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Genetic diversity and relationship of chicory (*Cichorium intybus* L.) using sequence-related amplified polymorphism markers

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ABSTRACT Chicory is a crop with economically important roles and is cultivated worldwide. The genetic diversity and relationship of 80 accessions of chicories and endives were evaluated by sequence-related amplified polymorphism (SRAP) markers to provide a theoretical basis for future breeding programs in China. The polymorphic rate was 96.83%, and the average polymorphic information content was 0.323, suggesting the rich genetic diversity of chicory. The genetic diversity degree of chicory was higher (G_S = 0.677) than that of endive (G_S = 0.701). The accessions with the highest genetic diversity (effective number of alleles, N_E = 1.609; Nei's genetic diversity, H = 0.372; Shannon information index, I = 0.556) were from Italy. The richest genetic diversity was revealed in a chicory line (N_E = 1.478, H = 0.289, I = 0.443) among the

3 types (line, wild, and cultivar). The chicory genetic structure of 8 geographical groups showed that the genetic differentiation coefficient (G_{ST}) was 14.20% and the number of immigrants per generation (N_m) was 3.020. A G_{ST} of 6.80% and an N_m of 6.853 were obtained from different types. This observation suggests that these chicory lines, especially those from the Mediterranean region, have potential for providing rich genetic resources for further breeding programs, that the chicory genetic structure among different countries obviously differs with a certain amount of gene flow, and that SRAP markers could be applied to analyze genetic relationships and classifications of *Cichorium intybus* and *C. endivia*.

Key words: Chicory; Cluster analysis; Genetic diversity; Genetic relationship; Sequence-related amplified polymorphism

INTRODUCTION

Chicory (*Cichorium intybus*; Asteraceae) is a diploid (2n = 18) and self-incompatible taproot perennial herb plant that is distributed in temperate and semi-arid regions of the world (Kiers et al., 2000; Hauser et al., 2012). It is widely cultivated as forage, vegetables, coffee substitutes, and traditional herbal medicine. Therefore, it is considered to be an important economic plant with extreme potential for future development (Bais and Ravishankar, 2001; de Kraker et al., 2001; Sulas, 2004; Nandagopal and Kumari, 2007). As forage, chicory is rich in nutrition, yields, palatability, digestibility, resistance to adversity, and pest resistance (de Kraker et al., 2002; Sulas, 2004; Ivarsson et al., 2011). Research has shown that the nutrition of chicory is virtually equivalent to and even surpasses some varieties of alfalfa (Sulas, 2004).

Compared with other animal husbandry in developed countries such as Italy, New Zealand, and Australia, forage chicory cultivated in China has a rather short history that began in the 1980s. Currently, there are no registered chicory varieties with independent intellectual property rights in China, and only 3 introduced forage chicory varieties are legally registered by Chinese authority. However, forage chicory planting areas have been expanding year by year, and the planting area of only one cultivar, Commander, reached 2600 ha in China in 2012. Some chicory varieties introduced are poor in adaptability to the local environment, which limits the improvement of yield and quality. Therefore, it is urgent to breed high-quality and high-yield forage chicory varieties. In recent years, a large number of chicory lines have been introduced from abroad for forage. The level of genetic diversity, genetic relationships, and genetic distances among and within chicory are unclear but would be of great interest for forage chicory breeding programs in China.

Molecular markers could be used to identify genetic relationships to help determine crop crossbreeding combinations of parents and the ideal traits in hybrid progeny (Ali et al., 2008). Considering the limited morphological traits and isozymes to identify genetic variations of chicory, molecular markers can be used to research the genetic variation of chicory (Kiers et al., 2000). Sequence-related amplified polymorphism (SRAP) markers are a newly developed molecular maker technology based on polymerase chain reaction (PCR). Compared

with other molecular marker technologies, SRAP combines the advantages of simplicity, reliability, reasonable throughput rate, codominance, clear and high-intensity bands that rarely overlap, cost effectiveness, and multi-loci and multi-allele applications, giving this technique potential to be efficient for genetic diversity, gene mapping, and fingerprinting analyses (Li and Quiros, 2001; Aneja et al., 2012). SRAP has been successfully applied in genetic diversity analysis, cultivar identification, genetic linkage map construction, and gene cloning for many plants (Aneja et al., 2012).

The aims of this study were to evaluate the molecular variation and structure of this population to explore the effectiveness for SRAP to analyze and identify *C. intybus* and *C. endivia*, and to determine whether there is a relatively high genetic diversity within chicory for further breeding programs.

MATERIAL AND METHODS

Plant materials

Eighty accessions of *Cichorium* consisted of 2 species, *C. intybus* and *C. endivia*. Seventy-five accessions were kindly provided by the National Plant Germplasm System and originated from 15 countries, which included 4 accessions of endive, and the other 5 accessions were from China. The detailed information on the type of materials, origins, and serial numbers is listed in Table 1. All materials were cultivated in the Regional Forage Experimental Station of China in Hongya County, Sichuan Province, China.

DNA extraction

Young leaves of 15 monoclonal chicory with similar phenotypes at the 5- or 6-leaf stage were equally selected and mixed to obtain 100 mg to extract general DNA utilizing the cetyltrimethylammonium bromide method. The concentration and purity of the DNA were evaluated by 0.8% agarose gel electrophoresis and ultraviolet spectrophotometry. The working solutions of DNA were then diluted to 10 ng/μL and stored at -20°C.

Primer selection and PCR amplification

Sixty-six primer pairs (Budak et al., 2004; Guo and Luo, 2006) were used to screen by SRAP markers. Thirty-six polymorphic primer pairs that generated clear and reproducible banding patterns were selected for further analysis. The PCR system was 15 μL, which included 1 μL DNA, 0.3 μL 2.5 U/μL Taq DNA polymerase, 0.8 μL 10 mM forward primer, 0.8 μL 10 mM reverse primer, 2 μL 10X PCR buffer, 2 μL 25 mM Mg^{2+}, 2.4 μL 2.5 mM dNTPs, and 5.7 μL sterile water. The PCR was conducted under the following conditions: 4 min at 94°C; 5 cycles of 94°C for 1 min, 35°C for 1 min, and 72°C for 1 min; 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min; extension of 10 min at 72°C; and a final storage at 4°C. PCR amplification products were visualized on 6.0% polyacrylamide gels by electrophoresis in 1X Tris, borate, and ethylenediaminetetraacetic acid electrophoresis buffer solution at 400 V for 2 h. The gel product was silver stained and photographed by a digital camera.

Table 1. Information on *Cichorium* accessions studied.

Accession No.	Species	Origin	Material type	Accession No.	Species	Origin	Material type
PI651889	*C.endivia* L	North Holland, Netherlands	Cultivar	PI503594	*C.endivia* L.	Peloponnese, Greece	Line
PI503588	*C.endivia* L.	Calabria, Italy	Line	J-2	*C.endivia* L.	China	Line
PI503599	*C.endivia* L.	Calabria, Italy	Line	PI651999	*C.intybus* L.	Piedmont, Italy	Line
PI651946	*C.intybus* L.	North Holland, Netherlands	Line	PI652035	*C.intybus* L.	Veneto, Italy	Cultivar
PI651947	*C.intybus* L.	North Holland, Netherlands	Line	PI652037	*C.intybus* L.	Veneto, Italy	Cultivar
PI651990	*C.intybus* L.	North Holland, Netherlands	Line	PI652038	*C.intybus* L.	Veneto, Italy	Cultivar
PI651992	*C.intybus* L.	North Holland, Netherlands	Line	PI652039	*C.intybus* L.	Veneto, Italy	Cultivar
PI651950	*C.intybus* L.	South Holland, Netherlands	Line	PI652040	*C.intybus* L.	Veneto, Italy	Cultivar
PI651951	*C.intybus* L.	South Holland, Netherlands	Line	PI652041	*C.intybus* L.	Trentino-Alto Adige, Italy	Cultivar
PI651984	*C.intybus* L.	South Holland, Netherlands	Line	PI652045	*C.intybus* L.	Trentino-Alto Adige, Italy	Cultivar
PI652005	*C.intybus* L.	South Holland, Netherlands	Line	PI652046	*C.intybus* L.	Trentino-Alto Adige, Italy	Cultivar
PI651969	*C.intybus* L.	Limburg, Netherlands	Line	PI652047	*C.intybus* L.	Trentino-Alto Adige, Italy	Cultivar
PI651971	*C.intybus* L.	Limburg, Netherlands	Line	PI652042	*C.intybus* L.	Lombardy, Italy	Line
PI651986	*C.intybus* L.	Limburg, Netherlands	Line	PI255565	*C.intybus* L.	Friuli-Venezia, Italy	Cultivar
PI651987	*C.intybus* L.	Limburg, Netherlands	Line	PI652043	*C.intybus* L.	Emilia-Romagna, Italy	Cultivar
PI651930	*C.intybus* L.	USA	Line	PI652044	*C.intybus* L.	Emilia-Romagna, Italy	Cultivar
NSL6407	*C.intybus* L.	California, USA	Cultivar	PI652007	*C.intybus* L.	Bydgoszcz, Poland	Wild
NSL69921	*C.intybus* L.	Pennsylvania, USA	Cultivar	PI652008	*C.intybus* L.	Suwalki, Poland	Wild
PI651954	*C.intybus* L.	Germany	Cultivar	PI652009	*C.intybus* L.	Zamosc, Poland	Wild
PI651931	*C.intybus* L.	Maine-et-Loire, France	Line	PI652025	*C.intybus* L.	Lublin, Poland	Wild
PI651932	*C.intybus* L.	Essonne, France	Line	PI652051	*C.intybus* L.	Przemysl, Poland	Wild
PI651936	*C.intybus* L.	Essonne, France	Line	PI652019	*C.intybus* L.	Switzerland	Wild
PI651940	*C.intybus* L.	Essonne, France	Line	PI652020	*C.intybus* L.	Hungary	Wild
PI651949	*C.intybus* L.	Essonne, France	Line	PI652021	*C.intybus* L.	Hungary	Wild
PI651975	*C.intybus* L.	Essonne, France	Line	PI652022	*C.intybus* L.	Hungary	Wild
PI652015	*C.intybus* L.	Yvelines, France	Cultivar	PI531291	*C.intybus* L.	Hungary	Wild
PI652017	*C.intybus* L.	Yvelines, France	Cultivar	PI531292	*C.intybus* L.	Hungary	Wild
PI652018	*C.intybus* L.	Yvelines, France	Cultivar	PI652026	*C.intybus* L.	Mazandaran, Iran	Wild
PI393816	*C.intybus* L.	France	Line	PI652028	*C.intybus* L.	Krasnodar, Russian Federation	Wild
PI393822	*C.intybus* L.	France	Line	PI652030	*C.intybus* L.	Former Serbia and Montenegro	Wild
PI261776	*C.intybus* L.	Ville-de-Paris, France	Line	PI652033	*C.intybus* L.	Coimbra, Portugal	Wild
PI651955	*C.intybus* L.	Baden-Wurttemberg, Germany	Line	PI652050	*C.intybus* L.	Coimbra, Portugal	Wild
PI652023	*C.intybus* L.	Saxony-Anhalt, Germany	Wild	PI393821	*C.intybus* L.	Belgium	Line
PI652024	*C.intybus* L.	Saxony-Anhalt, Germany	Wild	PI432336	*C.intybus* L.	Cyprus	Line
PI504468	*C.intybus* L.	Germany	Line	PI491197	*C.intybus* L.	Greece	Line
PI651957	*C.intybus* L.	Italy	Line	A-1	*C.intybus* L.	China	Line
PI651958	*C.intybus* L.	Italy	Line	A-2	*C.intybus* L.	China	Wild
PI652048	*C.intybus* L.	Italy	Cultivar	P-2	*C.intybus* L.	China	Line
PI651961	*C.intybus* L.	Piedmont, Italy	Line	J-1	*C.intybus* L.	China	Line
PI651995	*C.intybus* L.	Piedmont, Italy	Line				
PI651997	*C.intybus* L.	Piedmont, Italy	Line				

Data analysis

The raw data matrix was manually scored in accordance with band presence (1) and absence (0). The total number of bands, number of polymorphic bands, polymorphic rate, and polymorphism information content (PIC) (Roldán-Ruiz et al., 2000) were calculated. The NTsys-pc 2.10e software was used to calculate the Jaccard similarity coefficient using the SimQual program. A similarity matrix was calculated for the intraspecific genetic similarity coefficient (G_S). A clustering dendrogram was obtained by unweighted pair-group method using arithmetic averages (UPGMA) of the SHAN program through the Treeplot module. PopGen32 (Nei, 1973) was used to calculate genetic diversity indexes and the genetic structure of chicory groups, including the group Shannon information index (I), observed number of alleles, effective number of alleles (N_E), Nei's gene diversity (H), number of polymorphic loci, percentage of polymorphic loci, total genetic diversity, genetic diversity within popula-

tions, Nei's genetic differentiation between populations (G_{ST}), and number of immigrants per generation (N_m, $N_m = 0.5 \times (1 - G_{ST})/G_{ST}$).

RESULTS

Chicory polymorphism

A total of 36 primer combinations were used to evaluate the genetic diversity of the 75 chicory accessions. The total bands, polymorphic bands, polymorphic rates, and PIC values per primer pair were presented in Table 2. These 36 primer combinations totally produced 985 clear bands with a mean of 27.4 and a range from 15 to 41 bands per primer combination, including 951 polymorphic bands with a polymorphic rate that ranged from 86.67 to 100% and had a mean of 96.83%. The high genetic diversity of the materials studied was demonstrated. Each primer combination produced an average of 26.4 polymorphic bands. The PIC ranged from 0.211 (me1 + em1) to 0.376 (me7 + em7) and had a mean of 0.323.

Table 2. Polymorphic amplification of SRAP primer combinations on chicory.

Primer combination	Total number of bands (N)	Polymorphism bands (PB)	Polymorphic rate (P) (%)	Polymorphism information content (PIC)
me1 + em1	15	15	100	0.211
me1 + em2	19	18	94.74	0.278
me1 + em3	32	31	96.88	0.370
me1 + em12	26	26	100	0.367
me1 + em10	18	18	100	0.279
me1 + em13	23	21	91.30	0.297
me2 + em2	24	24	100	0.368
me2 + em3	28	28	100	0.362
me2 + em7	25	23	92	0.345
me2 + em15	24	23	95.83	0.307
me2 + em17	23	20	86.96	0.337
me2 + em18	28	28	100	0.349
me3 + em13	32	32	100	0.312
me3 + em15	23	20	100	0.249
me3 + em17	29	29	100	0.351
me4 + em2	23	22	95.65	0.290
me5 + em9	30	29	96.67	0.303
me7 + em3	30	26	86.67	0.293
me7 + em4	24	22	91.67	0.373
me7 + em7	33	32	96.97	0.376
me8 + em4	30	30	100	0.310
me8 + em13	24	24	100	0.248
me8 + em16	31	28	90.32	0.281
me9 + em7	30	29	96.67	0.372
me9 + em9	34	32	94.12	0.309
me10 + em4	26	25	96.15	0.316
me10 + em6	34	33	97.06	0.342
me10 + em9	33	32	96.97	0.338
me10 + em16	28	28	100	0.370
me10 + em5	21	21	100	0.320
me11 + em4	27	26	96.30	0.294
me11 + em8	27	26	96.30	0.337
me11 + em11	30	29	96.67	0.357
me11 + em16	32	32	100	0.316
me12 + em4	28	28	100	0.325
me12 + em19	41	41	100	0.368
Total	985	951		
Mean	27.4	26.4	96.83	0.323

Genetic variation based on SRAP markers

Analysis of the G_S among 80 accessions of tested materials showed a rich genetic diversity and far relationship between intraspecific and interspecific samples (data not shown). The in-pair G_S values of the 75 chicory accessions ranged from 0.519 to 0.903 and had a mean of 0.677. The G_S of PI 652005, which originated from South Holland of the Netherlands, and J-1, which originated from China, was the lowest, which indicated the farthest genetic relationship. The G_S values of the 5 accessions of endive ranged from 0.565 to 0.843 and had a mean of 0.701. Compared with the G_S values of interspecific samples, the average G_S value of endive was higher than that of chicory, which indicated that the genetic diversity of chicory was higher than that of endive.

At least 3 accessions of chicory from original countries were divided into 8 geographic groups to determine the genetic diversity based on structure analysis. The Shannon index varied between 0 and 1, where a number closer to 1 indicated that the genetic diversity was increasingly abundant (Estopa et al., 2006). Among the 8 groups, the richest genetic diversity was observed in Italy (N_E = 1.609, H = 0.372, I = 0.556), followed by France, and the last was the United States (Table 3). Based on the genetic diversity analyses for 3 types of materials (line, wild, and cultivar), the genetic differences among the 3 types of materials were large, and lines had the richest genetic diversity (N_E = 1.478, H = 0.289, I = 0.443). However, wild materials had the lowest genetic diversity (N_E = 1.378, H = 0.232, I = 0.359), showing that wild materials had genetic stability and a narrow genetic basis (Table 3). In addition, Nei's analysis for the genetic structure of the 8 geographical groupings showed that the average genetic diversity within a group was the richest (0.340) with a genetic differentiation coefficient (G_{ST}) of 14.20% and N_m of 3.020, suggesting that the chicory genetic structure among different countries obviously differed with a certain amount of gene flow (Table 4). The G_{ST} of 6.80% and N_m of 6.853 were used to analyze the genetic structure of 3 types of materials in a population, which indicated that the genetic structure among different types of materials has a small, frequent gene flow (Table 4).

Table 3. Genetic diversity indices of chicory from different grouping.

Group	N	K	P (%)	N_A	N_E	H	I
Country							
Holland	12	984	99.80	1.999 (0.045)	1.535 (0.222)	0.335 (0.099)	0.511 (0.117)
France	12	984	99.80	1.999 (0.045)	1.588 (0.227)	0.357 (0.097)	0.537 (0.115)
Italy	20	986	100.00	2.000 (0.000)	1.609 (0.171)	0.372 (0.069)	0.556 (0.077)
Germany	5	936	94.93	1.949 (0.220)	1.607 (0.294)	0.354 (0.134)	0.525 (0.172)
Poland	5	915	92.80	1.928 (0.259)	1.548 (0.291)	0.329 (0.138)	0.494 (0.183)
Hungary	5	960	97.36	1.974 (0.160)	1.574 (0.266)	0.345 (0.119)	0.519 (0.148)
China	4	842	85.40	1.854 (0.353)	1.559 (0.340)	0.323 (0.166)	0.479 (0.227)
America	3	763	77.38	1.774 (0.419)	1.536 (0.371)	0.305 (0.185)	0.449 (0.259)
Mean	66			2.000 (0.000)	1.630 (0.106)	0.384 (0.040)	0.572 (0.044)
Type							
Cultivar	19	832	84.38	1.844 (0.363)	1.425 (0.348)	0.255 (0.179)	0.391 (0.244)
Wild	19	795	80.63	1.806 (0.395)	1.378 (0.338)	0.232 (0.176)	0.359 (0.244)
Line	37	942	95.54	1.955 (0.207)	1.478 (0.326)	0.289 (0.160)	0.443 (0.208)
Mean	75			1.959 (0.197)	1.463 (0.317)	0.283 (0.155)	0.437 (0.203)

N = sample number; K = number of polymorphic loci; P = percentage of polymorphic loci; N_A = number of alleles; N_E = effective number of alleles; H = Nei's gene diversity; I = Shannon's information index.

Table 4. Genetic structure of chicory from different grouping.

Groups	N	H_T	H_S	G_{ST}	N_m
Country	66	0.396	0.340	14.20%	3.020
Type	75	0.278	0.259	6.80%	6.853

N = sample number; H_T = total genetic diversity; H_S = genetic diversity within populations; G_{ST} = genetic differentiation coefficient; N_M = gene flow estimates.

Genetic relationships revealed by clustering of SRAP markers

The UPGMA tree generated from genetic distance coefficients grouped the 80 materials tested into 7 groups with a genetic similarity coefficient of 0.698 (Figure 1). The dendrogram showed that samples from the same country could mostly be clustered together, but clustering did not exactly follow geographic origins. In contrast, the 5 accessions of endive species were clustered in 2 groups. Cluster I contained PI 651889 (*C. endivia*). Cluster II contained 29 accessions of *C. intybus*; the first subgroup included 16 accessions originated from Italy, the Netherlands, France, and Germany except cultivars PI 652048 and PI 651954, and the second subgroup revealed a clear organization that included 13 accessions from Italy. Of the 19 wild chicories, 16 were grouped in cluster III; materials from Poland and Hungary showed a clear geographic grouping. Group IV comprised 23 accessions of chicory with diversified types. Most of the accessions from the Netherlands and France were clustered in the same subgroup, while other samples from various regions overlapped in distribution from the dendrogram. Two accessions from the USA were clustered separately in group V. Group VI contained 4 endive accessions, and 2 endives from Italy were placed in one subgroup, while 2 endives from Greece and China were clustered in another subgroup. PI 652050 from Portugal, PI 432336 from Cyprus, and A-1 and J-1 from China were clustered in group VII, and A-1 and J-1 were clustered in one subgroup.

DISCUSSION

Effectiveness of SRAP markers to study genetic diversity and relationships in *Cichorium*

Genetic differences evaluated using molecular markers will help to determine appropriate selection ratios and to maintain enough variation to select the breeding cycles (Azevedo et al., 2011). Based on our SRAP polymorphic analysis, chicory had a high level of genetic diversity compared with other species (Chang et al., 2012), and the higher level of sample polymorphism by SRAP was because of differences in the species, number of primers (Ullah et al., 2012), type of materials, number of samples, and PCR system. Additionally, SRAP molecular markers could be utilized to detect variations among and within populations of chicory, differentiate the 2 species of chicory and endive within the genus, and reveal the genetic relationship between species as effectively as amplified fragment length polymorphism (Kiers et al., 2000) and restriction fragment length polymorphism molecular markers (Vermeulen et al., 1994). It may be inferred that SRAP markers can be used for system classification studies in *Cichorium*, but further study is needed to identify interspecific relationships of other species in *Cichorium*.

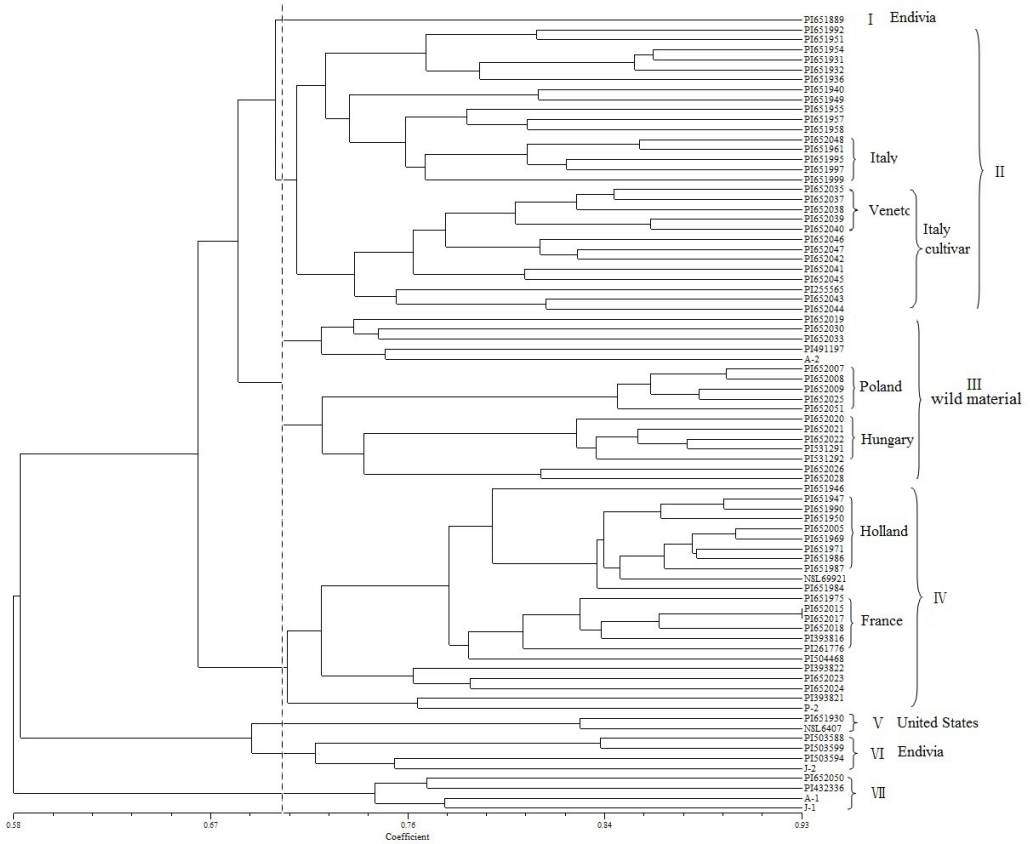

Figure 1. Unweighted pair-group method using arithmetic averages (UPGMA)-derived dendrogram of 80 accessions based on genetic identity (Nei, 1973). The accession designations refer to those listed in Table 1.

Analysis of the genetic diversity of chicory and endive and its significance in breeding

Chicory has accumulated a large amount of genetic variation, and it contains abundant genetic diversity, which is controlled by a number of factors including outcrossing mechanisms, environments, and natural and artificial selection (Kiær et al., 2009). The in-pair G_S mean value of the 75 chicory accessions was 0.677 in this study, while a high level of diversity was detected in the 2 species. This could be largely due to the observation that *C. endivia* is self-compatible, while *C. intybus* is self-incompatible (Kiers et al., 2000). In addition, this finding could also be caused by the morphological diversity of the 2 species: chicory contains 2 types of leaves and roots, but endive has only 1 leaf type (Lucchin et al., 2008). In addition, de Proft et al. (2003) suggested that negative characteristics probably appear as selection continues because of the narrow genetic basis of endive.

The genetic variation distribution is not random within populations, which are determined by the reproductive system, geographic distribution, effective scale of populations, pattern of reproduction, gene flow through the spread of pollen, and evolutionary factors that act

on the genetic diversity (Azevedo et al., 2011). Among the 8 countries, the degree of genetic diversity of chicory from Italy was the highest. This was probably because Italy is one of the essential origins of chicory (Kiær et al., 2009) and these tested materials covered most of the material types, or because of the uneven number of tested samples from different countries. Therefore, it is necessary to protect the biodiversity of chicory germplasm and consider introducing more lines from regions with high genetic diversity for breeding programs. Of the 3 types, wild materials had the lowest genetic diversity level, indicating the narrow genetic background and the loss of genetic diversity; this result was different from other research results (Van Cutsem et al., 2003). However, a broader genetic basis and potential gene resources with resistances to biological and abiotic stresses can be obtained through utilizing wild varieties (Singh et al., 2008). Thus, we should study the resistance characteristics of wild materials further. The results of this research showed that the varieties had a low level of genetic variation, probably because modern breeding practices led to a significant decrease of genetic diversity in modern varieties (Martos et al., 2005). As intermediate breeding materials, lines of chicories had the highest genetic diversity probably because of the wide source, large size of the sample, numerous functions, and direct domestication from wild material or hybrids. This also suggests that these lines have potential to provide further gains from selection within a breeding program. The genetic diversity level of different populations of chicory is high based on the genetic structure analysis in this study, but the diversity level of the geographic groups is higher than that of the type groups. The genetic structure analysis for type groups also showed a high N_m (6.853) and low G_{ST} (6.80%). Therefore, frequent gene flow is an essential reason for the low genetic differentiation level among the materials of different types of chicory. However, more samples should be included to provide a more exact estimation of genetic distances (Moura and de Oliveira, 2012).

Relationship between the clustering and geographic origin of *Cichorium*

UPGMA was known to be the hierarchical method of producing dendrograms with the maximum cophenetic correlation (Bertini et al., 2006), and it was the most efficient method to represent dissimilarities among genotypes. Clustering analysis showed that 75 chicory accessions from different countries could mostly be clustered together in a dendrogram, but the clustering was not exactly consistent with geographic origins. Chicory germplasm that originated from Italy, Poland, Hungary, and other countries gathered separately in the same group according to the geographical origin as expected because of the geographical isolation. Especially, Italian varieties except PI652048 were clustered in the same group, suggesting that Italy contains a core germplasm; this result was similar to that obtained by Koch and Jung (1997). The reason for this may be the frequent gene penetration among different varieties that originated from Italy or mixed cultivation, which resulted in natural hybridization and natural gene penetration (Kiers et al., 1999; Kiær et al., 2009). However, genetic relationships based on molecular markers and pedigree information were not always consistent (Ali et al., 2008). Accessions from Germany, France, China, and other countries had a staggered distribution in the dendrogram. Sarwat et al. (2008) indicated that populations with geographic and genetic differences cannot be completely isolated, but they can exchange genetic information, probably because of similar climates (mainly continental and oceanic climates), geographic neighborhoods, difficult gene isolation, and frequent gene flow in the countries with materials by

exchanging germplasms from different regions (Choudhary et al., 2012). China and Belgium are far apart and have large climate differences, but some of their chicories had close genetic relationships, probably because China introduced a large deal of chicory germplasm from Belgium since the 1980s. Most wild materials cluster together in a group, and a few cluster in other groups. This situation indicated the strong geographic distribution and wide ecological adaptability of chicory genotype through long-term natural selection. Meanwhile, this also suggests that gene penetration not only occurs between the domesticated materials but also spread to wild populations from the cultivated chicory (Sørensen et al., 2007).

Relationship between chicory and endive based on clustering

In addition, 5 accessions of endive were clustered separately in groups I and VI, indicating obvious genetic distance between the *C. endivia* and *C. intybus*. However, their distances were not very large. This was probably because natural hybridization can lead to a very close genetic relationship among populations of chicory and endive. Furthermore, artificial hybridization may cause gene flow and intraspecific and interspecific interpenetration. For example, variegated chicory is formed as a result of controlled or random crossbreeding between *C. intybus* var. foliosum and *C. endivia* var. latifolium (Biesiada and Tomczak, 2012), which indicated interspecific spontaneous gene flow. Therefore, out-crossing between *C. intybus* and *C. endivia* could be considered to promote the breeding success of the chicory breeding process.

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