. Group II-4 was composed of one accession (CSY46) of weedy var. *frutescens* from Southern China. Group III was comprised of three accessions of cultivated var. *crispa* from Northern China and one weedy accession (CSY48) of var. *crispa* from Southern China.

In our analysis, most *Perilla* accessions collected in Northern China were clearly distinguished from accessions collected in Southern China. For accessions from Northern China, all accessions of cultivated var. *frutescens* were clearly discriminated from the cultivated var. *crispa*, except for one accession (CH53) which was located in the group of cultivated var. *frutescens* in the phylogenetic tree (Fig. 2). Furthermore, in the case of accessions from Southern China, most accessions of cultivated var. *frutescens* was differentiated from weedy accessions of var. *frutescens*, but most accessions of weedy var. *crispa* were mixed with weedy var. *frutescens*.

Population structure

To identify the genetic structure among the 91 *Perilla* accessions from northern and southern areas of China, a model-based approach in the STRUCTURE software was employed to subdivide each accession into its corresponding subgroups. The ad hoc measure ΔK following the method demonstrated by Evanno et al. (2005) was applied to overcome the difficulty in interpreting the real K values. The

Table 4 No. of alleles, genetic diversity, and polymorphic information content (PIC) obtained from each SSR locus in accessions of cultivated var. *frutescens* from northern and southern areas of China

Markers	Accessions of cultivated var. *frutescens* from Northern China ($n=41$)			Accessions of cultivated var. *frutescens* from Southern China ($n=13$)		
	No. of alleles	GD	PIC	No. of alleles	GD	PIC
GBPF201	3	0.135	0.130	1	0.000	0.000
GBPF179	5	0.679	0.623	4	0.643	0.585
GBPF172	3	0.316	0.279	3	0.439	0.386
GBPF135	6	0.724	0.692	4	0.531	0.483
GBPF75	3	0.176	0.166	4	0.622	0.547
GBPF70	3	0.176	0.166	3	0.255	0.240
KWPE25	3	0.214	0.199	5	0.673	0.632
KWPE26	4	0.255	0.240	5	0.724	0.685
GBPF203	4	0.408	0.357	8	0.827	0.806
KWPE39	3	0.285	0.255	3	0.500	0.427
KWPE48	4	0.218	0.208	1	0.000	0.000
KWPE57	9	0.778	0.749	7	0.745	0.716
KWPE56	3	0.509	0.402	3	0.561	0.465
KWPE53	7	0.655	0.627	5	0.653	0.602
KWPE58	5	0.220	0.214	6	0.765	0.731
KWPE19	8	0.702	0.676	5	0.704	0.657
KWPE29	8	0.641	0.603	4	0.653	0.599
GBPF111	5	0.297	0.285	5	0.551	0.521
GBPF155	5	0.220	0.214	4	0.704	0.646
GBPF204	9	0.799	0.774	5	0.612	0.571
GBPFM91	1	0.000	0.000	1	0.000	0.000
Mean	4.81	0.400	0.374	4.10	0.532	0.490

GD genetic diversity, *PIC* polymorphic information content

highest value of ΔK for the 91 *Perilla* accessions was for K = 2 (Fig. 3).

Clustering bar plots with K = 2 are shown in Fig. 4. At K = 2, all 91 *Perilla* accessions were divided into Group I, Group II, and an admixed group. There was no clear geographic structure among the 91 *Perilla* accessions from southern and northern areas of China, which has been revealed in the analysis of UPGMA (Figs. 2, 4). Therefore, we conducted an analysis following the method of Wang et al. (2008) according to a membership probability threshold of 0.8. The result showed that the 91 *Perilla* accessions were classified into Group I, Group II, and an admixed group. Group I included 32 accessions (CH3, CH35, CH38, CH46, CH62, CH64, CH65, CH66, CH72, CH75, CH79, CH45, CH82, CH83, CH16, CH23, CH32, CH33, CH14, CH42, CH5, CH8, CH34, CH37, CH30, CH39, CH50, CH31, CH6, CH48, CH26, and CH47) of cultivated var. *frutescens* from Northern China and 5 accessions (CSY18, CSY21, CSY22, CSY31, and CSY40) of cultivated var. *frutescens* from Southern China. Group II included 3 accessions (CH52, CH2, and CH54) of cultivated var. *crispa* from Northern China, 1 accession (CSY39) of cultivated var. *frutescens*, 26 accessions (CSY6, CSY9, CSY10, CSY11, CSY12, CSY13, CSY14, CSY15, CSY19, CSY24, CSY25, CSY28, CSY32, CSY41, CSY42, CSY44, CSY45, CSY46, CSY3, CSY26, CSY27, CSY29, CSY30, CSY2, CSY5, and CSY20) of weedy var. *frutescens*, and 6 accessions (CSY7, CSY8, CSY16, CSY17, CSY23, and CSY48) of weedy var. *crispa* from Southern China. The admixed group is comprised of nine accessions (CH24, CH29, CH51, CH44, CH12, CH10, CH9, CH25, and CH36) of cultivated var. *frutescens* and one accession (CH53) of cultivated var. *crispa* from Northern China, seven accessions (CSY4, CSY33, CSY34, CSY35, CSY36, CSY37, and CSY38) of cultivated var. *frutescens* and one accession (CSY1) of weedy var. *frutescens* from Southern China.

Discussion

Investigation of the genetic diversity among landraces or cultivars and their geographical distribution of a given crop are one of the basic approaches to the identification of the origin and of varietal differentiation of the crop. In East Asia, China is considered to be one center of diversity of *Perilla* crop, however, by this time, there is very little study for genetic diversity and genetic relationships on *Perilla*

Table 5 No. of alleles, genetic diversity, and polymorphic information content (PIC) obtained from each SSR locus in accessions of *Perilla* crop and their weedy types from China

Markers	Cultivated var. *frutescens* (*n* = 54)			Weedy var. *frutescens* (*n* = 27)			Cultivated and weedy var. *crispa* (*n* = 10)		
	No. of alleles	GD	PIC	No. of alleles	GD	PIC	No. of alleles	GD	PIC
GBPF201	3	0.104	0.102	3	0.135	0.131	1	0.000	0.000
GBPF179	5	0.686	0.627	4	0.360	0.331	5	0.711	0.672
GBPF172	3	0.327	0.284	4	0.526	0.447	3	0.314	0.292
GBPF135	6	0.766	0.731	9	0.824	0.802	4	0.645	0.579
GBPF75	5	0.344	0.324	5	0.370	0.354	4	0.645	0.579
GBPF70	4	0.170	0.164	4	0.610	0.536	4	0.446	0.420
KWPE25	5	0.342	0.318	5	0.610	0.566	4	0.645	0.579
KWPE26	6	0.397	0.374	5	0.643	0.600	4	0.694	0.637
GBPF203	9	0.580	0.550	5	0.462	0.433	6	0.727	0.697
KWPE39	4	0.342	0.320	4	0.311	0.292	3	0.314	0.292
KWPE48	4	0.170	0.164	3	0.196	0.186	3	0.314	0.292
KWPE57	12	0.841	0.823	6	0.740	0.696	4	0.678	0.623
KWPE56	3	0.512	0.398	3	0.390	0.337	3	0.512	0.444
KWPE53	8	0.654	0.624	6	0.546	0.512	6	0.760	0.726
KWPE58	7	0.403	0.387	6	0.724	0.686	4	0.612	0.555
KWPE19	8	0.702	0.673	6	0.689	0.643	5	0.744	0.702
KWPE29	8	0.684	0.639	4	0.199	0.193	5	0.711	0.672
GBPF111	8	0.538	0.509	5	0.658	0.599	3	0.430	0.385
GBPF155	6	0.425	0.404	5	0.755	0.713	5	0.744	0.702
GBPF204	10	0.827	0.805	6	0.699	0.649	5	0.645	0.608
GBPFM91	1	0.000	0.000	1	0.000	0.000	4	0.612	0.555
Mean	6	0.467	0.439	4.71	0.500	0.460	4.05	0.567	0.524

GD genetic diversity, *PIC* polymorphic information content

accessions from northern and southern areas of China. Genetic diversity was attributed to many genetic differences between individuals and may be manifested in differences in DNA polymorphism (e.g., RFLP, RAPD, AFLP, and SSR markers) (Powell et al. 1996; Park et al. 2009). Particularly, utilization of SSR markers for detecting genetic diversity and genetic relationships among landraces or cultivars has been well-established for many crops (Prasad et al. 2000 for Wheat; Ni et al. 2002 for rice; Hamza et al. 2004 for barley; Xia et al. 2005 for maize; Ali et al. 2008 for sweet sorghum; Sa et al. 2013, 2015 for *Perilla* crop).

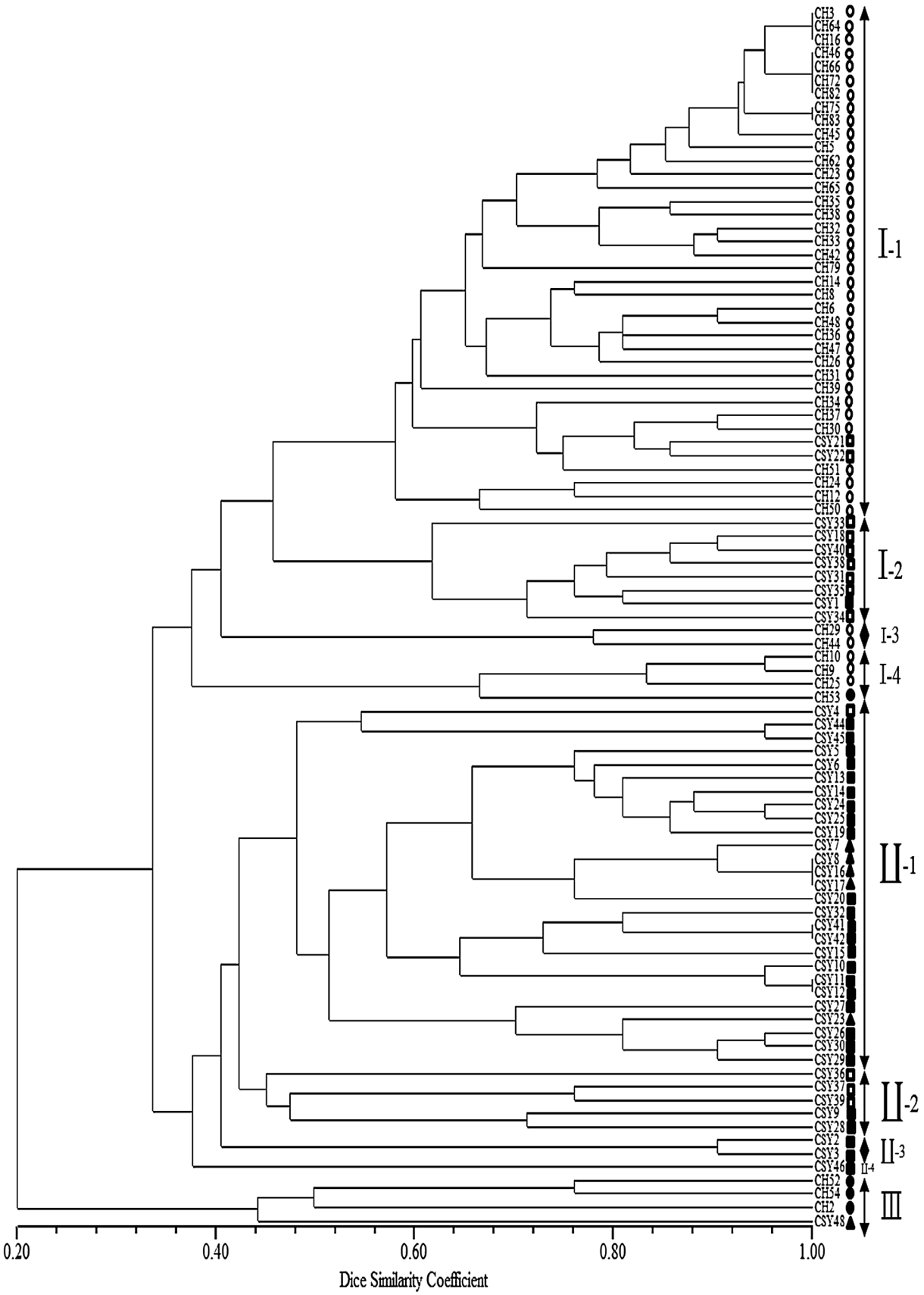
In this study, 21 SSR markers were applied for the assessment of genetic diversity among 91 *Perilla* accessions from southern and northern areas of China. According to our results, a total of 147 alleles were amplified by 21 SSRs from the 91 *Perilla* accessions from different areas of China, which produced an average of 7 alleles per locus. This value is a little lower than the effective number of alleles per SSR locus obtained in the results of previous studies. Lee and Kim (2007) detected 101 alleles among 70 *Perilla* accessions from East Asia using 11 SSRs, with an average allele number of 9.2 per locus. Sa et al. (2013) obtained 165 alleles using 18 SSR markers in 56 *Perilla* accessions from Korea and Japan with an average of 9.2 alleles yielded at each locus. Sa et al. (2015) produced 166 alleles by 18 SSR markers in 81 *Perilla* accessions from East Asia and other countries with an average of 9.8 alleles in each locus. Recently, Ma et al. (2017) obtained 95 alleles using 21 SSR markers in 77 *Perilla* accessions from northern areas of China with an average of 4.52 alleles yielded at each locus. In our study, the results showed a lower average value in genetic diversity than in previous studies by Lee and Kim (2007) and Sa et al. (2013, 2015), but showed a higher average value in genetic diversity than in previous studies by Ma et al. (2017). The reason for the low and high values of genetic diversity and allele numbers per locus obtained in the current study might be attributed to the lower or higher proportion of weedy accessions of var. *frutescens* and the narrow or wide collecting sites compared with the previous studies. For example, most *Perilla* accessions analyzed in this study are accessions of the cultivated var. *frutescens* collected from southern and northern areas of China. Unfortunately, weedy *Perilla* accessions were just collected from one province (Yunnan) in southern China. And also weedy *Perilla* accessions were not collected from northern areas compare to southern areas of China. In other studies, a higher proportion of weedy types of *Perilla* crop were used from several provinces of South China, Korea, Japan, and other countries (Lee and

Kim 2007; Sa et al. 2013, 2015). These researchers have reported that weedy *Perilla* accessions performed a higher genetic diversity level than cultivated *Perilla* accessions and suggested that domestication via direct or indirect selections from human or nature during the evolutionary stage from wild type to cultivated type caused some alleles to be lost, and led to the declined level of polymorphism and genetic diversity (Lee and Ohnishi 2003; Lee and Kim 2007; Sa et al. 2013, 2015). This conclusion was also proven in our study. *Perilla* accessions from Southern China comprising a high proportion of weedy *Perilla* accessions showed higher values of average number of alleles, GD, and PIC than *Perilla* accessions of Northern China consisting of 41 accessions of cultivated var. *frutescens* and four accessions of cultivated var. *crispa* (Table 3). A genetic diversity level for 27 accessions of weedy var. *frutescens* was higher than that of 54 accessions of cultivated var. *frutescens* (Table 5). Ten accessions of var. *crispa* exhibited the highest genetic diversity level compared with other types of *Perilla* accessions (Table 5). In the previous studies of Lee and Ohnishi (2001) and Sa et al. (2012, 2015), it was reported that the cultivated var. *frutescens* might be differentiable from the weedy var. *frutescens* but that the cultivated and weedy types of var. *crispa* would not prove sufficiently differentiable. Because the weedy var. *frutescens* and cultivated and weedy types of var. *crispa* had small seeds (less than 2 mm) with seed dormancy, whereas the cultivated var. *frutescens* had large seeds (larger than 2 mm) which are free from seed dormancy (Lee and Ohnishi 2001; Sa et al. 2012).

Considering seed size between cultivated and weedy types of var. *frutescens* and var. *crispa* indicated that further, Lee and Ohnishi (2001, 2003) suggested that the cultivated var. *frutescens* has been sufficiently differentiated from the weedy var. *frutescens*, whereas, the cultivated var. *crispa* has not yet sufficiently differentiated a domesticated form from the weedy or wild var. *crispa*, as well as previous reported by Lee and Ohnishi (2001, 2003). Although the wild species of the *Perilla* crop has not yet been reported in East Asia, our results suggest that the weedy types of *P. frutescens* including cultivated types of var. *frutescens* and var. *crispa* are the key taxa to understanding the origin of the cultivated type of var. *frutescens*, as mentioned in a previous report by Lee and Ohnishi (2001, 2003) and Sa et al. (2013, 2015).

On the other hand, accessions of the cultivated var. *frutescens* from southern areas of China showed much higher genetic diversity and PIC values than those of the cultivated var. *frutescens* from northern areas of China (Table 4), although accessions of the cultivated var. *frutescens* from southern areas of China had a smaller population size and narrow geographical distribution. In a previous report by Nitta et al. (2005), in China, *Perilla* accessions including cultivated and weedy types were widely distributed in village, roadside, rivers, or mountains. When we collected the *Perilla* genetic resources for the Yunnan Provinces in Southern China, these accessions of cultivated and weedy types of *Perilla* crop were founded in rivers or mountain areas. Various plant species collected from mountainous or plateau areas have been reported to perform a higher genetic diversity and variation, such as kiwi (Lai et al. 2015), strawberry (Zhang et al. 2016), cherry (Meng et al. 2015), and sugarcane (Zhang et al. 2013). The higher genetic diversity in cultivated accessions of var. *frutescens* from Southern China may be attributed to the complex topography and relatively weak interference of human activities in their collecting sites which is located on the Yunnan-Guizhou Plateau. Higher genetic diversity was formed for the adaptation to the higher altitude (1000–3000 m), complex topography, and climate. In contrast, the topography of the collecting sites in northern areas of China was major in the plain, where the environment is relatively softer and tends to be prone to habitat destruction due to industrialization. In addition, gene drift usually occurs under the stress of farmers' selection and also genetic bottleneck due to the expansion of cultivation from southern to northern regions, which may decrease the genetic diversity of local *Perilla* accessions in China. Therefore, the genetic diversity level of cultivated accessions of var. *frutescens* from Northern China was lower in spite of a larger population size and a wider geographical distribution of collecting sites.

The phylogenetic tree was constructed by UPGMA analysis and shown in Fig. 2, 91 *Perilla* accessions were classified into three main groups. Most cultivated *Perilla* accessions were clearly separated from weedy accessions, implying that artificial selection led to the genetic differences between cultivated and weedy *Perilla* accessions. For *Perilla* accessions in Group I, most cultivated accessions of var. *frutescens* from Southern China were situated in Group I-2 and obviously separated from cultivated accessions of var. *frutescens* from Northern China under the genetic similarity of 46%, indicating that gene flow occurring between cultivated accessions of var. *frutescens* from Southern and Northern China was limited significantly as a result of the long distance between collecting sites in southern and northern areas of China. Meanwhile, ambiguous classification was also found in this phylogenetic tree. For example, one accession (CSY1) of weedy var. *frutescens* and one accession (CH53) of cultivated var. *crispa* were grouped with the accessions of cultivated var. *frutescens*. Most accessions of weedy var. *crispa* were mixed with the accessions of weedy var. *frutescens*. Several accessions of cultivated var. *frutescens* (CSY4, CSY36, CSY37, and CSY39) from Southern China were observed in the group of weedy var. *frutescens*. Based on the morphological measurement, some special accessions such as CSY2, CSY26, CSY27, CSY29, and CSY30, which emitted a specific fragrance to var. *frutescens* and exhibited similar morphological traits with var. *crispa*, such as purple color of flower, leaves, and


CH3
CH64
CH16
CH46
CH66
CH72
CH82
CH75
CH83
CH45
CH5
CH62
CH23
CH65
CH35
CH38
CH32
CH33
CH42
CH79
CH14
CH8
CH6
CH48
CH36
CH47
CH26
CH31
CH39
CH34
CH37
CH30
CSY21
CSY22
CH51
CH24
CH12
CH50
CSY33
CSY18
CSY40
CSY38
CSY31
CSY35
CSY1
CSY34
CH29
CH44
CH10
CH9
CH25
CH53
CSY4
CSY44
CSY45
CSY5
CSY6
CSY13
CSY14
CSY24
CSY25
CSY19
CSY7
CSY8
CSY16
CSY17
CSY20
CSY32
CSY41
CSY42
CSY15
CSY10
CSY11
CSY12
CSY27
CSY23
CSY26
CSY30
CSY29
CSY36
CSY37
CSY39
CSY9
CSY28
CSY2
CSY3
CSY46
CH52
CH54
CH2
CSY48
I-1
I-2
I-3
I-4
II-1
II-2
II-3
II-4
III
0.20
0.40
0.60
0.80
1.00
Dice Similarity Coefficient

◂**Fig. 2** UPGMA dendrogram based on the SSR markers. The accessions of *Perilla* crop from northern and southern areas of China are shown in Table 1. White circle: cultivated var. *frutescens* from Northern China, black circle: cultivated var. *crispa* from Northern China, white square: cultivated var. *frutescens* from Southern China, black square: weedy var. *frutescens* from Southern China, black up-pointing triangle: weedy var. *crispa* from Southern China

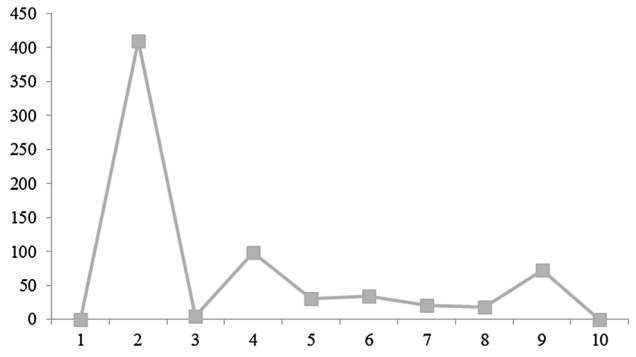


Fig. 3 Magnitude of ΔK as a function of K. The peak value of ΔK was at $K=2$, suggesting two genetic clusters in *Perilla* accessions from northern and southern areas of China. X-axis is K value and Y-axis is ΔK value

stem, were found. These accessions were grouped with weedy accessions of var. *frutescens* in the phylogenetic tree of this study. Therefore, these special accessions should be classified as weedy accessions of var. *frutescens*. Admixed groups were also reported in the studies of Lee and Ohnishi (2003) and Sa et al. (2013) on the basis of AFLP and SSR markers, respectively. Based on the conclusion of Lee et al. (2002); Lee and Kim (2007), Lee and Ohnishi (2001, 2003), Nitta and Ohnishi (1999), and Sa et al. (2013, 2015), it was assumed that CSY1 might be an escaped form from cultivation with small and hard seeds. CH53 might be the hybrid originating from natural hybridization between var. *frutescens* and var. *crispa*. These weedy accessions of var. *crispa* were classified ambiguously in the phylogenetic tree, which might have originated from the hybrids between these two weedy types of *Perilla frutescens*. The two accessions (CSY21 and CSY22) of cultivated var. *frutescens* from Southern China were situated in the group of cultivated var. *frutescens* from Northern China, which might be attributed to human business activities transferring the seeds of CSY21 and CSY22 from Northern China to Southern China. A population structure analysis in Fig. 4 showed that Group I was only composed of accessions of the var. *frutescens* from Northern and Southern China. Group II comprised most accessions of weedy var. *frutescens* and var. *crispa* from Southern China except three accessions (CH2, CH52, and CH54) of cultivated var. *crispa* from Northern China and one accession (CSY39) of cultivated var. *frutescens* from Southern China. However, the geographical locations of these *Perilla* accessions were not coincidental with their positions in the UPGMA dendrogram and population structure, indicating that the diffusion of Chinese *Perilla* accessions in China might be conducted through multiple routes and might neither be directional nor gradual, as in a previous report by Lee and Ohnishi (2003).

Development of SSR markers are essential for the investigations on genetic diversity, population structure, and collection and conservation of germplasm resources. In this study, 21 SSR markers have performed high efficiency in the differentiation between cultivated and weedy *Perilla* accessions, even in the classification of accessions from different areas in China. Valuable information on the genetic structures and relationships of *Perilla* accessions from northern and southern areas of China will assist breeders to make appropriate strategies for *Perilla* crop breeding and germplasm conservation.

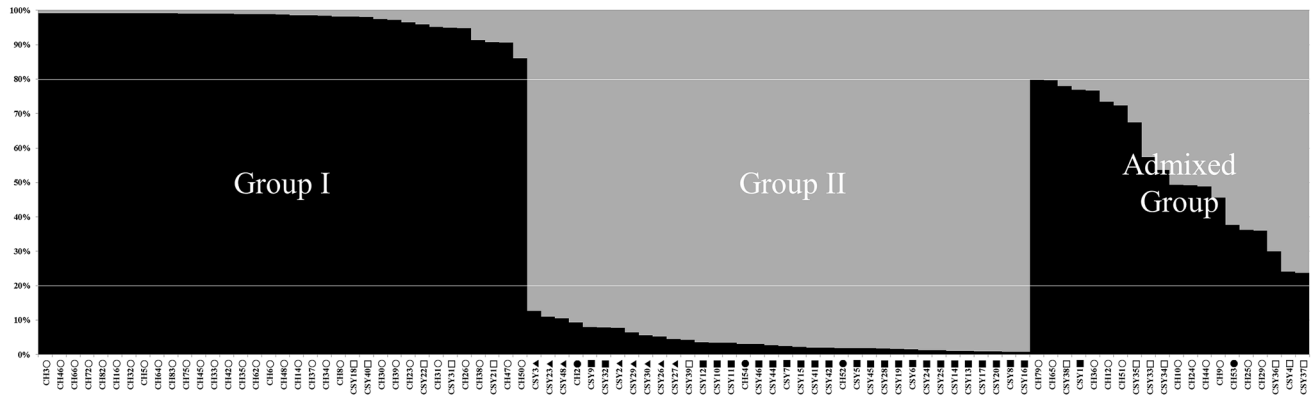


Fig. 4 Population structure of 91 accessions from northern and southern areas of China based on 21 SSRs for K = 2. The two lines are the criteria for distinguishing three groups, Group I, Group II, and an admixed group. White circle: cultivated var. *frutescens* from Northern China, black circle: cultivated var. *crispa* from Northern China, white square: cultivated var. *frutescens* from Southern China, black square: weedy var. *frutescens* from Southern China, black up-pointing triangle: weedy var. *crispa* from Southern China

Acknowledgements This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (2016R1D1A1B01006461), and a Grant from the Regional Subgenebank Support Program (PJ012923012017) of the Rural Development Administration (RDA).

Compliance with ethical standards

Conflict of interest The author declares that they have no conflict of interest.

Ethical approval This article does not contain any studies with human subjects or animals performed by any of the above authors.

References

Ali ML, Rajewski JF, Baenziger PS, Gill KS, Eskridge KM, Dweikat I (2008) Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm by SSR markers. Mol Breed 21:497–509

Da Cunha C, Resende F, Zucchi M, Pinheiro J (2014) SSR-based genetic diversity and structure of garlic accessions from Brazil. Genetica 142:419–431

Dice LR (1945) Measures of the amount of ecologic association between species. Ecology 26:297–302

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Ganapathy KN, Gnanesh BN, Byre Gowda M, Venkatesha SC, Gomashe SS, Channamallikarjuna V (2011) AFLP analysis in pigeonpea (*Cajanus cajan* (L.) Millsp.) revealed close relationship of cultivated genotypes with some of its wild relatives. Genet Resour Crop Evol 58:837–847

Hamza S, Hamida WB, Rebai A, Harrabi M (2004) SSR-based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits. Euphytica 135:107–118

Hu Y, Sun LW, Neo MC, Zhang YX, Wen CX, Xie XL, Liu YJ (2010) Primary identifications and palynological observations of *Perilla* L. in China. J Syst Evol 48:133–145

Kwon SJ, Lee JK, Kim NS, Yu JW, Dixit A, Cho EG, Park YJ (2005) Isolation and characterization of SSR markers in *Perilla frutescens* Britt. Mol Ecol Notes 5:454–456

Lai JJ, Li ZZ, Man YP, Lei R, Wang YC (2015) Genetic diversity of five wild *Actinidia arguta* populations native to China as revealed by SSR markers. Sci Hortic 191:101–107

Lee JK, Kim NS (2007) Genetic diversity and relationships of cultivated and weedy types of *Perilla frutescens* collected from East Asia revealed by SSR markers. Korean J Breed Sci 39:491–499

Lee JK, Ohnishi O (2001) Geographical differentiation of morphological characters among *Perilla* crops and their weedy types in East Asia. Breed Sci 51:247–255

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, Nitta M, Kim NS, Park CH, Yoon KM, Shin YB, Ohnishi O (2002) Genetic diversity of *Perilla* and related weedy types in Korea determined by AFLP analyses. Crop Sci 42:2161–2166

Li HL (1969) The vegetables of ancient China. Econ Bot 23:235–260

Liu K, Muse SV (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21:2128–2129

Liu YX, Zhang WM (1998) Classification and resources distribution of *Perilla*. Chin Wild Plant Resour 17:1–4

Liu YX, Zhang WM, Qian XS (1996) Research and utilization of *Perilla*. Chin Wild Plant Resour 3:24–27

Ma SJ, Lee JK (2017) Morphological variation of two cultivated types of *Perilla* crop from different areas of China. Korean J Hortic Sci Biotechnol 35:510–522

Ma SJ, Sa KJ, Hong TK, Lee JK (2017) Genetic diversity and population structure analysis in *Perilla frutescens* from Northern areas of China based on simple sequence repeats. Genet Mol Res 16(3):gmr16039746

Makino T (1961) Makino's new illustrated flora of Japan. Hokuryukan, Tokyo **(in Japanese)**

Meng F, Liu L, Peng M, Wang ZK, Wang C, Zhao Y (2015) Genetic diversity and population structure analysis in wild strawberry (*Fragaria nubicola* L.) from Motuo in Tibet Plateau based on simple sequence repeats (SSRs). Biochem Syst Ecol 63:113–118

Ni J, Colowit PM, Mackill DJ (2002) Evaluation of genetic diversity in rice subspecies using microsatellite markers. Crop Sci 42:601–607

Nitta M (2001) Origin *Perilla* crops and their weedy type. Ph.D. Thesis, Kyoto University, Kyoto, p 78

Nitta M, Ohnishi O (1999) Genetic relationships among two *Perilla* crops, shiso and egoma, and the weedy type revealed by RAPD markers. Jpn J Genet 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

Nitta M, Lee JK, Kobayashi H, Liu D, Nagamine T (2005) Diversification of multipurpose plant, *Perilla frutescens*. Genet Resour Crop Evol 52:663–670

Palmer JD, Jansen RK, Michaels HJ, Chase MW, Manhart JR (1988) Chloroplast DNA variation and plant phylogeny. Ann Missouri Bot Gard 75:1180–1206

Park YJ, Dixit A, Ma KH, Lee JK, Lee MH, Chung CS, Nitta M, Okuno K, Kim TS, Cho EG, Rao VR (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

Park JY, Ramekar RV, Sa KJ, Lee JK (2015) Genetic diversity, population structure, and association mapping of biomass traits in maize with simple sequence repeat markers. Genes Genom 37:725–735

Powell W, Morgante M, Andre C, Hanafey M, Vogelet J, Tingey S, Rafalski A (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

Pritchard JK, Wen W (2003) Documentation for STRUCTURE software: version 2. Available from http://pritch.bsd.uchicago.edu

Rao VR, Hodgkin T (2002) Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell Tissue Organ Cult 68:1–19

Rohlf FJ (1998) NTSYS-pc: numerical taxonomy and multivariate analysis system. Version: 2.02. Exter Software, Setauket

Sa KJ, Kim JA, Lee JK (2012) Comparison of seed characteristics between the cultivated and the weedy types of *Perilla* species. Hortic Environ Biotechnol 53(4):310–315

Sa KJ, Choi SH, Ueno M, Park KC, Park YJ, Ma KH, Lee JK (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

Song JY, Lee JR, Oh S, Kim CY, Bae CH, Lee GA, Ma KH, Choi YM, Park HJ, Lee MC (2012) Assessment of genetic diversity and fatty acid composition of *Perilla* (*Perilla frutescens* var. *frutescens*) germplasm. Korean J Plant Res 25(6):762–772

Tan M, Yan M, Wang L, Wang L, Yan X (2012) Research progress on *Perilla frutescens*. Chin J Oil Crop Sci 34:225–231

Wang S, Guo F (2012) Genetic diversity of *Perilla frutescens* from Yunnan based on ISSR. Chin J Oil Crop Sci 34:372–376

Wang R, Yu Y, Zhao J, Shi Y, Song Y, Wang T, Li Y (2008) Population structure and linkage disequilibrium of a mini core set of maize inbred lines in China. Theor Appl Genet 117:1141–1153

Wei Z, Li H, Feng B, Lin T, Lin W (2015) Studies on the germplasm resource investigation and utilization of *Perilla frutescens* (L.) in Guizhou. Seed 34:58–60

Xia XC, Reif JC, Melchinger AE, Frisch M, Hoisington DA, Beck D, Pixley K, Warburton ML (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

Yook MJ, Lim SH, Song JS, Kim JW, Zhang CJ, Lee EJ, Ibaragi Y, Lee GJ, Nah G, Kim DS (2014) Assessment of genetic diversity of Korean *Miscanthus* using morphological traits and SSR markers. Biomass Bioenergy 66:81–92

Yu H, Qiu JF, Ma LJ, Hu YJ, Li P, Wang JB (2016) Phytochemical and phytopharmacological review of *Perilla frutescens* L. (Labiatae), a traditional edible-medicinal herb in China. Food Chem Toxicol. https://doi.org/10.1016/j.fct.2016.11.023

Zeven AC, de-Wet JMJ (1982) Dictionary of cultivated plants and their regions of diversity. Centre for Agricultural Publishing and Documentation, Wageningen, p 34

Zhang X, Wu W, Zheng YL, Chen L, Cai Q (2009) Essential oil variations in different *Perilla* L. accessions: chemotaxonomic implications. Plant Syst Evol 281:1–10

Zhang JB, Yan JJ, Zhang YW, Ma X, Bai SQ, Wu YQ, Dao ZX, Li DX, Zhang CB, Zhang Y, You MH, Yang FY, Zhang J (2013) Molecular insights of genetic variation in *Erianthus arundinaceus* Populations native to China. PLoS One 11:e80388. https://doi.org/10.1371/journal.pone.0080388

Zhang J, Chen T, Wang J, Chen Q, Luo Y, Zhang Y, Tang H (2016) Genetic diversity and population structure in cherry [*Cerasus pseudocerasus* (Lindl). G. Don] along Longmenshan Fault Zones in China with newly developed SSR markers. Sci Hortic 212:11–19