

Variation for Morphological Characters in Cultivated and Weedy Types of *Perilla frutescens* Britt. Germplasm

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Abstract - Morphological variation between cultivated and weedy types of *Perilla frutescens* var. *frutescens* and *P. frutescens* var. *crispa* were studied in 327 germplasm by examining 17 morphological characters. The germplasm between the two varieties were varied for their qualitative and quantitative characters. The seed coat color of cultivated *P. frutescens* var. *frutescens* is commonly light brown and brown while deep brown color was observed in the weedy type *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa*. The leaf size, cluster length, plant height, flower number per cluster and seed weight in cultivated *P. frutescens* var. *frutescens* were significantly ($P < 0.05$) different from weedy type *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa*. The cultivated *P. frutescens* var. *frutescens* exhibited significantly higher plant height (158.6 cm) compared to the weedy *P. frutescens* var. *crispa* (133.8 cm). Likewise, seed weight was significantly higher in cultivated (1.9 g) than in the weedy type of *P. frutescens* var. *frutescens* (1.6 g) and *P. frutescens* var. *crispa* (1.4 g). Principal component analysis (PCA) result showed that the first and second principal component cumulatively explained 86.6% of the total variation. The cultivated type *P. frutescens* var. *frutescens* and its weedy accessions were not clearly separated with *P. frutescens* var. *crispa* by PCA. Hence it requires the use of molecular markers for better understanding of their genetic diversity.

Key words - *Perilla* species, Plant genetic resources, Principal component, Qualitative and quantitative characters, Variation

Introduction

Perilla (*Perilla frutescens* Britt.) is a self-fertilizing crop in Labiatae which is widely cultivated in East Asia. There are two varieties of *Perilla*, classified based on their morphology and utilization. In Korea, *P. frutescens* var. *frutescens* Britt. is an oil crop and contains the highest content of α -linolenic acid which is one of the essential nutrients in diet (Choi, 2015). The essential oils content in perilla leaves, stem and roots were also reported by Lee *et al.* (1997). Leaves of *P. frutescens* var. *frutescens* are also used as a vegetable and for making pickles in Korea. In addition to two cultivated varieties, weedy type plants in both *frutescens* and *crispa* had also been reported in perilla (Nitta and Ohnishi, 1999; Lee

and Ohnishi, 2001). *Perilla* is supposed to be originated in East Asia (Nitta *et al.*, 2003) but it is probably originated from China (Nitta, 2001; Lee and Ohnishi, 2003). Generally, weedy types of both perilla types are found in such habitats as roadsides, wasteland, and marginal lands around farmer's field in East Asia (Lee and Ohnishi, 2001).

Perilla is one of the important cash crops in Korea. Due to increased meat consumption, the consumer's demand for perilla fresh leaves is increasing. The seeds of *P. frutescens* var. *frutescens* are commonly used as a flavor for traditional food in Korea, Japan, China and Nepal, but *P. frutescens* var. *crispa* is a Chinese medicine and vegetable in the Far East region (Nitta *et al.*, 2003). In addition to food value, perilla crop has some beneficial effects on soil properties. Previous study (Nam *et al.*, 2003) reported that inclusion of perilla in cropping system of barley and safflower improved the soil

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physical properties. *P. frutescens* var. *crispa* is continuously grown in Japan, Vietnam and South Asia. *P. frutescens* var. *frutescens* is grown by sowing the seed in spring in field or nursery bed for the seedlings preparation, but seedlings of *P. frutescens* var. *crispa* that germinated in the spring from naturally shattered seeds in the previous fall are transplanted into the main field. Sowing the seeds in fields and high temperature treatment and long-day treatment before harvest in *P. frutescens* var. *crispa* is a recent technique for leaf production (Lee and Ohnishi, 2001). The two perilla crops have different morphological characters. *P. frutescens* var. *frutescens* has large seed size and has soft seeds, has green leaves and stems, and a specific fragrance whereas *P. frutescens* var. *crispa* is smaller, hard seeds with its specific fragrance. The two varieties are cross-fertile (Honda *et al.*, 1994). Many taxonomic studies have been done so far to distinguish between the two perilla crops, however; it is still difficult to distinguish between the crops of cultivated and weedy types because of morphological similarity. In the study of Koezuka *et al.* (1985, 1986) and Honda *et al.* (1990), they mentioned that essential oils, anthocyanins, color and hardness of the seeds cannot be used as differentiation characters. Song *et al.* (2012) reported the genetic diversity among weedy and landraces of perilla (*P. frutescens* var. *frutescens*) using molecular markers.

National Agrobiodiversity Center (NAC) of RDA has been collecting perilla genetic resources from Korea in recent years. However, morphological variation and relationship among these accessions is still unknown. Despite the wide use of molecular markers in germplasm characterization in the present context, use of morphological descriptors has still important in genetic diversity analysis. In addition, morphological markers could greatly facilitate for the selection in breeding program and also used to develop quantitative estimates of genetic similarity and relationship between the cultivar of relatives (Key, 1988). The success of genetic conservation program also depends on the understanding the amount and distribution of genetic variation present in the gene pool. Therefore, to maximize the use of perilla germplasm for breeding program in Korea, it is necessary to study the morphological variation among the cultivated types of perilla and their weedy types. In this paper, we reported the result of

the morphological variation by studying eight qualitative and nine quantitative characters in 327 perilla (cultivated and weedy types) germplasm of Korea.

Materials and Methods

Plant materials

A total of 327 accessions of perilla germplasm (186 cultivated types of *P. frutescens* var. *frutescens*, 75 weedy types of *P. frutescens* var. *frutescens*, and 66 weedy types of *P. frutescens* var. *crispa*) were received from National Agrobiodiversity Center (NAC), RDA in 2015 (Table 1). All accessions were collected from Korea and germplasm were classified either cultivated or weedy types based on plant passport data. About 30 seeds of each accession were sown in plug trays in June 4, 2015 and kept in a glasshouse for a month and then, ten plants of each accession were transplanted in a field in July 6, 2015 at NAC, Jeonju, Jeollabuk Do. Qualitative traits evaluated were hypocotyl color, leaf color at surface and reverse surface, stem color, pubescence distribution, flower, anther and seed coat color. Quantitative traits evaluated were leaf length and width, plant height, flower cluster length, days to flowering, flower number per cluster, internodes per plant, branch per plant, and seed weight. The morphological characters (qualitative and quantitative), their measurement time and units are given in Table 2. Measurement of eight qualitative and nine quantitative characters were taken on three individual for each accession. Morphological characters (qualitative and quantitative) were recorded according to RDA descriptors developed in perilla crop (Table 2).

Statistical analysis

Frequency and percentage were used to analyze the qualitative characters whereas mean and standard deviation were used for quantitative data analysis. One-way analysis of variance (ANOVA) was done followed by Student-Newman-Keuls test for multiple comparisons of means among the accessions of cultivated types of perilla crop and their weedy types. Principal component analysis (PCA) was used to see the morphological similarity/or difference among the accessions of perilla species. The univariate and multivariate analysis

Table 1. Accessions of cultivated types of perilla crop and their weedy types collected from Korea

S. N.	Accessions	Type	S. N.	Accessions	Type
1	K126154	Cultivated type of var. <i>frutescens</i>	49	K248264	Cultivated type of var. <i>frutescens</i>
2	K126183	Cultivated type of var. <i>frutescens</i>	50	K248265	Cultivated type of var. <i>frutescens</i>
3	K141327	Cultivated type of var. <i>frutescens</i>	51	K248266	Cultivated type of var. <i>frutescens</i>
4	K141353	Cultivated type of var. <i>frutescens</i>	52	K248267	Cultivated type of var. <i>frutescens</i>
5	K141354	Cultivated type of var. <i>frutescens</i>	53	K248269	Cultivated type of var. <i>frutescens</i>
6	K141355	Cultivated type of var. <i>frutescens</i>	54	K248270	Cultivated type of var. <i>frutescens</i>
7	K141360	Cultivated type of var. <i>frutescens</i>	55	K248271	Cultivated type of var. <i>frutescens</i>
8	K151664	Cultivated type of var. <i>frutescens</i>	56	K248272	Cultivated type of var. <i>frutescens</i>
9	K177361	Cultivated type of var. <i>frutescens</i>	57	K248275	Cultivated type of var. <i>frutescens</i>
10	K178502	Cultivated type of var. <i>frutescens</i>	58	K248277	Cultivated type of var. <i>frutescens</i>
11	K178550	Cultivated type of var. <i>frutescens</i>	59	K248279	Cultivated type of var. <i>frutescens</i>
12	K204413	Cultivated type of var. <i>frutescens</i>	60	K248283	Cultivated type of var. <i>frutescens</i>
13	K204421	Cultivated type of var. <i>frutescens</i>	61	K248287	Cultivated type of var. <i>frutescens</i>
14	K227321	Cultivated type of var. <i>frutescens</i>	62	K248288	Cultivated type of var. <i>frutescens</i>
15	K227327	Cultivated type of var. <i>frutescens</i>	63	K248289	Cultivated type of var. <i>frutescens</i>
16	K227328	Cultivated type of var. <i>frutescens</i>	64	K248290	Cultivated type of var. <i>frutescens</i>
17	K227333	Cultivated type of var. <i>frutescens</i>	65	K248296	Cultivated type of var. <i>frutescens</i>
18	K227335	Cultivated type of var. <i>frutescens</i>	66	K248297	Cultivated type of var. <i>frutescens</i>
19	K227339	Cultivated type of var. <i>frutescens</i>	67	K248299	Cultivated type of var. <i>frutescens</i>
20	K227453	Cultivated type of var. <i>frutescens</i>	68	K248301	Cultivated type of var. <i>frutescens</i>
21	K227458	Cultivated type of var. <i>frutescens</i>	69	K248304	Cultivated type of var. <i>frutescens</i>
22	K248218	Cultivated type of var. <i>frutescens</i>	70	K248309	Cultivated type of var. <i>frutescens</i>
23	K248219	Cultivated type of var. <i>frutescens</i>	71	K248312	Cultivated type of var. <i>frutescens</i>
24	K248220	Cultivated type of var. <i>frutescens</i>	72	K248313	Cultivated type of var. <i>frutescens</i>
25	K248221	Cultivated type of var. <i>frutescens</i>	73	K248318	Cultivated type of var. <i>frutescens</i>
26	K248228	Cultivated type of var. <i>frutescens</i>	74	K248330	Cultivated type of var. <i>frutescens</i>
27	K248231	Cultivated type of var. <i>frutescens</i>	75	K248332	Cultivated type of var. <i>frutescens</i>
28	K248233	Cultivated type of var. <i>frutescens</i>	76	K248333	Cultivated type of var. <i>frutescens</i>
29	K248235	Cultivated type of var. <i>frutescens</i>	77	K248335	Cultivated type of var. <i>frutescens</i>
30	K248236	Cultivated type of var. <i>frutescens</i>	78	K248337	Cultivated type of var. <i>frutescens</i>
31	K248238	Cultivated type of var. <i>frutescens</i>	79	IT104267	Cultivated type of var. <i>frutescens</i>
32	K248240	Cultivated type of var. <i>frutescens</i>	80	K130830	Cultivated type of var. <i>frutescens</i>
33	K248242	Cultivated type of var. <i>frutescens</i>	81	IT108680	Cultivated type of var. <i>frutescens</i>
34	K248243	Cultivated type of var. <i>frutescens</i>	82	IT109136	Cultivated type of var. <i>frutescens</i>
35	K248244	Cultivated type of var. <i>frutescens</i>	83	IT113199	Cultivated type of var. <i>frutescens</i>
36	K248246	Cultivated type of var. <i>frutescens</i>	84	IT117082	Cultivated type of var. <i>frutescens</i>
37	K248247	Cultivated type of var. <i>frutescens</i>	85	IT117110	Cultivated type of var. <i>frutescens</i>
38	K248248	Cultivated type of var. <i>frutescens</i>	86	IT117126	Cultivated type of var. <i>frutescens</i>
39	K248249	Cultivated type of var. <i>frutescens</i>	87	IT117136	Cultivated type of var. <i>frutescens</i>
40	K248250	Cultivated type of var. <i>frutescens</i>	88	IT117156	Cultivated type of var. <i>frutescens</i>
41	K248251	Cultivated type of var. <i>frutescens</i>	89	IT117160	Cultivated type of var. <i>frutescens</i>
42	K248253	Cultivated type of var. <i>frutescens</i>	90	IT117175	Cultivated type of var. <i>frutescens</i>
43	K248256	Cultivated type of var. <i>frutescens</i>	91	IT117209	Cultivated type of var. <i>frutescens</i>
44	K248258	Cultivated type of var. <i>frutescens</i>	92	IT157406	Cultivated type of var. <i>frutescens</i>
45	K248259	Cultivated type of var. <i>frutescens</i>	93	IT157423	Cultivated type of var. <i>frutescens</i>
46	K248260	Cultivated type of var. <i>frutescens</i>	94	IT157426	Cultivated type of var. <i>frutescens</i>
47	K248261	Cultivated type of var. <i>frutescens</i>	95	IT157488	Cultivated type of var. <i>frutescens</i>
48	K248263	Cultivated type of var. <i>frutescens</i>	96	IT157491	Cultivated type of var. <i>frutescens</i>

Table 1. Continued

S. N.	Accessions	Type	S. N.	Accessions	Type
97	IT157495	Cultivated type of var. <i>frutescens</i>	147	IT185660	Cultivated type of var. <i>frutescens</i>
98	IT157498	Cultivated type of var. <i>frutescens</i>	148	IT195493	Cultivated type of var. <i>frutescens</i>
99	IT157502	Cultivated type of var. <i>frutescens</i>	149	IT195496	Cultivated type of var. <i>frutescens</i>
100	IT157506	Cultivated type of var. <i>frutescens</i>	150	IT195498	Cultivated type of var. <i>frutescens</i>
101	IT157559	Cultivated type of var. <i>frutescens</i>	151	IT207962	Cultivated type of var. <i>frutescens</i>
102	IT157584	Cultivated type of var. <i>frutescens</i>	152	IT208811	Cultivated type of var. <i>frutescens</i>
103	IT157586	Cultivated type of var. <i>frutescens</i>	153	IT208894	Cultivated type of var. <i>frutescens</i>
104	IT157593	Cultivated type of var. <i>frutescens</i>	154	IT208938	Cultivated type of var. <i>frutescens</i>
105	IT160582	Cultivated type of var. <i>frutescens</i>	155	IT221549	Cultivated type of var. <i>frutescens</i>
106	IT160588	Cultivated type of var. <i>frutescens</i>	156	IT221550	Cultivated type of var. <i>frutescens</i>
107	IT175835	Cultivated type of var. <i>frutescens</i>	157	IT221555	Cultivated type of var. <i>frutescens</i>
108	IT175849	Cultivated type of var. <i>frutescens</i>	158	IT103179	Cultivated type of var. <i>frutescens</i>
109	IT178637	Cultivated type of var. <i>frutescens</i>	159	IT104583	Cultivated type of var. <i>frutescens</i>
110	IT178647	Cultivated type of var. <i>frutescens</i>	160	IT108761	Cultivated type of var. <i>frutescens</i>
111	IT180485	Cultivated type of var. <i>frutescens</i>	161	IT111050	Cultivated type of var. <i>frutescens</i>
112	IT208917	Cultivated type of var. <i>frutescens</i>	162	IT112893	Cultivated type of var. <i>frutescens</i>
113	IT209212	Cultivated type of var. <i>frutescens</i>	163	IT113112	Cultivated type of var. <i>frutescens</i>
114	IT209215	Cultivated type of var. <i>frutescens</i>	164	IT117096	Cultivated type of var. <i>frutescens</i>
115	IT209216	Cultivated type of var. <i>frutescens</i>	165	IT117125	Cultivated type of var. <i>frutescens</i>
116	IT209217	Cultivated type of var. <i>frutescens</i>	166	IT117196	Cultivated type of var. <i>frutescens</i>
117	IT209335	Cultivated type of var. <i>frutescens</i>	167	IT117205	Cultivated type of var. <i>frutescens</i>
118	IT213778	Cultivated type of var. <i>frutescens</i>	168	IT157402	Cultivated type of var. <i>frutescens</i>
119	IT213791	Cultivated type of var. <i>frutescens</i>	169	IT157469	Cultivated type of var. <i>frutescens</i>
120	IT214471	Cultivated type of var. <i>frutescens</i>	170	IT157515	Cultivated type of var. <i>frutescens</i>
121	IT214477	Cultivated type of var. <i>frutescens</i>	171	IT157542	Cultivated type of var. <i>frutescens</i>
122	IT220498	Cultivated type of var. <i>frutescens</i>	172	IT157589	Cultivated type of var. <i>frutescens</i>
123	IT261880	Cultivated type of var. <i>frutescens</i>	173	IT178655	Cultivated type of var. <i>frutescens</i>
124	IT104597	Cultivated type of var. <i>frutescens</i>	174	IT180966	Cultivated type of var. <i>frutescens</i>
125	IT109580	Cultivated type of var. <i>frutescens</i>	175	IT180978	Cultivated type of var. <i>frutescens</i>
126	IT111080	Cultivated type of var. <i>frutescens</i>	176	IT195351	Cultivated type of var. <i>frutescens</i>
127	IT117061	Cultivated type of var. <i>frutescens</i>	177	IT195335	Cultivated type of var. <i>frutescens</i>
128	IT117062	Cultivated type of var. <i>frutescens</i>	178	IT185664	Cultivated type of var. <i>frutescens</i>
129	IT117072	Cultivated type of var. <i>frutescens</i>	179	IT185659	Cultivated type of var. <i>frutescens</i>
130	IT117090	Cultivated type of var. <i>frutescens</i>	180	IT185646	Cultivated type of var. <i>frutescens</i>
131	IT117127	Cultivated type of var. <i>frutescens</i>	181	IT185639	Cultivated type of var. <i>frutescens</i>
132	IT117129	Cultivated type of var. <i>frutescens</i>	182	IT209213	Cultivated type of var. <i>frutescens</i>
133	IT117149	Cultivated type of var. <i>frutescens</i>	183	IT209916	Cultivated type of var. <i>frutescens</i>
134	IT117161	Cultivated type of var. <i>frutescens</i>	184	IT214486	Cultivated type of var. <i>frutescens</i>
135	IT117202	Cultivated type of var. <i>frutescens</i>	185	K141326	Cultivated type of var. <i>frutescens</i>
136	IT157412	Cultivated type of var. <i>frutescens</i>	186	IT177137	Cultivated type of var. <i>frutescens</i>
137	IT157433	Cultivated type of var. <i>frutescens</i>	187	K015896	Weedy type of var. <i>frutescens</i>
138	IT157462	Cultivated type of var. <i>frutescens</i>	188	K015902	Weedy type of var. <i>frutescens</i>
139	IT157466	Cultivated type of var. <i>frutescens</i>	189	K135813	Weedy type of var. <i>frutescens</i>
140	IT157477	Cultivated type of var. <i>frutescens</i>	190	K135827	Weedy type of var. <i>frutescens</i>
141	IT157541	Cultivated type of var. <i>frutescens</i>	191	K041482	Weedy type of var. <i>frutescens</i>
142	IT157578	Cultivated type of var. <i>frutescens</i>	192	K169088	Weedy type of var. <i>frutescens</i>
143	IT175845	Cultivated type of var. <i>frutescens</i>	193	K169093	Weedy type of var. <i>frutescens</i>
144	IT175906	Cultivated type of var. <i>frutescens</i>	194	K169113	Weedy type of var. <i>frutescens</i>
145	IT175963	Cultivated type of var. <i>frutescens</i>	195	K169117	Weedy type of var. <i>frutescens</i>
146	IT178770	Cultivated type of var. <i>frutescens</i>	196	K169131	Weedy type of var. <i>frutescens</i>

Table 1. Continued

S. N.	Accessions	Type	S. N.	Accessions	Type
197	K169175	Weedy type of var. <i>frutescens</i>	246	K248311	Weedy type of var. <i>frutescens</i>
197	K169175	Weedy type of var. <i>frutescens</i>	247	K248314	Weedy type of var. <i>frutescens</i>
198	K178470	Weedy type of var. <i>frutescens</i>	247	K248314	Weedy type of var. <i>frutescens</i>
199	K178479	Weedy type of var. <i>frutescens</i>	248	K248319	Weedy type of var. <i>frutescens</i>
200	K178485	Weedy type of var. <i>frutescens</i>	249	K248320	Weedy type of var. <i>frutescens</i>
201	K178536	Weedy type of var. <i>frutescens</i>	250	K248321	Weedy type of var. <i>frutescens</i>
202	K178538	Weedy type of var. <i>frutescens</i>	251	K248325	Weedy type of var. <i>frutescens</i>
203	K227340	Weedy type of var. <i>frutescens</i>	252	K248326	Weedy type of var. <i>frutescens</i>
204	K227344	Weedy type of var. <i>frutescens</i>	253	K248327	Weedy type of var. <i>frutescens</i>
205	K227345	Weedy type of var. <i>frutescens</i>	254	K248334	Weedy type of var. <i>frutescens</i>
206	K227346	Weedy type of var. <i>frutescens</i>	255	K248336	Weedy type of var. <i>frutescens</i>
207	K227347	Weedy type of var. <i>frutescens</i>	256	K248340	Weedy type of var. <i>frutescens</i>
208	K227349	Weedy type of var. <i>frutescens</i>	257	K248341	Weedy type of var. <i>frutescens</i>
209	K227350	Weedy type of var. <i>frutescens</i>	258	K248347	Weedy type of var. <i>frutescens</i>
210	K227372	Weedy type of var. <i>frutescens</i>	259	K130985	Weedy type of var. <i>frutescens</i>
211	K227387	Weedy type of var. <i>frutescens</i>	260	K135849	Weedy type of var. <i>frutescens</i>
212	K227393	Weedy type of var. <i>frutescens</i>	261	K178518	Weedy type of var. <i>frutescens</i>
213	K227394	Weedy type of var. <i>frutescens</i>	262	K130956	Weedy type of var. <i>crispa</i>
214	K227400	Weedy type of var. <i>frutescens</i>	263	K130990	Weedy type of var. <i>crispa</i>
215	K227402	Weedy type of var. <i>frutescens</i>	264	K131010	Weedy type of var. <i>crispa</i>
216	K227404	Weedy type of var. <i>frutescens</i>	265	K131036	Weedy type of var. <i>crispa</i>
217	K227405	Weedy type of var. <i>frutescens</i>	266	K131044	Weedy type of var. <i>crispa</i>
218	K227406	Weedy type of var. <i>frutescens</i>	267	K131075	Weedy type of var. <i>crispa</i>
219	K227425	Weedy type of var. <i>frutescens</i>	268	K131084	Weedy type of var. <i>crispa</i>
220	K227432	Weedy type of var. <i>frutescens</i>	269	K131094	Weedy type of var. <i>crispa</i>
221	K227434	Weedy type of var. <i>frutescens</i>	270	K175269	Weedy type of var. <i>crispa</i>
222	K227435	Weedy type of var. <i>frutescens</i>	271	K178540	Weedy type of var. <i>crispa</i>
223	K227440	Weedy type of var. <i>frutescens</i>	272	K248241	Weedy type of var. <i>crispa</i>
224	K227446	Weedy type of var. <i>frutescens</i>	273	K248316	Weedy type of var. <i>crispa</i>
225	K227451	Weedy type of var. <i>frutescens</i>	274	K248317	Weedy type of var. <i>crispa</i>
226	K227456	Weedy type of var. <i>frutescens</i>	275	K130983	Weedy type of var. <i>crispa</i>
227	K248225	Weedy type of var. <i>frutescens</i>	276	K126185	Weedy type of var. <i>crispa</i>
228	K248226	Weedy type of var. <i>frutescens</i>	277	K126202	Weedy type of var. <i>crispa</i>
229	K248229	Weedy type of var. <i>frutescens</i>	278	K126218	Weedy type of var. <i>crispa</i>
230	K248230	Weedy type of var. <i>frutescens</i>	279	K130988	Weedy type of var. <i>crispa</i>
231	K248237	Weedy type of var. <i>frutescens</i>	280	K130995	Weedy type of var. <i>crispa</i>
232	K248239	Weedy type of var. <i>frutescens</i>	281	K135749	Weedy type of var. <i>crispa</i>
233	K248245	Weedy type of var. <i>frutescens</i>	282	K135759	Weedy type of var. <i>crispa</i>
234	K248252	Weedy type of var. <i>frutescens</i>	283	K135769	Weedy type of var. <i>crispa</i>
235	K248254	Weedy type of var. <i>frutescens</i>	284	K135804	Weedy type of var. <i>crispa</i>
236	K248262	Weedy type of var. <i>frutescens</i>	285	K135805	Weedy type of var. <i>crispa</i>
237	K248274	Weedy type of var. <i>frutescens</i>	286	K135821	Weedy type of var. <i>crispa</i>
238	K248280	Weedy type of var. <i>frutescens</i>	287	K135842	Weedy type of var. <i>crispa</i>
239	K248285	Weedy type of var. <i>frutescens</i>	288	K135862	Weedy type of var. <i>crispa</i>
240	K248293	Weedy type of var. <i>frutescens</i>	289	K135879	Weedy type of var. <i>crispa</i>
241	K248294	Weedy type of var. <i>frutescens</i>	290	K135892	Weedy type of var. <i>crispa</i>
242	K248298	Weedy type of var. <i>frutescens</i>	291	K135903	Weedy type of var. <i>crispa</i>
243	K248300	Weedy type of var. <i>frutescens</i>	292	K135906	Weedy type of var. <i>crispa</i>
244	K248307	Weedy type of var. <i>frutescens</i>	293	K135923	Weedy type of var. <i>crispa</i>
245	K248308	Weedy type of var. <i>frutescens</i>	294	K169077	Weedy type of var. <i>crispa</i>

Table 1. Continued

S. N.	Accessions	Type	S. N.	Accessions	Type
295	K169107	Weedy type of var. <i>crispa</i>	312	K248291	Weedy type of var. <i>crispa</i>
296	K169111	Weedy type of var. <i>crispa</i>	313	K248295	Weedy type of var. <i>crispa</i>
297	K169133	Weedy type of var. <i>crispa</i>	314	K248302	Weedy type of var. <i>crispa</i>
298	K169144	Weedy type of var. <i>crispa</i>	315	K248303	Weedy type of var. <i>crispa</i>
299	K178467	Weedy type of var. <i>crispa</i>	316	K248315	Weedy type of var. <i>crispa</i>
300	K178529	Weedy type of var. <i>crispa</i>	317	K248328	Weedy type of var. <i>crispa</i>
301	K178549	Weedy type of var. <i>crispa</i>	318	K248329	Weedy type of var. <i>crispa</i>
302	K178551	Weedy type of var. <i>crispa</i>	319	K248331	Weedy type of var. <i>crispa</i>
303	K178556	Weedy type of var. <i>crispa</i>	320	K248338	Weedy type of var. <i>crispa</i>
304	K227343	Weedy type of var. <i>crispa</i>	321	K248339	Weedy type of var. <i>crispa</i>
305	K248227	Weedy type of var. <i>crispa</i>	322	K248343	Weedy type of var. <i>crispa</i>
306	K248234	Weedy type of var. <i>crispa</i>	323	K248346	Weedy type of var. <i>crispa</i>
307	K248268	Weedy type of var. <i>crispa</i>	324	K131007	Weedy type of var. <i>crispa</i>
308	K248273	Weedy type of var. <i>crispa</i>	325	K131054	Weedy type of var. <i>crispa</i>
309	K248276	Weedy type of var. <i>crispa</i>	326	K178500	Weedy type of var. <i>crispa</i>
310	K248281	Weedy type of var. <i>crispa</i>	327	K227341	Weedy type of var. <i>crispa</i>
311	K248282	Weedy type of var. <i>crispa</i>			

Table 2. Characters used in the morphological analysis of perilla germplasm

Characters	Growth stage	Unit or category
Qualitative characters		
Hypocotyl color	at seedling stage	1-green, 2-light purple, and 3-purple
Color of leaf surface	at vegetative stage	1-green, 2-deep green, 3-light green, 4-green purple, and 5- purple
Color of reverse side of leaf	at vegetative stage	1-green, 2 -light green, 3-green purple, 4-purple and 5- purple green
Pubescence distribution	at flowering stage	1-normal, 2-few, and 3-many
Stem color	at flowering stage	1-green, 2-light green, 3-deep green, 4-light purple, 5-green purple, 6-purple green, 7-purple and 8-yellow green
Flower color	at flowering stage	1-white, 2-purple, and 3-light pink
Anther color	at flowering stage	1-white and 2-purple
Seed coat color	after harvest	1-brown, 2-deep brown, 3-light brown, 4-grey white, 5-grey and 6-mixed
Quantitative characters		
Leaf length	at flowering stage	cm
Leaf width	at flowering stage	cm
Plant height	at harvest	cm
Flower cluster length	at flowering stage	cm
Days to flowering	at flowering stage	day
Flower/cluster	at flowering stage	no.
Internodes/plant	at flowering stage	no.
Branch/plant	at harvest	no.
Seed weight	at harvest	g

were performed using the Microsoft Excel Statistical Analysis System (version 10.0, Microsoft, Redmond, WA, USA).

Results and Discussion

Qualitative characters

Variation on qualitative characters such as hypocotyl, leaf color (upper and reverse surface), pubescence distribution, stem, flower, anther and seed coat color was observed in 327 perilla accessions of cultivated and weeds types of *P. frutescens* var. *frutescens* and *crispa*, respectively. Hypocotyl color was green in 83.3% of total accessions in *P. frutescens* var. *frutescens* whereas light purple and purple color contained in 73.3 and 69.9% of accessions in weedy types of *P. frutescens* var. *frutescens* and *crispa*, respectively (Fig. 1A). Leaf color at upper surface was green (95.1 and 93.3% of accession) in cultivated and weedy types *P. frutescens* var. *frutescens* but the frequency of green purple in weedy types of *P. frutescens* var. *crispa* was 54.5% (Fig. 1B). Similarly, green leaf color at reverse surface was observed in 94.6, 93.3 and 69.6% of total accession in cultivated, weedy types of *P. frutescens* var. *frutescens* and *crispa*, respectively (Fig. 1C). Frequency distribution of light green stem color was the highest (68.3% of accession) in cultivated *P. frutescens* var. *frutescens* but the green and green purple colors were 48.0 and 33.3% of total accession in weedy of *P. frutescens* var. *frutescens* and var. *crispa* (Fig. 1D). Few leaf pubescences were predominantly distributed in all the studied perilla species (Fig. 1E). White flower distributed in cultivated and weedy *P. frutescens* var. *frutescens* was 98.9 and 90.6 %, respectively, whereas, purple color was distributed in 53% of total accessions in weedy types of *P. frutescens* var. *crispa* (Fig. 1F). Frequency of white anther color was observed 98.9% accession of cultivated type *P. frutescens* var. *frutescens* while purple anther was observed in 48.0 and 74.6% of accessions of weedy type *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa*, respectively (Fig. 1G). Cultivated *P. frutescens* var. *frutescens* had 46.7% light brown seed coat color. Weedy type of *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa* had deep brown (85.3% and 92.4 % of accession) seed coat color. Kim *et al.* (2005) reported 55% of perilla local germplasm containing brown seed coat color. We observed

six types of seed color i.e. brown, deep brown, light brown, grey white, grey and mixed in cultivated type *P. frutescens* var. *frutescens* but Jung *et al.* (2008) reported four different seed coat color i.e. white, gray, brown and dark brown type. Morphological descriptors had been used to distinguish between the cultivated and weed types of *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa* by previous studies (Makino, 1961; Lee and Ohnishi, 2001; Jung *et al.*, 2008).

Quantitative characters

Nine quantitative characters measured in 327 accessions of cultivated and weedy types of *P. frutescens* var. *frutescens*, and *P. frutescens* var. *crispa* were summarized in Table 3. Accessions of cultivated *P. frutescens* var. *frutescens* exhibited an average leaf length of 16.4±1.2 cm, with a range from 12.7 to 21.3 cm. Weedy accessions of *P. frutescens* var. *frutescens* showed an average leaf length of 14.7±2.5 cm with a range from 11.1 to 28.3 cm while weedy accessions of var. *crispa* were averaged 13.6±2.0 cm had a range from 9.7 to 18.6 cm. Average leaf width of cultivated *P. frutescens* var. *frutescens* was higher than weedy type of *P. frutescens* var. *frutescens* and *crispa*. Accessions of cultivated *P. frutescens* var. *frutescens* had an average flower cluster length of 9.6±2.3 cm with a range from 6.1 to 25.0 cm while weedy accessions of *P. frutescens* var. *frutescens* were 12.8±2.8 cm with a range from 5.2 to 18.2 cm. Accession of weedy *P. frutescens* var. *crispa* showed an average cluster length of 14.9±4.9 cm with a range from 8.0 to 34.0 cm. Nam *et al.* (2004) were also reported the flower cluster length ranging from 5.1 to 9 cm in local perilla germplasm. Flower cluster length ranging from 6 to 20 cm was reported in perilla germplasm (Nam *et al.* 2003). Tall plants (158.6±15.1 cm) were measured in cultivated type of *P. frutescens* var. *frutescens* with a range from 96.7 to 188.3 cm followed by the weedy type of *P. frutescens* var. *frutescens* (151.1±18.6 cm) with a range from 88.3 to 181.7 cm. Cultivated accessions *P. frutescens* var. *frutescens* showed average flowering day of 82.3±5.2 with a range from 57.0 to 94.0 while accessions of weedy type *P. frutescens* var. *frutescens* were averaged 86.1±7.5 flowering day with a range from 63.0 to 104.0. Flowering days exhibited high variability in weedy type of *P. frutescens* var. *frutescens*. Accession of cultivated type *P. frutescens* var. *frutescens*

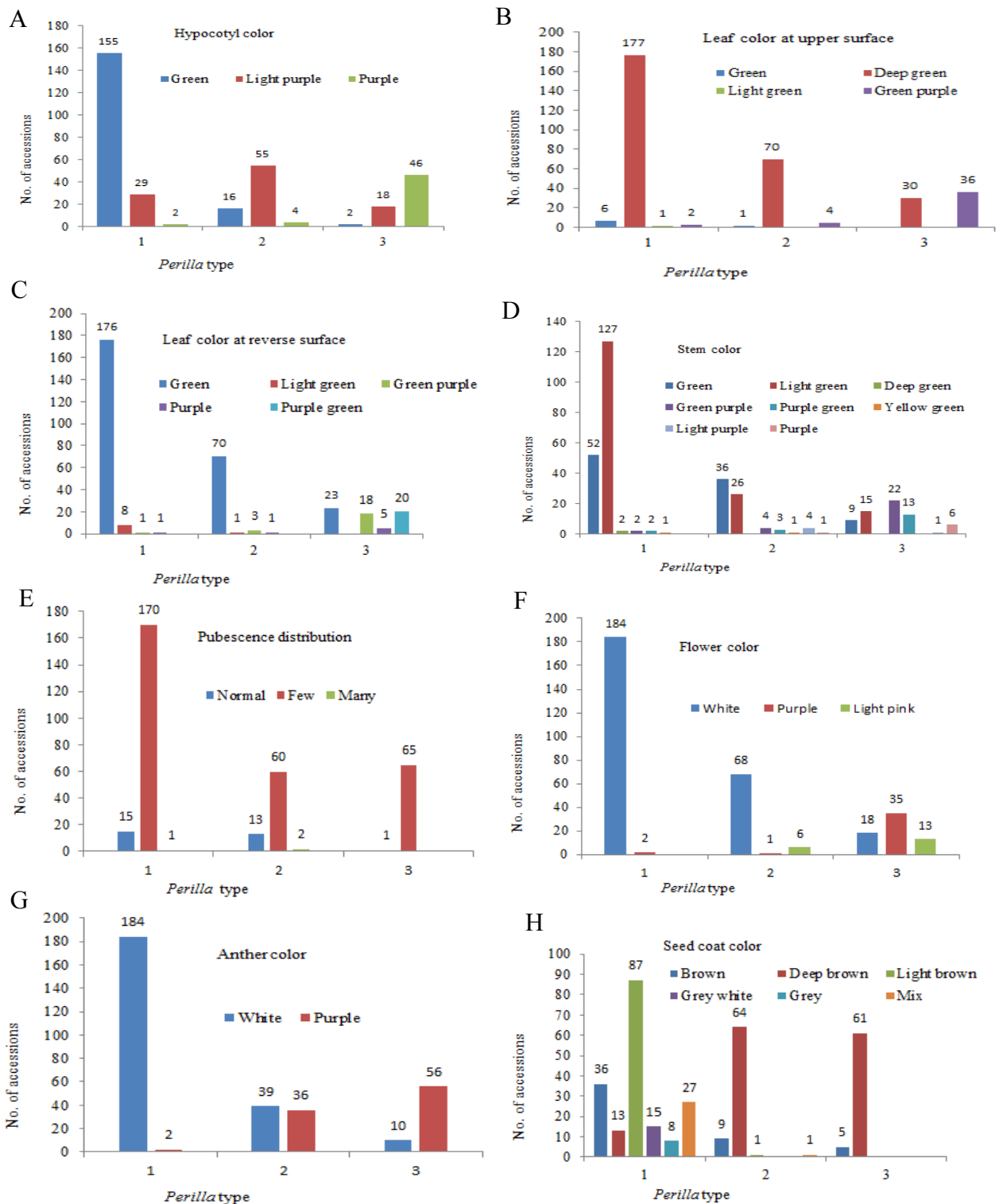


Fig. 1. Frequency distribution of qualitative characters A; Hypocotyl color, B; Leaf color at upper surface, C; Leaf color at reverse surface, D; Stem color, E; Pubescence distribution, F; Flower color, G; Anther color, and H; Seed coat color. Perilla type 1, 2, and 3 indicate cultivated and weedy type of *P. frutescens* var. *frutescens*, and *P. frutescens* var. *crispa*, respectively.

Table 3. Mean, standard deviation and range for nine quantitative characters of cultivated and weedy types of 327 perilla crop accessions in Korea

Characters	<i>Perilla frutescens</i> var. <i>frutescens</i>		<i>Perilla frutescens</i> var. <i>crispa</i>
	Cultivated (N = 186) ^z	Weedy (N = 75) ^z	Weedy (N = 66) ^z
Leaf length (cm)	16.4 ± 1.2a ^y (12.7-21.3)	14.7 ± 2.1b (11.1-28.3)	13.6 ± 2.0c (9.7-18.6)
Leaf width (cm)	12.9 ± 1.2a (8.2-17.0)	11.9 ± 1.3b (9.0-14.8)	10.8 ± 1.6c (7.0-15.6)
Cluster length (cm)	9.6 ± 2.3c (6.1-25.0)	12.8 ± 2.8b (5.2-18.2)	14.9 ± 4.9a (8.0-34.0)
Plant height (cm)	158.6 ± 15.1a (96.7-188.3)	151.1 ± 18.6b (88.3-181.7)	133.8 ± 15.1c (98.3-168.3)
Days to flowering	82.3 ± 5.2ab (57.0-94.0)	86.1 ± 7.5a (63.0-104.0)	80.5 ± 7.2bc (62.0-96.0)
Flower/cluster (no.)	31.0 ± 6.1c (16.3-58.3)	35.2 ± 6.5ab (17.0-56.7)	37.6 ± 9.2a (19.7-65.7)
Internodes/branch (no.)	15.1 ± 2.0ab (9.7-20.0)	16.0 ± 2.4a (9.3-21.0)	14.8 ± 2.1bc (7.7-19.3)
Branch /plant (no.)	22.8 ± 3.5ab (10.3-33.0)	23.3 ± 4.2a (11.3-34.7)	21.4 ± 3.8c (9.7-28.7)
Seed weight (g)	1.9 ± 0.4a (1.0-3.0)	1.6 ± 0.2b (1.2-2.0)	1.4 ± 0.2c (1.0-1.7)

^zN = Number of germplasm.

^yValues are presented as mean ± SD (standard deviation). Means in the rows for each character followed by different letter are significantly different at $P < 0.05$.

showed average flower cluster number (31.0±6.1) with a range from 16.3 to 58.3. Meanwhile, weedy type *P. frutescens* var. *frutescens* had an average number of 35.2±6.5 flower per cluster with a range from 17.0 to 56.7. Weedy type of *P. frutescens* var. *crispa* had an average number of 37.6±9.2 flower per cluster with a range from 19.7 to 65.7. Accessions of cultivated type *P. frutescens* var. *frutescens* had averaged 15.1±2.0 internodes with a range from 9.7 to 20.0 while weedy type *P. frutescens* var. *frutescens* had an average value of 16.0±2.4 with a range from 9.3 to 21.0 cm. Weedy type of *P. frutescens* var. *crispa* exhibited an average of 14.8 internodes with a range from 7.7 to 19.3. Cultivated accessions *P. frutescens* var. *frutescens* showed an average value of 22.8±3.5 branches with a range from 10.3 to 33.0 while weedy accessions *P. frutescens* var. *frutescens* were averaged the value of 23.3±4.2 with a range from 11.3 to 34.7. Weedy type *P. frutescens* var. *crispa* had an average of 21.4±3.8 with a range from 9.7 to 28.7. Seed weight of cultivated type *P. frutescens* var. *frutescens* showed an average value of 1.9±0.4

g with a range from 1.0 to 3.0 g while accessions of weed type *P. frutescens* var. *frutescens* had an average of 1.6±0.2 g weight with a range from 1.1 to 2.0 g. Seed weight ranged from 1.7 to 3.8 g in local perilla germplasm was also reported by Nam *et al.* (2004). In another study of Nam *et al.* (2003), they reported the seed weight from 3.1 to 4.0 g in the perilla collections in southern region of Korea. As compared to cultivated and weedy type of *P. frutescens* var. *frutescens*, the seed weight was lower (1.4 ± 0.2 g) in weedy type of *P. frutescens* var. *crispa*. The small seed size is a characteristic of weedy types of *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa* (Lee *et al.*, 2002). Quantitative variation was observed in the perilla germplasm of China and Japan (Seong *et al.*, 2015). In the study of Kim *et al.* (2005), they reported the average seed weight of 3.9 g in Hamyang perilla collections, while it was averaged 1.7 g in Pyeongchang collections.

Statistical comparisons of means for nine quantitative characters of each species are given in Table 3. There were

significant ($P < 0.05$) differences between cultivated and weedy type of *P. frutescens* var. *frutescens* for leaf length, width, cluster length, plant height, flower number per cluster, and seed weight. Likewise, significant differences were found between cultivated type *P. frutescens* var. *frutescens* and weedy type *P. frutescens* var. *crispa*, for leaf length, width, cluster length, plant height, flower number per cluster, branch number per plant and seed weight ($P < 0.05$). However, significant differences were found for leaf length, width, cluster length, plant height, days to flowering, internodes number per branch, branch number per plant and seed weight between weedy type *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa*. Days to flowering and internodes number per branch were not significantly different between cultivated type *P. frutescens* var. *frutescens*, weedy type of *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa*. But branch number per plant was not significantly different between cultivated *P. frutescens* var. *frutescens* and weedy type of *P. frutescens* var. *frutescens*. Flower number per cluster was not significantly different between weedy types of *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa*. Overall, leaf length and width, plant height and seed weight were significantly higher in cultivated *P. frutescens* var. *frutescens* than others. Shortest cluster length found in cultivated *P. frutescens* var. *frutescens* which can be used to differentiate cultivated type *P. frutescens* var. *frutescens* from other species of perilla crop. In our study, cultivated type of *P. frutescens* var. *frutescens* showed the highest plant height and which in agreement with the previous reports (Jung *et al.*, 2008; Lee *et al.*, 2002). They suggested that plant height can be reliable characters for the discrimination between cultivated *P. frutescens* var. *frutescens* and cultivated *P. frutescens* var. *crispa* or between cultivated *P. frutescens* var. *frutescens* and its weedy type. In our study, flower number per cluster showed the lowest in cultivated *P. frutescens* var. *frutescens* but Nam *et al.* (2004) reported the cluster per flower range from 23 to 120 in local perilla germplasm. The significant differences in seed weight between perilla species are the most reliable characters to discriminate between cultivated *P. frutescens* var. *frutescens* and weedy type of *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa*. Our study showed the significant

differences on days to flowering, internodes number per branch and branch number per plant between weedy type of *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa* but in the study of Jung *et al.* (2008), they were not reported the significant result of above characters among the perilla species. Lee and Ohnishi (2001) had also identified seed size, color, color of leaf surface and reverse leaf side as important traits to discriminate between cultivated and weedy type perilla.

Multivariate analyses for morphological characters

PCA analyses revealed that the first and second principal components accounted for 74.49 % and 12.48% of total variance, respectively (Table 4). Most of qualitative characters except seed coat color contributed in negative direction whereas most of quantitative characters except flower cluster length and flower number per cluster contributed in the positive direction in the first axis. Out of nine quantitative traits, plant height greatly contributed in the positive direction on the first axis. But flower number per cluster, flower cluster length and plant height greatly contributed in the positive direction in the second axis. But third principal component explained 7.63% of total variance and days to flowering contributed greatly to this variation. Thus, the first axis could mainly be utilized for distinction between cultivated *P. frutescens* var. *frutescens* and weedy *P. frutescens* var. *crispa*. Along the first axis of PCA analysis (Fig. 2), *P. frutescens* var. *frutescens*, and *P. frutescens* var. *crispa* exhibited a clear separation and most of the accessions of cultivated *P. frutescens* var. *frutescens* formed a group on the positive side and its few accessions were scattered in negative side. Most of the accessions of weedy type *P. frutescens* var. *frutescens* were in positive side, closely mixed with cultivated *P. frutescens* var. *frutescens* and few accessions of its weedy type were negative side, closely adjoined with weedy type *P. frutescens* var. *crispa*. Because of morphological similarity of weedy type *P. frutescens* var. *frutescens*, many accessions were found to be closely related with cultivated *P. frutescens* var. *frutescens* and its few accessions found to be closely related to *P. frutescens* var. *crispa*. All the accessions of weedy type *P. frutescens* var. *crispa* were grouped in negative side. Few weedy accessions of *P. frutescens* var. *frutescens*

Table 4. Eigenvector and eigenvalues generated by PCA applied on 17 morphological characters of 327 perilla crop accessions

Morphological characters	Eigenvectors				
	PC 1	PC 2	PC 3	PC 4	PC 5
Hypocotyl color	-0.018	0.012	0.045	0.022	0.142
Color of leaf surface	-0.014	0.006	0.027	0.024	0.085
Color of reverse side of leaf	-0.026	0.008	0.034	0.039	0.184
Stem color	-0.041	0.015	0.043	0.014	0.244
Pubescence distribution	0.000	0.002	-0.001	0.001	0.012
Flower color	-0.013	0.001	0.023	0.016	0.075
Anther color	-0.011	0.007	0.032	0.011	0.082
Seed coat color	0.015	-0.025	-0.050	-0.021	-0.142
Leaf length	0.053	-0.044	-0.043	-0.123	-0.301
Leaf width	0.034	-0.040	-0.052	-0.139	-0.287
Plant height	0.969	0.175	-0.125	-0.058	0.085
Flower cluster length	-0.083	0.363	0.053	0.062	0.717
Days to flowering	0.141	-0.217	0.917	-0.288	0.032
Flower/cluster	-0.111	0.885	0.235	-0.047	-0.352
Internodes/plant	0.055	-0.027	0.150	0.386	-0.024
Branch/plant	0.105	-0.042	0.222	0.848	-0.160
Seed weight	0.005	-0.005	-0.018	-0.030	-0.037
Eigenvalue	365.16	61.21	37.44	10.9	5.4
Total variation explained (%)	74.49	12.48	7.63	2.23	1.11

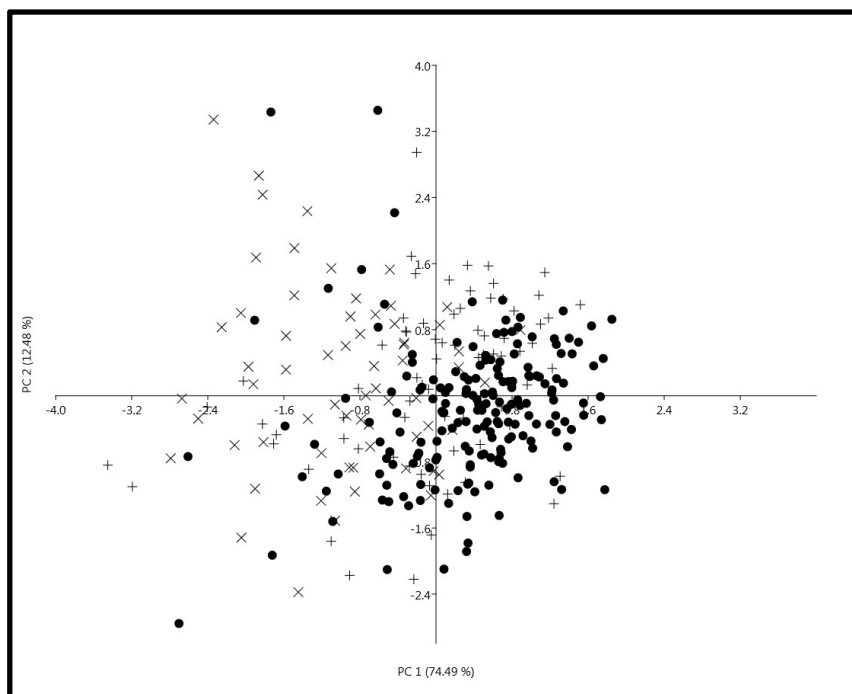


Fig. 2. Scatter diagram of 327 accessions perilla for the first (PC1) and second (PC2) principal component produced by analysis of 17 morphological characters. ● = cultivated type of *P. frutescens* var. *frutescens*; + = weedy type of *P. frutescens* var. *frutescens*; and × = weedy type of *P. frutescens* var. *crispa*.

were so closely grouped with weedy samples of *P. frutescens* var. *crispa* that these two weedy types could not be discriminated and such weedy type of *P. frutescens* var. *frutescens* may be originated from different multiple sources. Lee and Ohnishi (2003) reported the similar results in the study of cultivated and weedy types of perilla. Our result also could not discriminate the few accessions of cultivated type *P. frutescens* var. *frutescens*, its weedy type and weedy type of *P. frutescens* var. *crispa* using the morphological descriptors. The cultivated type of *P. frutescens* var. *crispa* might not be completely domesticated and a wild form has been used for cultivation. Generally, seed dormancy is an important character that is strongly selected against during domestication and it should be present in cultivated crops (Hancock, 1992). Overall, PCA analysis did not separate clearly between the cultivated, weedy type of *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa* and similar findings had been reported by Kim *et al.* (2013) and Jung *et al.* (2008).

In this study, most of the accessions belonging to cultivated *P. frutescens* var. *frutescens* tended to group together and were closed with weedy type *P. frutescens* var. *frutescens*. But some of the accessions of weedy *P. frutescens* var. *frutescens* were much closed with weedy type *P. frutescens* var. *crispa*. In the study of Nitta *et al.* (2003), they reported that the weedy forms were similar to *P. frutescens* var. *crispa* and *P. frutescens* var. *frutescens* are genetically closely related to *P. frutescens* var. *crispa*. This might be reason of some cultivated *P. frutescens* var. *frutescens* was closely related to *P. frutescens* var. *crispa* type in this study. Also, these accessions may be the hybrids between *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa*, and these accessions need to be studied in more detail for their taxonomic position and origin. The analysis of the two perilla crops and their weedy form indicated that the cultivated varieties are genetically well differentiated from weedy type and that few accessions of the weedy type *P. frutescens* var. *frutescens* form are genetically intermediate between the two crops, however, it is still obscure whether the weedy forms result from crossing between the two crops, escaped crops or ancestor of the crops (Nitta and Ohnishi, 1999).

To conclude, hypocotyl, leaf, flower and seed coat color, plant height, cluster length and seed weight found the major

morphological traits to discriminate between cultivated, weedy types of *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa*. Our results based on morphological analysis revealed mostly the similarity between cultivated type *P. frutescens* var. *frutescens* and its weedy type but showed significant differences for leaf size, cluster length, plant height, flower number per cluster, branch number per plant and seed weight with the weedy *P. frutescens* var. *crispa*. This study could not clearly differentiate between varieties of *P. frutescens* var. *frutescens* and its weedy type, and weedy type of *P. frutescens* var. *frutescens* with weedy type *P. frutescens* var. *crispa* and, there might be some intraspecific relationship. Hence, in addition to morphological analysis, this study further recommends the use of molecular marker to assess the genetic diversity between cultivated, weedy type of *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa* in detail.

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