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Morphological variation of *Perilla* crop and their weedy types from northern and southern areas of China

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ABSTRACT. In this study, we detected the morphological variation of 91 *Perilla* accessions from Northern and Southern China by the measurement of 7 quantitative and 10 qualitative characters. The accessions of cultivated var. *frutescens* were significantly different from accessions of weedy var. *frutescens* and cultivated and weedy types of var. crispa in 7 quantitative traits such as plant height (QN1), internodes (QN2), the number of branches (QN3 and QN4), length of the largest inflorescence (QN5), the number of florets (QN6) and flowering time (QN7) etc. In addition, significant differences were found between accessions of cultivated var. *frutescens* from northern and southern areas of China for 7 quantitative traits. However, there were no significant differences between accessions of cultivated and weedy types of var. crispa. In principal components analysis (PCAs), 5 quantitative traits and 4 qualitative characters contributed in the positive direction on the first axis. 2 quantitative and 6 qualitative characters contributed in the negative direction on the first axis. The accessions of cultivated and weedy types of var. crispa were clearly separated from accessions of cultivated and weedy types of var. *frutescens* in the PCAs. In addition, most accessions of cultivated var. *frutescens* from Northern China were discriminated from accessions of cultivated and weedy types of var. *frutescens* from Southern China. These findings in this study will assist to better understand the morphological variation for two cultivated types of *Perilla* crop and their weedy types according to the geographical distribution in northern and southern areas of China.

Key words: *Perilla frutescens*; Cultivated and weedy types; Geographical distribution; Morphological traits; Principal components analysis

INTRODUCTION

Perilla frutescens Britt. (Labiatae) is a perennial crop with self-pollination and grows intensively in Himalayan mountain areas, Southeast Asia, and East Asia; i.e., China, Korea, and Japan (Makino, 1961; Lee and Ohnishi, 2001, 2003; Nitta et al., 2003). Recently, it has been introduced to Europe and North America due to its economic properties (Nitta et al., 2003). In East Asia, *Perilla* crop is cultivated in a large scale and used widely; therefore, East Asia is the birth place of *Perilla 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) 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 (Makino, 1961; Nitta and Ohnishi, 1999; Lee and Ohnishi, 2001, 2003). *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 are traditionally used in the same way as sesame seeds in China, Korea, and Japan from old times (Lee and Ohnishi, 2001, 2003; Nitta et al., 2003). Meanwhile, *P. frutescens* var. *crispa*, a Chinese medicine crop, is called Cha-jo-ki in Korean, Shiso in Japan, and Zisu in China (Lee and Ohnishi, 2001, 2003; Nitta et al., 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, 2001, 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 farmer's 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. In Yanbian area of Northeast China inhabited by Chinese-Korean particularly, var. *frutescens* is cultivated in a larger scale. The seeds of var. *frutescens* are used for flavouring in traditional foods, and *Perilla* seed oil has been used for vegetable oil or industrial purpose, while cultivated and weedy types of var. *crispa* are found intensively all over the provinces in South China (Tan et al., 2012; Wei et al., 2015). In there, var. *crispa* is used majorly due to their medicinal function (Tan et al., 2012; Wang and Guo, 2012). 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). Morphological variation of crop species within their geographic distribution areas has been essential for the study of the differentiation and evolution of crop species (Gould and Johnston, 1972; Wyatt and Antonovics, 1981). Domestication is an evolutionary process which led domesticated plants to be different from their wild ancestors in morphological and physiological features (Schwanitz, 1966; Harlan, 1992). Lee and Ohnishi (2001) performed morphological variation studies with 22 morphological traits of 60 *Perilla* accessions collected from East Asian areas for the better understanding of morphological variation among different types of *Perilla* crop. Cultivated var. *frutescens* has taller plant height, larger seed size (>2mm), green wrinkle-free leaves and stem, soft or hard seed, and fragrance specific to var. *frutescens*, while cultivated var. *crispa* has lower plant height, smaller seed size (<2mm), only hard seeds, red or green leaves and stem, and wrinkly or smooth leaves with fragrance specific to var. *crispa* (Lee and Ohnishi, 2001). In addition, germination rate is used as an index for the discrimination between cultivated var. *frutescens* and weedy var. *frutescens* and between cultivated var. *frutescens* and var. *crispa* (Kim et al., 2011; Sa et al., 2012; Jung et al., 2009). Based on the evaluation of the morphological traits of 54 *Perilla* accessions, cultivated var. *frutescens* was identified to be more domesticated than cultivated var. *crispa* (Sa et al., 2012). However, few studies regarding morphological variation of *Perilla* crop from different regions of China have been carried out in the past years. Therefore, the objective of this study was to investigate the differences among cultivated and weedy types of *Perilla* accessions from southern and northern areas of China based on 17 morphological traits.

MATERIAL AND METHODS

Plant materials

The materials for this study consist of 91 accessions (54 cultivated var. *frutescens*, 27 weedy var. *frutescens*, 4 cultivated var. *crispa,* and 6 weedy var. *crispa*) collected in different provinces of Southern and Northern China. The number of accessions and the name of the collection places for *Perilla* accessions collected in China are shown in Table 1 and Figure 1.

Table 1. *Perilla* accessions from different regions of China used for morphological 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*
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.

Morphological character detection

To assess the morphological variation among the accessions of cultivated and weedy types of *Perilla* crop from northern and southern areas of China, ten individuals of each accession were grown in a field at the College of Agriculture and Life Sciences, Kangwon National University, Chuncheon, Gangwon-do, in Korea. Approximately twenty seeds from each accession were sown in a nursery bed in early May and kept in a glass house for a month. Ten seedlings of each accession were then transplanted into the field in early June. We examined seven quantitative and 10 qualitative characters that were selected based on the previous report by Lee and Ohnishi (2001) and evaluated at the appropriate growth stages as described in detail in Table 2. Measurements of the seven quantitative characters and observation of 10 qualitative characters were made on 10 individuals for each accession.

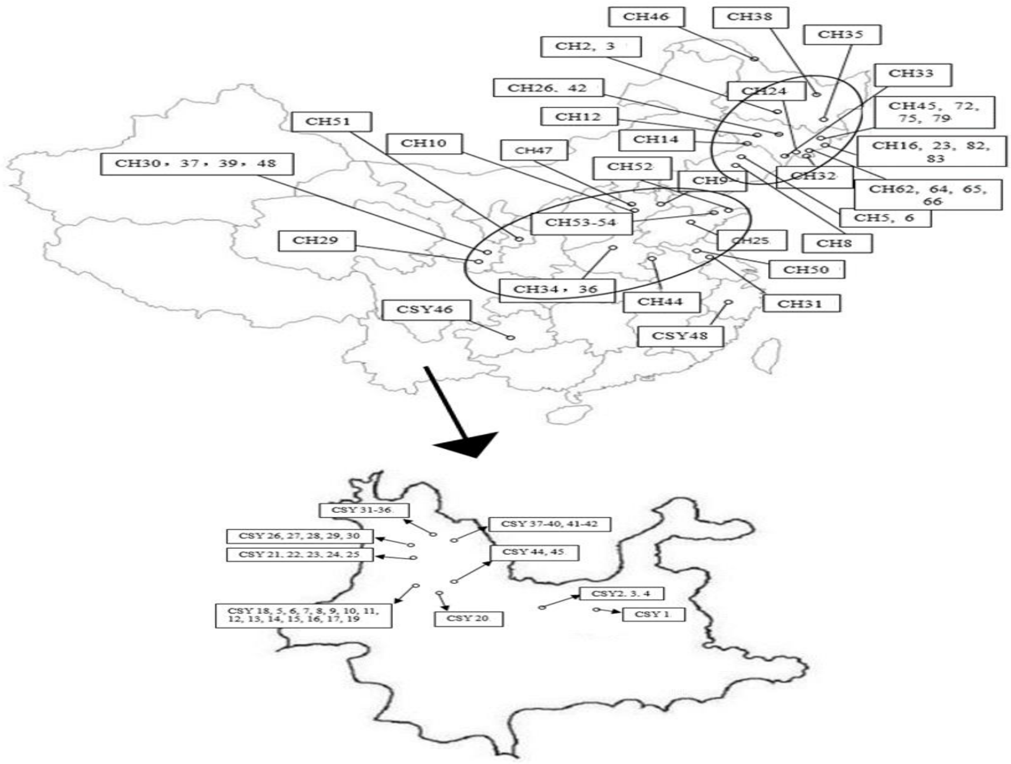

Figure 1. Collecting sites of 91 *Perilla* accessions gathered from Northern and Southern China.

Table 2. Quantitative and qualitative characters used in the morphological analysis of *Perilla* accessions.

Abbreviatio	Character	When/how measured	Unit or category
QN1	Plant height	Before Harvest	cm
QN2	Number of internodes	Before Harvest	Number
QN3	Number of branches	Before Harvest	Number
QN4	Effective number of branches	Before Harvest	Number
QN5	Length of the largest inflorescence	After harvest (The average of 10 largest inflorescences per accession)	cm
QN6	Number of floret of the largest inflorescence	After harvest (The average of 10 largest inflorescences per accession)	Number
QN7	Days from seedling to flowering	The day of more than 50% flowering per plant	day
QL1	Fragrance of plant	At Flowering Stage	*Frutescens*-1, *Crispa*-2
QL2	Color of leaf surface	At Flowering Stage	Green-1, Light Purple-2, Pruple-3, Deep Green-4
QL3	Color of reverse side of leaf	At Flowering Stage	Green-1, Light Purple-2, Pruple-3, Deep Green-4
QL4	Color of stem	At Flowering Stage	Green-1, Light Purple-2, Pruple-3, Deep Green-4
QL5	Degree of pubescence	At Flowering Stage	Slightly Pubescent-1, Pubescent-2, Heavily Pubescent-3
QL6	Color of flower	At Flowering Stage	White-1, Purple-2
QL7	Shape of leaf	Before Ears Come Out	Non-Wrinkle -1, Wrinkle-2
QL8	Color of seed	After Harvest	White-1, Gray-2, Brown-3, Dark Brown-4
QL9	Seed size	After Harvest	Small-1, Big-2
QL10	Hardness of seed	After Harvest	Soft-1, Hard-2

Data analysis

The measurements of each trait were compared among the accessions using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test for multiple comparison of means among the accessions of cultivated var. *frutescens*, weedy var. *frutescens*, cultivated var. *crispa* and weedy var. *crispa* collected from different areas of China. Principal component analyses (PCAs) were conducted to detect differences among accessions. Geographical comparison of the accessions collected from northern and southern areas of China was also made by using univariate and multivariate analyses. These comparisons were made only for accessions of cultivated var. *frutescens* because there was no sample of weedy var. *frutescens* from Northern China in this study. Microsoft Excel Statistical Analysis System Program was used for univariate and multivariate analyses.

RESULTS

Morphological differences among cultivated types of *Perilla* crop and their weedy types from China

The average values, standard deviation, range of seven quantitative traits, and morphological characteristics of 10 qualitative traits for accessions of cultivated and weedy types of var. *frutescens* and var. *crispa* were respectively summarized in Table 3. Based on the survey of quantitative traits, the average of plant height (QN1) was 123.3 cm (39.0-189.7 cm), 145.8 cm (95.0-178.0 cm), 123.5 cm (115.0-137.0 cm), and 137.3cm (109.33-153.0 cm) for cultivated var. *frutescens*, weedy var. *frutescens*, cultivated var. *crispa*, and weedy var. *crispa*, respectively. The average number of internodes (QN2) showed 13.4 (6.0-21.0), 17.6 (14.0-23.0), 18.9 (16.3-21.5), and 16.9 (16.0-18.3) for cultivated var. *frutescens*, weedy var. *frutescens*, cultivated var. *crispa*, and weedy var. *crispa*, respectively. The average values of the effective number of branches (QN3) showed 21.0 (5.7-36.0), 31.1 (26.0-40.0), 32.9 (25.3-40.0), and 29.4 (27.3-34.0) for cultivated var. *frutescens*, weedy var. *frutescens*, cultivated var. *crispa*, and weedy var. *crispa*, respectively. The average of the effective number of branches (QN4) showed 20.1 (5.3-36.0), 27.6 (22.0-36.0), 30.42 (23.3-36.0), and 27.3 (25.3-31.3) for cultivated var. *frutescens*, weedy var. *frutescens*, cultivated var. *crispa*, and weedy var. *crispa*, respectively. The average length of the largest inflorescence (QN5) showed 12.9cm (3.6-30.0cm), 7.6cm (5.1-9.5cm), 9.21cm (7.6-11.3 cm), and 6.54 cm (5.57-9.17 cm) for cultivated var. *frutescens*, weedy var. *frutescens*, cultivated var. *crispa*, and weedy var. *crispa*, respectively. The average floret number of the largest inflorescence (QN6) showed 42.8 (20.5-83.3), 32.5 (24.0-42.7), 32.1 (24.0-47.7), and 31.0 (28.0-35.3) for cultivated var. *frutescens*, weedy var. *frutescens*, cultivated var. *crispa*, and weedy var. *crispa*, respectively. The average days from seedling to flowering (QN7) showed 71.7 (23.0-107.0), 100.0 (92.0-106.0), 89.3 (84.0-91.0), and 93.7 (92.0-94.0) for cultivated var. *frutescens*, weedy var. *frutescens*, cultivated var. *crispa*, and weedy var. *crispa*, respectively.

Table 3. Mean values, standard deviation and range for 7 quantitative and 10 qualitative characters among 91 accessions of two cultivated types of *Perilla* crop and their weedy types from northern and southern areas of China

Traits	Cultivated var. *frutescens*, n=54	Weedy var. *frutescens*, n=27	Cultivated var. *crispa*, n=4	Weedy var. *crispa*, n=6	Statistical comparison*
QN1	123.3 ± 39.2 (39.0--189.7)	145.8 ± 20.9 (95.0--178.0)	123.5 ± 9.03 (115.0--137.0)	137.3 ± 14.73 (109.33--153.0)	A≠B, B≠C, B=D, C≠D, A≠C, A≠D
QN2	13.4 ± 3.8 (6.0--21.0)	17.6 ± 2.0 (14.0--23.0)	18.9 ± 2.11 (16.3--21.5)	16.94 ± 0.93 (16.0--18.33)	A≠B=C=D
QN3	21.0 ± 8.0 (5.7--36.0)	31.1 ± 3.8 (26.0--40.0)	32.9 ± 5.48 (25.3--40.0)	29.44 ± 2.26 (27.3--34.0)	A≠B=C=D
QN4	20.1 ± 7.0 (5.3--36.0)	27.6 ± 3.6 (22--36)	30.42 ± 4.87 (23.3--36.0)	27.33 ± 2.04 (25.3--31.3)	A≠B=C=D
QN5	12.9 ± 7.1 (3.6--30.0)	7.6 ± 1.1 (5.1--9.5)	9.21 ± 1.48 (7.6--11.3)	6.54 ± 1.22 (5.57--9.17)	A≠B, B≠C, B=D, C≠D, A≠C, A≠D
QN6	42.8 ± 15.9 (20.5--83.3)	32.5 ± 4.2 (24.0--42.7)	32.1 ± 9.15 (24.0--47.7)	31.0 ± 2.32 (28.0--35.3)	A≠B=C=D
QN7	71.7 ± 25.2 (23--107)	100 ± 4.2 (92--106)	89.25 ± 3.03 (84--91)	93.67 ± 0.75 (92--94)	A≠B≠C=D
QL1	*Frutescens* (54)	*Frutescens* (27)	*Crispa* (4)	*Crispa* (6)	
QL2	Green (32), Deep Green (22)	Green (5), Purple (5), Light Purple (1), Deep Green (16)	Green (1), Purple (3)	Purple (1), Deep Green (5)	
QL3	Green (26), Deep Green (19), Light Purple (1), Purple (8)	Green (3), Purple (24)	Green (1), Purple (3)	Purple (6)	
QL4	Green (26), Deep Green (19), Light Purple (6), Purple (3)	Green (20), Purple (7)	Green (1), Purple (3)	Purple (6)	
QL5	Pubescent (23), Heavily Pubescent (31)	Pubescent (15), Heavily Pubescent (12)	Slightly Pubescent (4)	Slightly Pubescent (6)	
QL6	White (45), Purple (9)	White (2), Purple (25)	Purple (4)	Purple (6)	
QL7	Non-Wrinkle (54)	Non-Wrinkle (27)	Wrinkle (4)	Wrinkle (6)	
QL8	White (5), Gray (30), Brown (9), Dark Brown (10)	Gray (22), Brown (5)	Brown (4)	Gray (5), Brown (1)	
QL9	Big (54)	Small (27)	Small (4)	Small (6)	
QL10	Soft (45), Hard (9)	Hard (27)	Hard (4)	Hard (6)	

Statistical comparisons of means (P<0.05) revealed that accessions of cultivated var. *frutescens* were significantly different from accessions of weedy var. *frutescens*, cultivated and weedy var. *crispa* in plant height (QN1), number of internodes (QN2), number of branches (QN3), effective number of branches (QN4), length of the largest inflorescence (QN5), number of florets (QN6), and flowering time (QN7). No significant differences between weedy var. *crispa* and weedy var. *frutescens* were found in quantitative traits except flowering time (QN7). Significant differences between weedy var. *frutescens* and cultivated var. *crispa* were found in plant height (QN1), length of inflorescence (QN5), and flowering time (QN7). In addition, significant differences between cultivated and weedy var. *crispa* were found in plant height (QN1) and length of inflorescences (QN5).

Based on the detection of qualitative traits, 54 accessions of cultivated var. *frutescens* and 27 accessions of weedy var. *frutescens* performed the specific fragrance of var. *frutescens*, and 10 accessions of cultivated and weedy types of var. *crispa* exhibited the specific fragrance of var. *crispa*. For the leaf surface color (QL2), 32 accessions of cultivated var. *frutescens*, five accessions of weedy var. *frutescens*, and one accession of cultivated var. *crispa* had a green color of leaf surface. 22 accessions of cultivated var. *frutescens*, 16 accessions of weedy var. *frutescens*, and five accessions of weedy var. *crispa* exhibited a deep green color on the leaf surface. Three accessions of cultivated var. *crispa* and five accessions of weedy var. *frutescens* performed the purple color of leaf surface. Only one accession of weedy var. *crispa* had the purple color of leaf surface. For the color of reverse side of leaves (QL3), the accessions of cultivated var. *frutescens* had green (26 accessions), deep green (19 accessions), light purple (1 accession), and purple (8 accessions) colors. The accessions of weedy var. *frutescens* had green (3 accessions) and purple (24 accessions) colors, while the cultivated accessions of var. *crispa* had green (1 accessions) and purple (3 accessions) colors, while six accessions of weedy var. *crispa* had only purple color. The stem color (QL4) of the accessions of cultivated var. *frutescens* included green (26 accessions), deep green (19 accessions), light purple (6 accession), and purple (3 accessions) colors. The accessions of weedy var. *frutescens* had green (20 accessions) and purple (7 accessions) colors, while the cultivated accessions of var. *crispa* had green (1 accession) and purple (3 accessions) colors, and all accessions of weedy var. *crispa* had only purple (6 accessions) color. In terms of pubescent degree (QL5), all accessions of cultivated and weedy var. *crispa* were slightly pubescent, 23 accessions of cultivated var. *frutescens* and 15 accessions of weedy var. *crispa* were pubescent and 31 accessions of cultivated var. *frutescens* and 12 accessions of weedy var. *frutescens* were heavily pubescent. For flower color (QL6), the 45 cultivated accessions and 2 weedy accessions of var. *frutescens* were white, while in all accessions of cultivated and weedy types of var. *crispa*, 9 cultivated accessions and 25 weedy accessions of var. *frutescens* had purple color. For the leaf shape (QL7), all accessions of cultivated and weedy types of var. *frutescens* were unwrinkled, while all accessions of cultivated and weedy types of var. *crispa* had wrinkled leaves. The seed color (QL8) of the accessions of cultivated var. *frutescens* included white (5 accessions), gray (30 accessions), brown (9 accessions), and dark brown (10 accessions). The accessions of weedy var. *frutescens* were gray (22 accessions) and brown (5 accessions) colors. All accessions of cultivated var. *crispa* showed only brown hue, and the accessions of weedy var. *crispa* had gray (5 accessions) and brown (1 accessions) colors. As for seed size (QL9) and hardness (QL10), all accessions of cultivated var. *frutescnes* were large, while all accessions of weedy var. *frutescnes* and cultivated and weedy types of var. *crispa* were small. Seed hardness (QL10) in the accessions of cultivated var. *frutescnes* was found to be both soft (45 accessions) and hard (9 accessions), while all accessions of weedy var. *frutescnes* and cultivated and weedy types of var. *crispa* had only hard seed type.

Geographical differences in cultivated var. *frutescens* between northern and southern China

To understand the geographical difference between the accessions of cultivated var. *frutescens* from Northern and Southern areas of China, we performed a statistical analysis of the seven quantitative characters on the accessions of cultivated var. *frutescens* collected from these regions. The mean values, standard deviation, and range of the seven quantitative traits are summarized in Table 4.

Table 4. Mean values, standard deviation and range for 7 quantitative characters among 54 accessions of cultivated type of var. *frutescens* from northern and southern areas of China

Morphological traits	Cultivated type of var. *frutescens* (northern areas of China, n=41)	Cultivated type of var. *frutescens* (southern areas of China, n=13)	Statistical comparison *
QN1	114.5 ± 37.8 (39.0--177.5)	150.7 ± 29.8 (85.3--189.7)	A≠B
QN2	12.6 ± 3.8 (6.0--21.0)	16.0 ± 2.0 (10.7--19.0)	A≠B
QN3	19.4 ± 8.3 (5.7--36.0)	26.2 ± 4.0 (18.7--34.0)	A≠B
QN4	19.0 ± 7.4 (5.3--36.0)	23.7 ± 3.8 (16.7--32.0)	A≠B
QN5	14.1 ± 7.3 (3.6--30.0)	8.9 ± 4.2 (6.0--23.0)	A≠B
QN6	45.6 ± 15.8 (20.5--83.3)	34.0 ± 12.7 (24.7--76.0)	A≠B
QN7	64.1 ± 22.6 (23.0--107.0)	95.7 ± 16.6 (43.0--106.0)	A≠B

Note: *: Test of statistical significant difference between the two areas of cultivated var. *frutescens*. Comparison was performed with ANOVA followed by Student-Newman-Keul test ($P<0.05$). A= Cultivated type of var. *frutescens* from northern areas of China; B= Cultivated type of var. *frutescens* from southern areas of China.

As shown in Table 4, QN1 was 114.5 cm (39.0-177.5cm) and 150.7 cm (85.3-189.7 cm) for accessions of cultivated var. *frutescens* from northern and southern areas of China, respectively. QN2 was 12.6 (6.0-21.0) and 16.0 (10.7-19.0) in accessions of cultivated var. *frutescens* from northern and southern areas of China, respectively. QN3 was 19.4 (5.7-36.0) and 26.2 (18.7-34.0) in accessions of cultivated var. *frutescens* from northern and southern areas of China, respectively. QN4 was 19.0 (5.3-36.0) and 23.7 (16.7-32.0) in accessions of cultivated var. *frutescens* from northern and southern areas of China, respectively.

QN5 was 14.1 (3.6-30.0) and 8.9 (6.0-23.0) in accessions of cultivated var. *frutescens* from northern and southern areas of China, respectively. QN6 was 45.6 (20.5-83.3) and 34.0 (24.7-76.0) in accessions of cultivated var. *frutescens* from northern and southern areas of China, respectively. QN7 was 64.1 (23.0-107.0) and 95.7 (43.0-106.0) in accessions of cultivated var. *frutescens* from northern and southern areas of China, respectively.

In addition, the results of the statistical comparison of means ($p < 0.05$) were as follows: significant differences were found between accessions of cultivated var. *frutescens* from northern and southern areas of China for all 7 quantitative traits.

Multivariate analysis based on quantitative and qualitative characteristics in *Perilla* accessions from northern and southern China

In this study, principal components analysis (PCA) was used to detect the morphological variation among the *Perilla* accessions from Northern and Southern China. The first (principal component 1) and second (principal component 2) components accounted for 39.3% and 19.5% of the total variance, respectively (Table 5).

Five quantitative traits (QN1, QN2, QN3, QN4, and QN7) and 4 qualitative characters (QL1, QL6, QL7, and QL10) contributed in the positive direction on the first axis (Table 5). The other quantitative and qualitative characters contributed in the negative direction on the first axis.

Table 5. Cumulative variances of first and second principal components and loadings of 7 quantitative and 10 qualitative traits on each principal component

Morphological traits	Eigen vectors	
	C1	C2
QN3	0.914142	-0.08316
QN4	0.886108	-0.11906
QN7	0.863996	-0.24747
QL10	0.722351	0.484047
QL7	0.698328	0.521077
QN1	0.605418	-0.31594
QN2	0.467202	-0.02807
QL1	0.400927	0.579008
QL6	0.400927	0.579008
QL8	-0.07443	-0.11797
QL2	-0.19904	0.776409
QL3	-0.31001	0.808069
QL5	-0.53285	-0.23629
QN6	-0.65211	0.168466
QL4	-0.66873	0.580149
QL9	-0.72317	-0.4793
QN5	-0.75984	0.206002
Cumulative variance (%)	39.30	19.54

As shown in Figure 2, most accessions of cultivated var. *frutescens* from northern and southern areas of China were clearly separated by the first axis. Namely, most accessions of cultivated var. *frutescens* from Northern and Southern China were respectively situated to the left and right on the first axis, except for several accessions (Figure 2). In addition, all accessions of cultivated and weedy types of var. *crispa* were clearly separated with the accessions of cultivated and weedy types of var.

frutescens in the first axis (Figure 2), while the accessions of cultivated and weedy types of var. *crispa* were not clearly separated by the first axis. In addition, the accessions of cultivated and weedy types of var. *frutescens* from Southern China were not clearly separated by the first axis, except for several accessions (Figure 2). Along axis 2, the accessions of cultivated and weedy types of var. *frutescens* from Southern China were clearly separated. Most accessions of cultivated var. *frutescens* were in the lower part of the plane, whereas most accessions of weedy var. *frutescens* were in the upper part of the plane, except for several accessions (Figure 2).

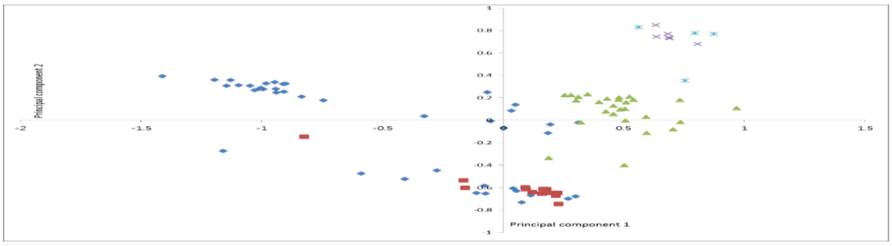

Figure 2. Projection of 91 accessions of *Perilla* crops collected from China in the first and second principal components (PC1 and PC2), with “red squares” standing for accessions of cultivated type of var. *frutescens* from southern areas of China, “blue rhomb” representing accessions of cultivated type of var. *frutescens* from northern areas of China, and “green triangles” indicating weedy type of var. *frutescens* from southern areas of China. “Purple cross” is the weedy accessions of var. crispa from southern areas of China. “Blue asterisk” means the cultivated type of var. crispa from northern areas of China.

DISCUSSION

Morphological variation among cultivated and weedy types of *Perilla* crop from China

In the long-term process of evolution, the changes in micro-evolution under different conditions led to the morphologically and physiologically variation among the accessions of plant species (Gould and Johnston, 1972). Generally, the process of crop domestication has been closely associated with cultivation in controlled environments of plant species (Harlan, 1992; Zohary and Hopf, 1993).

In this study, 7 quantitative characters and 10 qualitative characters were examined for 91 *Perilla* accessions (54 cultivated var. *frutescens*, 27 weedy var. *frutescens*, 4 cultivated var. *crispa*, and 6 weedy var. *crispa*) from northern and southern areas of China. According to our results, significant differences between accessions of cultivated of var. *frutescens* and the other three types of *Perilla* crop were detected in all quantitative characters measured in this study (Table 3). The accessions of weedy var. *frutescens* were different significantly from the accessions of cultivated var. *crispa* in plant height (QN1), length of inflorescence (QN5) and flowering time (QN7), while significant differentiation between weedy var. *frutescens* and weedy var. *crispa* was revealed only in flowering time (QN7) (Table 3). The accessions of cultivated var. *crispa* were different significantly from the accessions of weedy var. *crispa* in plant height and length of inflorescence (Table 3).

In addition, based on the examination of qualitative traits in Table 3, morphological differences between accessions of cultivated var. *frutescens* and accessions of cultivated and weedy types of var. *crispa* were revealed in seed size (QL9), seed hardness (QL10), color of leaf (QL2, QL3), color of stem (QL4), shape of leaf (QL7), and pubescence degree (QL5). The differences between accessions of cultivated and weedy types of var. *frutescens* were mainly shown in two qualitative traits: seed size (QL9) and seed hardness (QL10). The main differences between accessions of weedy var. *frutescens* and accessions of cultivated and weedy types of var. *crispa* were leaf shape (QL7), leaf color (QL2), and pubescent degree (QL5). Therefore, these morphological traits may be considered as effective characters for the differentiation among accessions of cultivated and weedy types of *Perilla* crop. Lee and Ohnishi (2001) suggested that seed size, plant height, and branch number could be used as the effective morphological traits in distinguishing between cultivated and weedy types of var. *frutescens*; particularly, seed size was the most reliable trait in discriminating between var. *frutescens* and var. *crispa* or between cultivated and weedy var. *frutescens*. In our study, the accessions of cultivated var. *frutescens* were significantly different from the accessions of weedy var. *frutescens* in plant height and branch number. In addition, seed size is identified to be a reliable character for the differentiation between accessions of cultivated var. *frutescens* and weedy var. *frutescens*, and between accessions of cultivated var. *frutescens* and cultivated and weedy types of var. *crispa.*

In the PCA analysis, the accessions of cultivated and weedy types of var. *crispa* were situated far away from the accessions of cultivated and weedy types of var. *frutescens,* and most accessions of weedy var. *frutescens* were separated from the accessions of cultivated var. *frutescens* (Figure 2). These findings indicated that the combination of quantitative and qualitative traits can be used to discriminate the accessions of cultivated and weedy types of *Perilla* crop only through multivariate analysis. However, no significant difference was found between cultivated and weedy var. *crispa* in morphological traits except plant height and length of inflorescence. Furthermore, in the PCA analysis, cultivated accessions of var. *crispa* were not separated from weedy accessions of var. *crispa*, which was also reported by Lee and Ohnishi (2001) and Sa et al. (2012). These results indicated that var. *crispa* was not domesticated completely and its weedy form has been used for cultivation by farmers, as in the previous report of Lee and Ohnishi (2001). In addition,, among the accessions of weedy types of *Perilla* crop used this study, several accessions showed that intermediate types between cultivated var. *frutescens* and cultivated var. *crispa* were observed. Thus, these accessions were very difficult to distinguish between var. *frutescens* and var. *crispa* by their morphological characteristics. In the previous report by Lee et al. (2002), they suggested the possibility of inter-varietal crosses among the *Perilla* species and related weedy types, but so far it has not been clear. The outcrossing rate in *Perilla* crop is not available at this moment. Although the wild species of *Perilla* crop has not yet been identified, the above results might provide some evidence that the weedy types of *Perilla* crop may be the key taxon for our understanding of the origin of cultivated types of var. *frutescens* and var. *crispa* in East Asia, as well as the previous report of Lee and Ohnishi (2001, 2003) and Lee et al. (2002).

Geographical differences of cultivated var. *frutescens* between northern and southern areas of China

The morphological and physiological traits of local crop landraces have a close correlation with their habitats where these traits are formed, are developed, and have evolved under natural conditions (Rao and Hodgkin, 2002). Many morphological and physiological traits, such as the relative development rates, tolerance to biotic and abiotic stresses, edaphic responses and responses to soil fertility, and adaptation to cultivation and harvesting methods, can be affected by the ecological differentiation in local crop landraces (Rao and Hodgkin, 2002). Generally, adaptive genetic variation is quantitative and responds sensitively even to the slight differences in habitats. The close relationships between population characteristics and the environments have been illustrated by many studies (Aston and Bradshaw, 1966; Al-Hiyaly et al., 1993; Rao and Hodgkin, 2002). The interaction between the environment and genetic systems during the long periods led to the formation of the various local crop landraces (IPGRI, 1994; Brush, 1995).

The *Perilla* accessions analyzed in this study were collected in the northern and southern areas of China. Differences in ecological and geographic factors, such as latitude, altitude, moisture, rainfall, temperature, and topographic features, may be an intensive influence on the evolution and development of morphological traits of *Perilla* species from Northern and Southern China. The previous research by Lee and Ohnishi (2001) has found that the formation of morphological variation has a close relationship with the geographic origin of *Perilla* accessions. For instance, most accessions of cultivated var. *frutescens* from China showed significant differences from accessions of cultivated var. *frutescens* from Korea and Japan, such as in seed size, leaf size, plant height, internode number, and flowering time (Lee and Ohnishi, 2001). In addition, the accessions of cultivated var. *frutescens* from high-latitude areas (Northeast China) and middle-latitude areas (Northwest China and North China) of China showed significant differences in some quantitative traits such as branch number, internode number, floret number, length of largest inflorescence, and flowering time (Ma and Lee, 2017). In this study, the accessions of cultivated var. *frutescens* from Northern and Southern China also showed significant differences in morphological traits, plant height (QN1), internode number (QN2), branch number (QN3 and QN4), floret number (QN6), length of largest inflorescence (QN5), and flowering time (QN7) (Table 4). Most accessions of cultivated var. *frutescens* from Northern China showed lower plant height than those accessions from Southern China. Furthermore, these accessions of cultivated var. *frutescens* from northern areas have fewer internodes, long inflorescence length with much florets, and early flowering time. Based on the flowering time, Lee and Ohnishi (2001) classified *Perilla* accessions collected in East Asia into three types: early-maturity (flowering time <100 days), middle-maturity (100 days <flowering time <130 days), and late-maturity (flowering time >130 days). In this study, all accessions of cultivated var. *frutescens* from Northern China except one accession (CH44) belong to the early-maturity type with the flowering time varying from 23 days to 99 days, while eight accessions of cultivated var. *frutescens* from Southern China were middle-mature type with the flowering time range of 101 to 106 days. The other accessions were early-maturity type. Lee and Ohnishi (2001) found that all accessions of cultivated and weedy types of var. *frutescens* collected from South China belong to late-maturity type with a flowering time of more than 130 days. These findings imply that latitude with geographical distribution may be a critical factor that affects the flowering time in *Perilla* species because *Perilla* crop is well-known as one of the representative short-day crops. In addition, selection from natural factors and local farmers could be a major force affecting the formation of morphological variation among *Perilla* accessions from different parts of China. Growing days and temperature of crop cultivation in the northern regions of China are much shorter and lower than those in southern areas of China. In addition, seeds of cultivated var. *frutescens* were majorly used for *Perilla* seed oil in the northern area of China (Tan et al., 2012). Therefore, the accessions of cultivated var. *frutescens* with these traits such as long inflorescence length with much florets and early flowering time may have more probabilities to be selected and preserved by local farmers in northern regions of China.

CONCLUSION

PCA analysis showing most accessions of cultivated var. *frutescens* from Northern China were clearly differentiated from accessions of cultivated and weedy var. *frutescens* from Southern China. This indicates that a combination of quantitative and qualitative traits measured in this study can be used to discriminate *Perilla* accessions from different regions in China only through multivariate analysis (Figure 2). However, several accessions of var. *frutescens* from Northern China were mixed with accessions of var. *frutescens* from Southern China in the PCA analysis (Figure 2). This result shows that the gene flow, which is caused by seeds, pollens, and other natural ways, may be limited by natural conditions due to the long distance between the collecting sites in Northern and Southern China. Therefore, the ambiguous classification might be attributed to the interference from human activities. Gene flow can significantly influence the differentiation and population structure of plants (Duminil et al., 2009), and may be affected by human activities (Meng et al., 2015). Many plant species collected in mountainous terrains usually exhibited a high level of morphological differentiation among and within populations because of the limited gene flow (Meng et al., 2015; Chen et al., 2016; Zhang et al., 2016). *Perilla* seeds from southern areas, because the southern area has a higher genetic diversity, might be transferred to northern areas of China through the expansion cultivation area of *Perilla* crop or sales of *Perilla* seeds by humans. Therefore, some accessions of var. *frutescens* from Northern China showed similar morphological properties with accessions of var. *frutescens* from Southern China, and have been grouped together with accessions of var. *frutescens* from Southern China in the PCA analysis (Figure 2).

In this study, we have provided the information regarding the morphological properties of *Perilla* accessions from Northern and Southern China. These findings demonstrated in this study could assist us to further understand the morphological differentiation of *Perilla* accessions with different geographical distributions in China and also would be beneficial in *Perilla* germ-plasm resource management and in formulating effective strategies for excellent cultivar breeding.

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