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한국에서 수집한 들깨종 작물의 재배형 및 잡초형들의 형태적 변이

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Morphological Variation of Cultivated and Weedy Types in *Perilla* Crop Collected from South Korea

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ABSTRACT

Background: *Perilla* (*Perilla frutescens* L.) has been widely cultivated and used for medicinal use, aromatic, functional food, and ornamental plant in South Korea. To understand the morphological variation in the cultivated and weedy types of *Perilla* (CWTP) collected from South Korea, 52 accessions collected from different areas were evlauated using one quantitative and 10 qualitative characteristics.

Methods and Results: Principal component analysis (PCA) was performed using the NTSYS-pc V2.1 program to detect the differences among the accessions of the CWTP. Multivariate analysis was performed using Microsoft Excel Statistical Analysis System. The cultivated *P. frutescens* var. *frutescens* and two weedy types of *Perilla* crop were accurately distinguished based on he 11 morphological characteristics, paricularly seed related traits, used in the study. PCA results showed that, most morphological characteristics such as weight per 100 seeds (QN1), color of leaf adaxial side (QL1), color of leaf abaxial side (QL2), stem color (QL4), flower color (QL5), seed size (QL9), and seed hardness (QL10) provided a significant contribution in the positive or negative direction on the first axis. These characteristics are considered useful for distinguishing between accessions of the CWTP collected from South Korea.

Conclusions: The accessions of weedy *P. frutescens* var. *frutescens* are located between the accessions of cultivated *P. frutescens* var. *frutescens* and the those of weedy *P. frutescens* var. *crispa* on the first axis. Although the cultivated and weedy types of *P. frutescens* var. *frutescens* and the two weedy types of *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa* cannot be completely distinguished, the results provide useful information for future studies on the identification and classification of CWTP germplasm accessions collected from different areas in South Korea.

Key Words: *Perilla* Crop, Cultivated and Weedy Types, Morphological Characteristics, Morphological Differentiation, Crop Evolution, Principal Component Analysis

INTRODUCTION

Perilla (*Perilla frutescens* L.) is an annual crop of the Lamiaceae family, and it can be divided into two cultivated types (or varieties) based on their morphological characteristics and usage conditions: *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa* (Makino, 1961).

This crop is considered to have originated in East Asia, including China, because in East Asia there is a long history of cultivation, large scale distribution and cultivation, and a wide range of uses (Makino, 1961; Li, 1969; Nitta, 2001; Nitta *et al.*, 2003).

Today, the two cultivated types of *Perilla* crop are most widely cultivated and used in East Asia (Li, 1969; Lee

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and Ohnishi, 2001; Lee and Ohnishi, 2003; Nitta *et al*., 2003) as follows: The cultivated var. *frutescens* is widely cultivated in South Korea, where it is mainly used for both oil and vegetable crop; and the cultivated var. *crispa* is widely cultivated in Japan, where it is mainly used for both herbal medicine and vegetable crop (Lee and Ohnishi, 2001; Nitta, 2001; Lee and Ohnishi, 2003; Nitta *et al*., 2003).

In recent years, *Perilla* has attracted worldwide attention as a multi-purpose cash crop with uses such as for oil, condiment, vegetable, herbal medicine, and ornamental plants (Park *et al*., 2021).

In particularly, *Perilla* leaves are rich in iron, vitamin B and C, and anthocyanin, and are preferred in salad vegetables and pickles eaten with meat and sashimi in South Korea and Japan (Lee and Ohnishi, 2001; Nitta *et al*., 2003).

Also, *Perilla* seed oil of *P. frutescens* var. *frutescens* contains polyunsaturated fatty acids such as ω-3 fatty acids (alpha-linolenic acid), ω-6 fatty acids (linoleic acid) and ω-9 fatty acids (Oleic acid), which are the most beneficial to human health in prevention and control of various diseases like cardiovascular disorders, cancer, inflammation, rheumatoid arthritis, and mental health condition (Asif, 2011; Hashimoto *et al*., 2020; Park *et al*., 2021).

Since the morphological characteristics of the two cultivated types of *Perilla* differ in many respects, the two cultivated types of *Perilla* are well differentiated based on morphological characteristics. Of these, the cultivated var. *frutescens* has large (more than 2 ㎜) soft seeds, green leaves and stems, and leaves without wrinkles. In contrast, the cultivated var. *crispa* has small (less than 2 ㎜) hard seeds, purple or green coloration on the leaves and stems, and wrinkly or non-wrinkly leaves (Makino, 1961; Lee and Ohnishi, 2001; Nitta, 2001; Nitta *et al*., 2003).

In addition, although the wild species of the two cultivated types of *Perilla* has not been identified in East Asia, its weedy types are commonly found in East Asia including in China and Korea in habitats such as wasteland, roadside, and around farmers' houses or farmers' fields (Lee and Ohnishi, 2001; Lee *et al*., 2002; Lee and Ohnishi, 2003; Nitta *et al*., 2003; Nitta *et al*., 2005; Sa *et al*., 2015; Ma *et al*., 2017; Sa *et al*., 2018; Ma *et al*., 2019; Ha *et al*., 2021).

Until now, many plant researchers have been conducting research to distinguish between the two cultivated and weedy types of *Perilla* (CWTP). Compared with the two cultivated types of *Perilla*, the weedy types are relatively indistinguishable based on morphological characteristics.

The two cultivated types of *Perilla* have the same number of chromosomes (2n = 40) (Yamane, 1950; Honda *et al*., 1994), they can be crossed by artificial crossing, and intermediate hybrid types exist in the natural habitat (Nagai, 1935; Honda *et al*., 1990, 1994; Lim *et al*., 2019, 2021; Kim *et al*., 2021). The presence of intermediate hybrid types is the reason why the two CWTP are difficult to distinguish completely (Lee and Ohnishi, 2001).

Modern biotechnology is now mainstream, and various molecular marker technologies such as random amplified polymorphic DNA (RAPD) (Nitta *et al*., 2003; Nitta and Ohnishi, 1999), amplified fragment length polymorphism (AFLP) (Lee and Ohnishi, 2003; Lee *et al*., 2002), and simple sequence repeats (SSR) (Park *et al*., 2009; Sa *et al*., 2013, Sa *et al*., 2018; Ha *et al*., 2021; Kim *et al*., 2021; Park *et al*., 2021; Sa *et al*., 2021; Fu *et al*., 2022; Park *et al*., 2022) have been applied successfully to identify and classify germplasm accessions of the CWTP.

However, the identification and description of phenotypic morphological traits with obvious and easily identifiable characteristics are still the most direct and basic methods and means of germplasm research, and they cannot be replaced completely by molecular marker technology (Liao *et al*., 2015).

Unlike China, which has a long history of cultivation and high genetic diversity of *Perilla* crop but few uses, South Korea, which has large-scale cultivation and multi-purpose use, is considered to be a secondary diffusion center of *Perilla* crop (Li, 1969; Lee and Ohnishi, 2001; Lee and Ohnishi, 2003; Nitta *et al*., 2003; Nitta *et al*., 2005).

In order to study and utilize comprehensively the *Perilla* crop germplasm resources collected from South Korea, it is necessary to identify and evaluate a variety of morphological characteristics of these germplasm resources, which will provide a research basis for future research and utilization of *Perilla* germplasm resources.

Therefore, in order to understand further the morphological variation among accessions of the CWTP, in this study we investigated the morphological characteristics of 52 accessions of the CWTP from South Korea using one quantitative and 10 qualitative characteristics. This study is expected to provide useful information for future taxonomic studies of CWTP germplasm accessions collected from South Korea.

MATERIALS AND METHODS

1. Plant materials

The 52 accessions of the CWTP (21 cultivated var. *frutescens*, 14 weedy var. *frutescens*, 17 weedy var. *crispa*) used in this study were selected from a previous study by Fu *et al.* (2022). In the present study, the 52 accessions of the CWTP used represented all regions of South Korea as much as possible, and the collection information is shown in Table 1. The subset of each collections was

Table 1. *Perilla* accessions from different areas of South Korea used for morphological characteristic analysis.

No.	Accession no.	City and province	Country	Type
1	103282	Samcheok, Gangwon-do	Korea	Cultivated type of var. *frutescens*
2	195496	Yanggu-gun, Gangwon-do	Korea	Cultivated type of var. *frutescens*
3	195498	Hwacheon-si, Gangwon-do	Korea	Cultivated type of var. *frutescens*
4	112893	Paju-si, Gyeonggi-do	Korea	Cultivated type of var. *frutescens*
5	157559	Suwon-si, Gyeonggi-do	Korea	Cultivated type of var. *frutescens*
6	195351	Yeoncheon-gun, Gyeonggi-do	Korea	Cultivated type of var. *frutescens*
7	105950	Miryang-si, Gyeongsangnam-do	Korea	Cultivated type of var. *frutescens*
8	195623	Sangcheong-gun, Gyeongsangnam-do	Korea	Cultivated type of var. *frutescens*
9	208641	Sangcheong-gun, Gyeongsangnam-do	Korea	Cultivated type of var. *frutescens*
10	209922	Changnyeong-gun, Gyeongsangnam-do	Korea	Cultivated type of var. *frutescens*
11	117160	Uiseong-gun, Gyeongsangbuk-do	Korea	Cultivated type of var. *frutescens*
12	157529	Gurye-gun, Jeollanam-do	Korea	Cultivated type of var. *frutescens*
13	216238	Gwangyang-si, Jeollanam-do	Korea	Cultivated type of var. *frutescens*
14	216239	Gangjin-gun, Jeollanam-do	Korea	Cultivated type of var. *frutescens*
15	216246	Hampyeong-gun, Jeollanam-do	Korea	Cultivated type of var. *frutescens*
16	157586	Namwon-si, Jeollabuk-do	Korea	Cultivated type of var. *frutescens*
17	117136	Nonsan-si, Chungcheongnam-do	Korea	Cultivated type of var. *frutescens*
18	157578	Asan-si, Chungcheongnam-do	Korea	Cultivated type of var. *frutescens*
19	207962	Hongseong-gun, Chungcheongnam-do	Korea	Cultivated type of var. *frutescens*
20	117161	Chungju-si, Chungcheongbuk-do	Korea	Cultivated type of var. *frutescens*
21	157515	Okcheon-gun, Chungcheongbuk-do	Korea	Cultivated type of var. *frutescens*
22	PF09-042	Yeongwol-gun, Gangwon-do	Korea	Weedy type of var. *frutescens*
23	PF15-018	Jeongseon-gun, Gangwon-do	Korea	Weedy type of var. *frutescens*
24	PF08-115	Pyeongchang-gun, Gangwon-do	Korea	Weedy type of var. *frutescens*
25	PF09-085	Hongcheon-gun, Gangwon-do	Korea	Weedy type of var. *frutescens*
26	PF18-043	Geochang-gun, Gyeongsangnam-do	Korea	Weedy type of var. *frutescens*
27	PF18-052	Hamyang-gun, Gyeongsangnam-do	Korea	Weedy type of var. *frutescens*
28	PF19-007	Andong-si, Gyeongsangbuk-do	Korea	Weedy type of var. *frutescens*
29	PF18-077	Gokseong-gun, Jeollanam-do	Korea	Weedy type of var. *frutescens*
30	PF17-027	Sunchang-gun, Jeollabuk-do	Korea	Weedy type of var. *frutescens*
31	PF18-016	Geumsan-gun, Chungcheongnam-do	Korea	Weedy type of var. *frutescens*
32	PF15-101	Dangjin-si, Chungcheongnam-do	Korea	Weedy type of var. *frutescens*
33	PF16-019	Yesan-gun, Chungcheongnam-do	Korea	Weedy type of var. *frutescens*
34	PF16-024	Hongseong-gun, Chungcheongnam-do	Korea	Weedy type of var. *frutescens*

Table 1. *Continued.*

No.	Accession no.	City and province	Country	Type
35	PF15-051	Eumseong-gun, Chungcheongbuk-do	Korea	Weedy type of var. *frutescens*
36	PF16-123	Muju-gun, Jeollabuk-do	Korea	Weedy type of var. *crispa*
37	PF16-126	Muju-gun, Jeollabuk-do	Korea	Weedy type of var. *crispa*
38	PF17-017	Imsil-gun, Jeollabuk-do	Korea	Weedy type of var. *crispa*
39	PF17-025	Sunchang-gun, Jeollabuk-do	Korea	Weedy type of var. *crispa*
40	PF17-039	Sunchang-gun, Jeollabuk-do	Korea	Weedy type of var. *crispa*
41	PF17-054	Damyang-gun, Jeollanam-do	Korea	Weedy type of var. *crispa*
42	PF17-062	Jangseong-gun, Jeollanam-do	Korea	Weedy type of var. *crispa*
43	PF17-068	Jangseong-gun, Jeollanam-do	Korea	Weedy type of var. *crispa*
44	PF18-006	Geumsan-gun, Chungcheongnam-do	Korea	Weedy type of var. *crispa*
45	PF18-007	Geumsan-gun, Chungcheongnam-do	Korea	Weedy type of var. *crispa*
46	PF18-011	Geumsan-gun, Chungcheongnam-do	Korea	Weedy type of var. *crispa*
47	PF18-012	Geumsan-gun, Chungcheongnam-do	Korea	Weedy type of var. *crispa*
48	PF19-001	Andong-si, Gyeongsangbuk-do	Korea	Weedy type of var. *crispa*
49	PF19-012	Andong-si, Gyeongsangbuk-do	Korea	Weedy type of var. *crispa*
50	PF19-045	Yeongcheon-si, Gyeongsangbuk-do	Korea	Weedy type of var. *crispa*
51	PF19-050	Yeongcheon-si, Gyeongsangbuk-do	Korea	Weedy type of var. *crispa*
52	PF19-054	Yeongcheon-si, Gyeongsangbuk-do	Korea	Weedy type of var. *crispa*

deposited in the National Agrobiodiversity Center, National Institute of Agricultural Sciences, Rural Development and Administration, Suwon, Republic of Korea, for permanent seed preservation.

2. Morphological characteristic survey

To evaluate the morphological variation between the *Perilla* and related weedy types from South Korea, the 52 accessions of the CWTP used for morphological investigation in this study were selected from germplasm accessions used for genetic variation analysis in a previous study by Fu *et al.* (2022).

To investigate the morphological characteristics, 10 seeds of each accession were sown in a seedling box at the end of May 2022 and kept in a glass greenhouse for one month. Then in early July 2022, five seedlings of each accession were transplanted into a field at the experimental farm of Kangwon National University.

One quantitative and 10 qualitative characteristics of each *Perilla* accession used in this study were selected on the basis of a previous report by Lee and Ohnishi (2001), and the specific information is shown in Table 2.

3. Data analysis

Principal component analysis (PCA) was performed using the NTSYS-pc V2.1 Program (Rohlf, 1998) to detect the differences among accessions of the CWTP.

Multivariate analysis was performed using the Microsoft Excel Statistical Analysis System program. IBM SPSS Statistics version 21 (IBM Co., Armonk, N.Y., USA) software was used to perform correlation analysis for the one quantitative and 10 qualitative characteristics in the 52 accessions of the CWTP.

RESULTS

1. Morphological characteristics among accessions of the CWTP collected from South Korea

The results of a survey of the morphological characteristics of the one quantitative and 10 qualitative characteristics of the 52 accessions of the CWTP collected from South Korea are shown in Table 3.

According to the survey results of the one quantitative trait, the average weight per 100 seeds (QN1) for the cultivated var. *frutescens*, the weedy var. *frutescens,* and

Table 2. Characteristics used in the morphological analysis of the CWTP collected from South Korea.

Abbreviation	Morphological character	Unit or Category
QN1	Weight per 100 seeds	g
QL1	Color of leaf adaxial side	Light green-1, green-3, deep green-5, green/purple-7, purple-9
QL2	Color of leaf abaxial side	Light green-1, green-3, deep green-5, green/purple-7, purple-9
QL3	Number of leaf teeth	Few-2, middle-3, many-4
QL4	Stem color	Light green-1, green-2, purple-3
QL5	Flower color	White-1, white/purple-2, purple-3
QL6	Plant fragrance	*Frutescens*-1, *crispa*-2, other-3
QL7	Leaf shape	Lanceolate-3, heart type-5, long oval-7, wrinkle-9
QL8	Seed color	Dark brown-1, brown-2, gray-3, white-4, mixed-5
QL9	Seed size	Large (> 2 ㎜)-1, small (2 ㎜ <)-2
QL10	Seed hardness	Hard-1, soft-2

*QN: quantitative character, QL: qualitative character.

Table 3. Mean values, standard deviation, range, and accession number for one quantitative and 10 qualitative characteristics among 52 accessions of the CWTP collected from different areas of South Korea.

Morphological Character	Cultivated var. *frutescens* (n = 21)	Weedy var. *frutescens* (n = 14)	Weedy var. *crispa* (n = 17)
QN1 (Weight per 100 seeds)	0.402±0.120 (0.297-0.721)	0.157±0.073 (0.095-0.307)	0.120±0.021 (0.091-0.166)
QL1 (Color of leaf adaxial side)	Light green (1*), green (17), deep green (3)	Green (7), deep green (7)	Green (1), deep green (3), green/purple (10), purple (3)
QL2 (Color of leaf abaxial side)	Light green (4), green (17)	Light green (3), green (8), purple (3)	Light green (1), purple (16)
QL3 (Number of leaf teeth)	Less (1), middle (13), more (7)	Less (9), middle (5), more (8)	Less (8), middle (7), more (2)
QL4 (Stem color)	Light green (12), green (7), purple (2)	Light green (5), green (4), purple (5)	Purple (17)
QL5 (Flower color)	White (21)	White (11), purple (3)	White (1), white/purple (3), purple (13)
QL6 (Plant fragrance)	*Frutescens* (19), other (2)	*Frutescens* (7), *crispa* (2), other (5)	*Frutescens* (1), *crispa* (13), other (3)
QL7 (Leaf shape)	Lanceolate (7), long oval (14)	Lanceolate (4), heart type (3), long oval (7)	Lanceolate (10), long oval (4), wrinkle (3)
QL8 (Seed color)	Dark brown (10), brown (7), gray (1), white (3)	Dark brown (12), brown (2)	Dark brown (12), brown (4), mixed (1)
QL9 (Seed size)	Large (21)	Small (14)	Small (17)
QL10 (Seed hardness)	Soft (21)	Hard (13), soft (1)	Hard (17)

*Accession number of 52 *Perilla* accessions for each character.

the weedy var. *crispa* was 0.402 g (0.297 g - 0.721 g), 0.157 g (0.095 g - 0.307 g), and 0.120 g (0.091 g - 0.166 g), respectively (Table 3).

Among the 10 qualitative characteristics, for the color of leaf adaxial side (QL1), one accession of cultivated var. *frutescens* was light green, 17 accessions were green, and three accessions were dark green; 7 accessions of weedy var. *frutescens* were green and 7 accessions were dark green; and the accessions of weedy var. *crispa* showed green (one accession), dark green (3 accessions), green/purple (10 accessions), and purple (3 accessions).

For color of leaf abaxial side (QL2), the accessions of cultivated var. *frutescens* showed light green (4 accessions) and green (17 accessions); the accessions of weedy var. *frutescens* showed light green (3 accessions), green (8 accessions), and purple (3 accessions); and the accessions of weedy var. *crispa* showed light green (1 accession) and purple (16 accessions).

For number of leaf teeth (QL3), the accessions of cultivated var. *frutescens* showed few (1 accession), middle (13 accessions), and many (7 accessions) types; the accessions of weedy var. *frutescens* showed few (9 accessions) and middle (5 accessions) types; and the accessions of weedy var. *crispa* showed few (8 accessions), middle (7 accessions), and many (2 accessions) types.

For stem color (QL4), the accessions of cultivated var. *frutescens* showed light green (12 accessions), green (7 accessions), and purple (2 accessions); the accessions of weedy var. *frutescens* showed light green (5 accessions), green (4 accessions), and purple (5 accessions); and the accessions of weedy var. *crispa* showed only purple (17 accessions).

For flower color (QL5), the accessions of cultivated var. *frutescens* showed only white (21 accessions); the accessions of weedy var. *frutescens* showed white (11 accessions) and purple (3 accessions); and the accessions of weedy var. *crispa* showed white (1 accession), white/purple (3 accessions), and purple (13 accessions).

For plant fragrance (QL6), the cultivated var. *frutescens* showed *frutescens* (19 accessions) and other (2 accessions) botanical aromas; the weedy var. *frutescens* showed *frutescens* (7 accessions), *crispa* (2 accessions), and other (5 accessions) botanical aromas; and the weedy var. *crispa* showed *frutescens* (1 accession), *crispa* (13 accessions), and other (3 accessions) botanical aromas.

For leaf shape (QL7), the cultivated var. *frutescens* showed lanceolate (7 accessions) and long oval (14 accessions) types; the weedy var. *frutescens* showed lanceolate (4 accessions), heart (3 accessions), and long oval (7 accessions) types; and the weedy var. *crispa* showed lanceolate (10 accessions), long oval (4 accessions),and wrinkle (3 accessions) types.

For seed color (QL8), the accessions of cultivated var. *frutescens* showed dark brown (10 accession), brown (7 accessions), gray (1 accession), and white (3 accessions); the accessions of weedy var. *frutescens* showed dark brown (12 accessions) and brown (2 accessions); and the accessions of weedy var. *crispa* showed dark brown (12 accessions), brown (4 accessions), and mixed (1 accession).

For seed size (QL9), all accessions of cultivated var. *frutescens* showed large seeds (21 accessions), while all weedy var. *frutescens* (14 accessions) and var. *crispa* (17 accessions) showed small seeds.

For seed hardness (QL10), the accessions of cultivated var. *frutescens* showed only soft (21 accessions) type, the accessions of weedy var. *frutescens* showed hard (13 accessions) and soft (1 accession) types, and the accessions of weedy var. *crispa* had only hard (17 accessions) type (Table 3).

2. Morphological differences among accessions of the CWTP collected from South Korea

Results of correlation analysis among the morphological characteristics of the 52 accessions of the CWTP collected from South Korea are shown in Table 4.

In our study, many morphological characteristics among the accessions of the CWTP showed statistically significant positive or negative correlation coefficients, with significance levels of 0.05 and 0.01.

The results showed that, among all morphological characteristics, the combinations between QN1 and QL10 (0.848^{**}); between QL1 and QL2 (0.838^{**}), QL4 (0.736^{**}), and QL5 (0.770^{**}); and between QL2 and QL4 (0.746^{**}) and QL5 (0.843^{**}) had the highest positive correlation coefficients compared with the other combinations, with significance levels of 0.01. Also the combinations between QN1 and QL9 (-0.838^{**}) and between QL9 and QL10 (-0.961^{**}) showed the highest negative correlation coefficients compared with the other combinations, with significance levels of 0.01.

In addition, the combinations between QL1 and QL6 (0.522^{**}) and QL9 (0.590^{**}); between QL2 and QL6 (0.557^{**}) and QL9 (0.583^{**}); between QL3 and QL10 (0.546^{**}); between QL4 and QL5 (0.630^{**}), QL6 (0.628^{**}), and QL9 (0.573^{**}); between QL5 and QL9 (0.606^{**}); and between QL6 and QL9 (0.511^{**}) showed comparatively higher positive correlation coefficients than the other combinations, with significance levels of 0.01.

Also the combinations between QN1 and QL1 (-0.553^{**}),

Table 4. Pearson correlation coefficient between one quantitative and 10 qualitative characteristics for 52 accessions of the CWTP.

Trait	QL1	QL2	QL3	QL4	QL5	QL6	QL7	QL8	QL9	QL10
QN1	–0.553**	–0.546**	0.364**	–0.499**	–0.598**	–0.303*	0.116	0.223	–0.838**	0.848**
QL1		0.838**	–0.281*	0.736**	0.770**	0.522**	0.021	–0.002	0.590**	–0.577**
QL2			–0.267	0.746**	0.843**	0.557**	–0.016	–0.121	0.583**	–0.602**
QL3				–0.401**	–0.239	–0.316*	–0.087	0.262	–0.540**	0.546**
QL4					0.630**	0.628**	–0.050	0.029	0.573**	–0.575**
QL5						0.418**	–0.035	–0.087	0.606**	–0.631**
QL6							0.173	–0.023	0.511**	–0.441*
QL7								0.112	–0.112	0.141
QL8									–0.282*	0.258
QL9										–0.961**

**; significant at $p < 0.01$. *; significant at $p < 0.05$ levels, respectively.

Table 5. Cumulative variances of first and second principal components and the loadings of one quantitative and 10 qualitative characteristics on each principal component.

Morphological character	Eigenvectors	
	C1	C2
QL9 (Seed size)	–0.884	–0.318
QL2 (Color of leaf abaxial side)	–0.854	0.286
QL1 (Color of leaf adaxial side)	–0.832	0.337
QL5 (Flower color)	–0.822	0.201
QL4 (Stem color)	–0.809	0.301
QL6 (Plant fragrance)	–0.641	0.339
QL7 (Leaf shape)	0.053	0.424
QL8 (Seed color)	0.206	0.672
QL3 (Number of leaf teeth)	0.530	0.323
QN1 (Weight per 100 seeds)	0.800	0.307
QL10 (Seed hardness)	0.883	0.330
Cumulative variance (%)	51.7	13.5

QL2 (–0.546**), and QL5 (–0.598**); between QL1 and QL10 (–0.577**); between QL2 and QL10 (–0.602**); between QL3 and QL9 (–0.540**); between QL4 and QL10 (–0.575**); between QL5 and QL10 (–0.631**); and QL9 and QL10 (–961**) showed comparatively higher negative correlation coefficients than the other combinations, with significance levels of 0.01. The remaining combinations showed lower positive or negative correlation coefficients, with significance levels of 0.01 and 0.05 (Table 4).

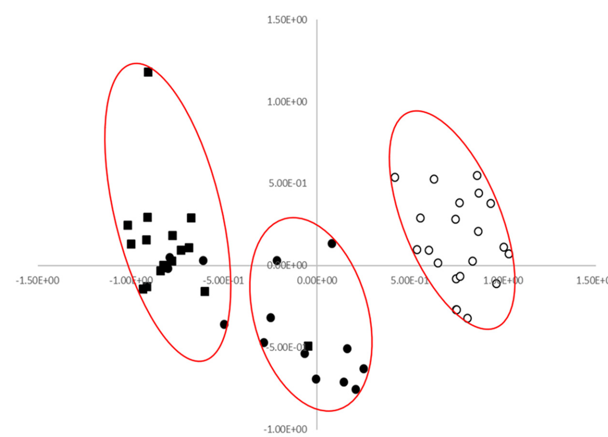

Fig. 1. Projection of 52 accessions of the CWTP collected from South Korea in the first (PC1) and second (PC2) principal components (○: accessions of cultivated var. *frutescens*; ●: accessions of weedy var. *frutescens*; ■: accessions of weedy var. *crispa*).

Meanwhile, with the PCA, the first and second principal components accounted for 51.7% and 13.5%, respectively, of the total variance (Table 5). For the first principal component in the PCA, one quantitative and most of the qualitative characteristics made a significant contribution in either the positive or negative direction of the first axis, such as QN1, QL1, QL2, QL4, QL5, QL9, and QL10 (Table 5).

Based on the first axis of the PCA (Fig. 1), all accessions of cultivated var. *frutescens* were located on the right side, while most accessions of weedy var. *frutescens* were located on the left side.

In addition, all accessions of weedy var. *crispa* were located on the left side of the first axis. Therefore, all accessions of cultivated var. *frutescens* were clearly separated from the accessions of weedy var. *crispa* on the first axis. In addition, all accessions of cultivated var. *frutescens* were relatively clearly separated from the accessions of weedy var. *frutescens*, and most of the accessions of weedy var. *frutescens* and var. *crispa* were clearly separated on the first axis by the PCA (Fig. 1).

DISCUSSION

In crop species, differences in morphological characteristic changes between native landraces and wild species in accordance with geographic distribution will provide taxonomically important clues to understanding the evolutionary or domestication process of a specific crop (Gould and Johnston, 1972; Wyatt and Antonovics, 1981; Ohnishi, 2001; Ma and Lee, 2017; Lee and Sa *et al.*, 2018).

In recent years, many taxonomic studies have been performed using morphological characteristics to distinguish CWTP accessions. Meanwhile, domestication is the evolutionary process from wild species to cultivated varieties, and domesticated crops are morphologically (such as seed size and plant size) and physiologically (such as seed dormancy and flowering time) different from their wild ancestors (Schwanitz, 1966; Hancock, 1992; Harlan, 1992; Ha *et al.*, 2021).

Morphological characteristics are easily affected by environmental conditions; therefore, relying on phenotypic traits is an inaccurate method for understanding the genetic variation within crop species. However, as described in the Introduction, molecular markers have been applied successfully for identifying and classifying plant germplasm resources.

Nonetheless, the identification and description of phenotypic morphological traits are still the most basic methods of genetic variation analysis among accessions of crop germplasm resources and cannot be replaced completely by molecular marker technology (Liao *et al.*, 2015).

Therefore, in this study, 52 accessions of *Perilla* and its weedy types collected from South Korea were used to investigate variation in morphological characteristics and classification of the CWTP.

In our study, one quantitative characteristic and 10 qualitative characteristics of 52 accessions of the CWTPC from South Korea were compared and analyzed as shown in Table 3.

For QN1, the accessions of cultivated var. *frutescens* showed significant differences compared with accessions of weedy var. *frutescens* and var. *crispa*. In recent years, *Perilla* seed oil, which contains various amino acids and has many health benefits, has attracted worldwide attention as a natural health care product.

Cultivated var. *frutescens* is widely cultivated and used in South Korea because it has much higher seed oil content than other oil crops. In particular, in East Asia the cultivated var. *frutescens*is considered as a highly domesticated crop by natural or human selection during the domestication process because the cultivated var. *frutescens* shows more variation in morphological characteristics such as seed size and weight compared with its weedy type (Lee and Ohnishi, 2003; Sa *et al.*, 2013). Thus, the significantly higher seed weight of cultivated var. *frutescens* compared with that of the two weedy types of *Perilla* was considered to be because of strong selection by farmers.

Meanwhile, the cultivated var. *crispa* is not cultivated at all in South Korea today, while the weedy var. *crispa* grows naturally. However, in Japan, the cultivated var. *crispa* is cultivated as a leaf vegetable by following traditional methods in which seedlings of cultivated var. *crispa* that sprouted in the field from seed shattering during autumn of the previous year are transplanted into fields for cultivation.

In South Korea, cultivated var. *frutescens* is cultivated by sowing the seeds harvested in the previous fall in the field (Nitta *et al.*, 2003; Sa *et al.*, 2013; Ha *et al.*, 2021). Therefore, the cultivation methods of the two cultivated types of *Perilla* in South Korea and Japan show a distinct difference.

The morphological differences between accessions of cultivated var. *frutescens* and weedy var. *crispa* (Table 3) were shown by the following qualitative characteristics: QL1, QL2, QL4, QL5, QL6, QL9, and QL10. The only significant differences between the cultivated and weedy types of var. *frutescens* were seed characteristics QL9 and QL10, and there were no significant morphological differences in the other remaining characteristics.

In addition, the morphological differences among the accessions of the two weedy types of var. *frutescens* and var. *crispa* were shown by QL1, QL2, and QL5. As suggested by Lee and Ohnishi (2001), the color of leaf adaxial side, color of leaf abaxial side, stem color, flower color, plant fragrance, seed size, and seed hardness were useful morphological characteristics for distinguishing between cultivated var. *frutescens* and cultivated var. *crispa*.

In particular, the cultivated var. *frutescens* and two weedy types of *Perilla* can be accurately distinguished based on the seed-related characteristics among these morphological characteristics. The different degrees of morphological difference among accessions of the CWTP collected from South Korea reflect the morphological diversity of the Korean *Perilla* germplasm accessions.

From the results of Pearson correlation coefficient analysis (Table 4), among the 11 morphological characteristics, the leaf characteristics showed the following correlation coefficients: the combinations between QL1 and QL2 (0.838^{**}), QL4 (0.736^{**}), and QL5 (0.770^{**}) and between QL2 and QL4 (0.746^{**}) and QL5 (0.843^{**}) showed the most statistically significant positive correlation coefficients compared with the other combinations at $p < 0.01$.

Among the seed characteristics, the combinations between QN1 and QL9 (-0.838^{**}) and QL10 (0.848^{**}) and between QL9 and QL10 (-0.961^{**}) showed the most statistically significant positive or negative correlation coefficients compared with the other combinations at $p < 0.01$.

In addition, the combinations between QL1 and QL6 (0.522^{**}) and QL9 (0.590^{**}); between QL2 and QL6 (0.557^{**}) and QL9 (0.583^{**}); between QL3 and QL10 (0.546^{**}); between QL4 and QL5 (0.630^{**}), QL6 (0.628^{**}), and QL9 (0.573^{**}); between QL5 and QL9 (0.606^{**}); and between QL6 and QL9 (0.511^{**}) showed statistically significant positive correlation coefficients. Also the combinations between QN1 and QL1 (-0.553^{**}), QL2 (-0.546^{**}), and QL5 (-0.598^{**}); between QL1 and QL10 (-0.577^{**}); between QL2 and QL10 (-0.602^{**}); between QL3 and QL9 (-0.540^{**}); between QL4 and QL10 (-0.575^{**}); and between QL5 and QL10 (-0.631^{**}) showed statistically significant negative correlation coefficients at a significance level of 0.01 (Table 4).

According to our current results, among the 11 morphological characteristics, most of the characteristics related to leaves and seeds showed statistically significant correlations among the accessions of the CWTP collected from South Korea. Therefore, traits related to leaves and seeds were found to be useful for distinguishing between accessions of the CWTP.

PCA can transform multiple variables into a few principal components. For further evaluation using the 11 morphological characteristics among the 52 accessions of the CWTP collected from South Korea, PCA was conducted (Table 5). Among all the principal components, the first and second principal components accounted for 51.7% and 13.5%, respectively, of the total variance (Table 5).

As the first principal component in the PCA, QN1, QL1, QL2, QL4, QL5, QL9, and QL10 contributed significantly to the positive or negative direction of the first axis (Table 5).

Therefore, as reported by Lee and Ohnishi (2001), these characteristics are considered useful characteristics for distinguishing between accessions of the CWTP.

In particular, on the first axis of the PCA, the accessions of cultivated var. *frutescens* and the two weedy types of *Perilla* collected from South Korea were relatively clearly separated. Also, most accessions of weedy var. *frutescens* and var. *crispa* were relatively clearly separated from each other by the first axis (Fig. 1).

The accessions of weedy var. *frutescens* are between the accessions of cultivated var. *frutescens* and weedy var. *crispa* on the first axis. Although a clear distinction was found by the PCA between accessions of cultivated and weedy types of *Perilla*, it was difficult to distinguish them clearly.

In recent years, there have been many studies of classification between var. *frutescens* and var. *crispa*. As reported in the Introduction, because there is an intermediate type between var. *frutescens* and var. *crispa*, it is impossible to distinguish fully between the two varieties of *Perilla* (Honda *et al.*, 1990; Lee and Ohnishi, 2001, 2003). The two cultivated types of var. *frutescens* and var. *crispa* can be crossed by artificial pollination (Nagai, 1935; Honda *et al.*, 1990; Honda *et al.*, 1994; Lim *et al.*, 2019; Kim *et al.*, 2021; Lim *et al.*, 2021).

In this study, there were a few special accessions of weedy var. *frutescens* similar to cultivated var. *frutescens* or weedy var. *crispa*. These accessions of weedy var. *frutescens* may be derived from escape forms from accessions of cultivated var. *frutescens*, or they may be hybrids between CWTP accessions (Nitta and Ohnishi, 1999; Lee *et al.*, 2002; Lee and Ohnishi, 2003; Lee and Kim, 2007; Sa *et al.*, 2013).

The morphological characteristics of the CWTP are highly variable and easily affected by environmental conditions; therefore, future taxonomic studies of *Perilla* species should be performed by combining molecular marker techniques and morphological characteristics.

This study provides useful information for future evaluation and classification of the CWTP collected from South Korea. Furthermore, based on morphological characteristics, we have observed that it is difficult to distinguish completely between the cultivated and weedy types of var. *frutescens*, as well as between the two weedy types of var. *frutescens* and var. *crispa*. Morphological characteristics can reflect the genetic variation of crop species.

In this study, by investigating the morphological characteristics of *Perilla* accessions collected from South Korea, we revealed the diversity of morphological characteristics among accessions of the CWTP. Therefore, the results of this study are expected to provide useful information for understanding the morphological variation of the CWTP genetic resources collected in Korea.

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