Genetic Relationship among Sweetpea Cultivars and Related Species by AFLP Analysis

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Sweetpea (*Lathyrus odoratus* L.) is an important ornamental crop belonging to the *Fabaceae* family. In Japan, commercial cut flower production under forced culture has flourished, but few cultivars are bred exclusively for cut flower forced culture. In order to obtain knowledge for the selection of breeding materials, we elucidated the genetic relationships among eighty-seven sweetpea cultivars and two related species using the amplified fragment length polymorphisms (AFLP). Sweetpea cultivars were grouped into two clusters. Cluster I consisted of four cultivars, including a wild cultivar, ‘Cupani’ and cultivars established from spontaneous mutation or natural hybridization, such as ‘Cupid’ and ‘Painted Lady’. The biggest cluster, cluster II, consisted of eighty-three sweetpea cultivars. No distinct subcluster shared common morphological or ecological characteristics, such as tendrill-less, plant type, or flowering habit in cluster II. The distinct genetic differentiation among cultivars highly seems to reflect sweetpea breeding history originating in the United Kingdom 300 years ago using limited genetic resources. Thus, hybridization with genetically distinct, exotic resources may contribute to the development of sweetpea cultivars.

Key Words: AFLP, cluster analysis, cultivar, relationship, sweetpea.

Introduction

Genetic resources used as breeding materials involve cultivars or lines bred by breeders, native species adapted to the natural conditions of specific areas, and wild species. These genetic resources have genetic variability or latent potentialities to spread variation (Ohkawa, 1995). It is very important to collect genetic resources, and maintain, reproduce, and examine their morphologies, reveal relationships, and to identify and classify such resources to be used in future plant breeding. Recently, DNA-based markers for genetic analysis have been used to examine relationships, and to identify and classify cultivars and lines (Tsuruta et al., 2002). Notably, amplified fragment length polymorphisms (AFLP) analysis is an efficient technique to reveal genetic relationships, and to identify and classify cultivars and lines. Hence, the AFLP technique is used already in many crops to determine genetic relationships (Lee and Han, 2006; Mace et al., 1999; Warwick et al., 2007).

Sweetpea is an important ornamental crop. In Japan, commercial cut flower production under forced culture has flourished (Motozu, 2006; Nakamura et al., 2008). Because sweetpea was improved in the United Kingdom and the United States and then introduced into Japan (Inoue, 2007; Nakamura et al., 2008), most cultivars used for cut flower production in Japan were selected and fixed from imported winter or spring flowering cultivars from the United States (Inoue, 2007; Nakamura et al., 2008). Therefore, few cultivars have been bred by hybridization specific for cut flower forced culture in Japan. Commercial cultivars for cut flowers need good characteristics, including not only flower color, flower size, flower form, long vase life, and tolerance to bud drop, but also high yielding ability, disease resistance, plant vigor stability, and heat tolerance. Hereafter, it is important to reveal the genetic relationships and examine the specific characteristics of each distinct genetic group in order to choose cultivars adapted to breeding objectives.

The sweetpea has a history of more than 300 years. Its characteristics, such as flower color, flower form, and flowering habit have diversified significantly as the result

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of breeding throughout its history. The morphological and ecological characteristics of sweetpea cultivars under normal culture were described previously by Beal (1914), Ito et al. (1991), and Hanada (2003). Murray et al. (1992) reported no evidence of change in chromosome structure and genome size throughout sweetpea development. On the other hand, Hanada and Hirai (2000) reported that the RAPD technique classified 15 cultivars into clusters corresponded to the flowering habit and the tendril-less trait. However, it is unknown whether there is a relationship between these characteristics and the genetic differentiation of other sweetpea cultivars.

We have been breeding sweetpea cultivars for commercial cut flower production under forced culture (Nakamura et al., 2008, 2006a, b). We have also been collecting cultivars used for not only cut flower production in Japan but also those used as summer flowering cultivars from the United Kingdom to obtain useful characteristics, and reported the morphological and ecological classification of cultivars under forced culture (Nakamura et al., 2010).

In this study, in order to obtain knowledge for the selection of breeding materials, we elucidated the genetic relationships among eighty-seven sweetpea cultivars and two related species using the AFLP technique.

Materials and Methods

Plant materials and DNA extraction

One accession each of 87 sweetpea cultivars and two out-group species, *L. belinensis* Maxted & Gloyd and *L. chloranthus* Boiss. were used in this study (Table 1). The plant materials were grown in the greenhouse of the Miyazaki Agricultural Research Institute. Young leaves (0.1 g) of each cultivar and species were frozen in liquid nitrogen and ground into a fine powder using a mortar and pestle. DNA was extracted from powder using the CTAB method, as described by Murray and Thompson (1980), and Nucleon PHYTOPURE (Amersham Life Science, Buckinghamshire, UK).

AFLP analysis

The amplification conditions adopted for AFLP analysis were initially described by Vos et al. (1995). The electrophoresis conditions and band detection procedures were adopted for high efficiency genome scanning (HEGS) system as described by Kawasaki and Murakami (2000) and Kawasaki et al. (2003). Genomic DNA (250 ng) was digested with *Mse* I (5 units) and *Xba* I (5 units). Digested fragments were ligated to each adaptor (Table 2) of the restriction enzyme combination. The first amplification was performed using pre-amplification primers (Table 2). The first PCR products were diluted 1/100 with 1/10 TE (1 mM Tris hydroxymethyl aminomethane, 0.1 mM disodium dihydrogen ethylenediamine tetraacetate dehydrate, pH 8.0) buffer and used as the template DNA for the second

amplification step. The second amplification was performed using primers (Table 2) in a 10 µL total reaction volume. Primer combinations for amplification were: *Mse* I-*caa/Xba* I-cgc, *Mse* I-*cac/Xba* I-ccg, *Mse* I-*cac/Xba* I-cgc, *Mse* I-*cag/Xba* I-cat, *Mse* I-*cag/Xba* I-cga, *Mse* I-*cag/Xba* I-cgt, *Mse* I-*cat/Xba* I-cgc, *Mse* I-*cat/Xba* I-cgg, *Mse* I-*cat/Xba* I-cgt, *Mse* I-*cat/Xba* I-cta, *Mse* I-*cca/Xba* I-cac, *Mse* I-*cca/Xba* I-cag, *Mse* I-*cca/Xba* I-ccg, *Mse* I-*cca/Xba* I-cgc, *Mse* I-*ccc/Xba* I-ccc, *Mse* I-*ccc/Xba* I-cgc, *Mse* I-*ccc/Xba* I-cgg, *Mse* I-*ccc/Xba* I-cgt, *Mse* I-*ccc/Xba* I-ctc, *Mse* I-*ccc/Xba* I-ctg, *Mse* I-*ccc/Xba* I-ctt. The PCR products of the second amplification were separated on 13% polyacrylamide gels at 300 V for 10 min and 450 V for 3 h in a small vertical electrophoresis unit of 26 × 26 cm (Nippon-eido, Tokyo, Japan). The gels were stained with SYBR Green I (BMA, Rockland, USA) for 30 min, and were detected with a fluorescent image analyzer (FLA-3000G; Fuji Film, Tokyo, Japan). Duplicate amplifications were analyzed to confirm the AFLP analyses.

Data analysis

Polymorphic AFLP markers were manually scored as binary data with presence of a band denoted as “1” and absence as “0”. Only clearly distinguishable bands were used in the genetic analysis. Genetic distances were estimated using the simple matching coefficient (Sokal and Michener, 1958). Similarity coefficient was calculated as $S_{ij} = (a + d) / (a + b + c + d)$: where S_{ij} is the similarity between two individuals i and j , a is the number of fragments in both i and j , b is the number of fragments present in i and absent in j , c is the number of fragments absent in i and present in j , and d is the number of fragments absent in both i and j . Conversion to genetic distances, D_{ij} , was obtained by the equation $D_{ij} = 1 - S_{ij}$. The genetic distances obtained were used for cluster analysis based on the neighbor-joining (NJ) method (Saitou and Nei, 1987) with 1000 bootstraps by using the software package TREECON (ver. 1.3b) (Van De Peer and De Wachter, 1994).

Results and Discussion

AFLP polymorphism

The 21 AFLP primer combinations used in this study amplified 1043 DNA fragments among eighty-seven sweetpea cultivars and two related species accessions (Table 3). The number of DNA fragments for each primer combination ranged from 30 to 71 (mean, 49.7). Out of 1043 amplified bands, 159 (15.2%) were found to be polymorphic among accessions, and each primer combination yielded 4 to 16 polymorphic bands. The primer combination of *Mse* I-*cat/Xba* I-cgg generated the most polymorphic bands; therefore, we suggest that AFLP analysis is a useful genetic technique to study relationships in sweetpea cultivars.

Table 1. Characteristics and origins of sweetpea cultivars and related two *Lathyrus* species used in this study.

Species and cultivar	Usage in Japan	Color of the standard ²		Flowering habit ³	Tendrils ⁴	Plant type ⁵	Origin
		Color code No.	Color name				
<i>Lathyrus odoratus</i> L.							
Aisome	Cut flower	8905	bright reddish purple	Spring	+	Tall	Bred in Miyazaki Agri. Res. Inst.
Anniversary	Garden	9501	pale purplish pink	Summer	+	Tall	Selfed and selected after introduced from seed company
Ayahime	Cut flower	1615	pale grayish brown	Spring	+	Tall	Bred in Miyazaki Agri. Res. Inst.
Benishikibu	Cut flower	0110	dark red	Spring	+	Tall	Bred in Miyazaki Agri. Res. Inst.
Blue Ripple	Garden	8615	light grayish purple	Summer	+	Tall	Mis. Noguchi
Celeste	Cut flower	8304	light bluish purple	Spring	+	Tall	Selfed and selected after introduced from seed company
Ceremony Lavender	Cut flower	8604	bright purple	Winter	-	Tall	Selfed and selected after introduced from seed company
Ceremony Pink	Cut flower	9704	strong purplish pink	Winter	-	Tall	Selfed and selected after introduced from seed company
Ceremony Scarlet	Cut flower	0714	deep yellowish red	Winter	-	Tall	Selfed and selected after introduced from seed company
Ceremony White	Cut flower	2501	yellowish white	Winter	-	Tall	Selfed and selected after introduced from seed company
Charlie's Angel	Garden	8304	light bluish purple	Summer	+	Tall	Selfed and selected after introduced from seed company
Christina	Cut flower	2901	yellowish white	Winter	+	Tall	Selfed and selected after introduced from seed company
Cupani	Wild type	0419	grayish red	Summer	+	Tall	Mis. Kurose
Cupid	Bedding	9505	strong purplish pink	Spring	+	Dwarf	Selfed and selected after introduced from seed company
Diana	Cut flower	0105	strong pink	Winter	+	Tall	Daichi Engei co.ltd.
Early Lavender	Cut flower	8604	bright purple	Winter	+	Tall	Selfed and selected after introduced from seed company
Early Salmon Pink	Cut flower	0111	pink	Spring	+	Tall	Selfed and selected after introduced from seed company
Early White	Cut flower	3301	yellowish white	Winter	+	Tall	Selfed and selected after introduced from seed company
Easter Parade	Cut flower	3301	yellowish white	Spring	+	Tall	Selfed and selected after introduced from seed company
Elizabeth	Cut flower	9704	strong purplish pink	Winter	+	Tall	Selfed and selected after introduced from seed company
Ellie	Cut flower	8604	bright pink	Winter	+	Tall	Selfed and selected after introduced from seed company
Emily	Cut flower	0404	strong pink	Winter	+	Tall	Selfed and selected after introduced from seed company
First Lady	Cut flower	9503	purplish pink	Spring	+	Tall	Hamayu agricultural cooperatives
Galatia	Cut flower	8310	strong bluish purple	Winter	+	Tall	Selfed and selected after introduced from seed company
Grace	Cut flower	8604	bright purple	Spring	+	Tall	Osuzu agricultural cooperatives
Honeymoon	Garden	8303	light bluish purple	Summer	+	Tall	Selfed and selected after introduced from seed company
Maifuji	Cut flower	8604	bright purple	Spring	+	Tall	Bred in Miyazaki Agri. Res. Inst.
Maihime	Cut flower	0114	strong red	Spring	+	Tall	Bred in Miyazaki Agri. Res. Inst.
Mammoth Deep Rose	Cut flower	9707	vivid purplish red	Winter	+	Tall	Selfed and selected after introduced from seed company
Mary	Cut flower	0104	strong pink	Winter	+	Tall	Selfed and selected after introduced from seed company
Mimi	Cut flower	0104	strong pink	Spring	+	Tall	Bred in Miyazaki Agri. Res. Inst.
Miranda	Cut flower	0105	strong pink	Winter	+	Tall	Selfed and selected after introduced from seed company
Miyazaki Sunny Peach	Cut flower	0702	pale yellowish pink	Spring	+	Tall	Bred in Miyazaki Agri. Res. Inst.
Mrs. Douglas MacArthur	Cut flower	0105	strong pink	Winter	+	Tall	Selfed and selected after introduced from seed company
Musica Purple	Cut flower	9208	vivid red purple	Winter	-	Tall	Bred in Miyazaki Agri. Res. Inst.
Musica Rose	Cut flower	9205	strong purplish pink	Spring	-	Tall	Bred in Miyazaki Agri. Res. Inst.
Neo Grace	Cut flower	8905	bright reddish purple	Spring	+	Tall	Hamayu agricultural cooperatives
Osuzu Royal White	Cut flower	3301	yellowish white	Spring	+	Tall	Osuzu agricultural cooperatives
Painted Lady	Wild type	0106	bright red	Summer	+	Tall	Dr. Inoue
Pansy Lavender Flash	Garden	8604	bright purple	Summer	+	Dwarf	Tompson & Morgan Seed
Patio Deep Purple	Bedding	9218	dark grayish purple	Spring	+	Dwarf	Selfed and selected after introduced from seed company
Patio Pink	Bedding	9705	strong purplish pink	Spring	+	Dwarf	Selfed and selected after introduced from seed company
Patio Rose	Bedding	9708	strong purplish pink	Spring	+	Dwarf	Selfed and selected after introduced from seed company
Patio Rose Pink	Bedding	9705	strong purplish pink	Spring	+	Dwarf	Selfed and selected after introduced from seed company
Pearl	Cut flower	9202	pale orchid	Spring	+	Tall	Hamayu agricultural cooperatives
Percy Thrower	Garden	9205	strong purplish pink	Summer	+	Tall	Selfed and selected after introduced from seed company
Red Arrow	Garden	0713	strong yellowish red	Summer	+	Tall	Selfed and selected after introduced from seed company
Rosé	Cut flower	9205	strong purplish pink	Spring	+	Tall	Osuzu agricultural cooperatives
Rose Pink	Cut flower	9205	strong purplish pink	Spring	+	Tall	Selfed and selected after introduced from seed company
Royal blue	Cut flower	8310	strong bruish purple	Spring	+	Tall	Selfed and selected after introduced from seed company
Royal Lavender	Cut flower	8304	light bluish purple	Spring	+	Tall	Selfed and selected after introduced from seed company
Royal Marion	Cut flower	2503	pale yellow	Spring	+	Tall	Selfed and selected after introduced from seed company
Royal Navy blue	Cut flower	8609	dark purple	Spring	+	Tall	Selfed and selected after introduced from seed company
Royal Rose Pink	Cut flower	9204	strong purplish pink	Spring	+	Tall	Selfed and selected after introduced from seed company
Royal Wedding	Cut flower	2903	pale greenish purple	Spring	+	Tall	Selfed and selected after introduced from seed company
Royal White	Cut flower	3302	yellowish white	Spring	+	Tall	Selfed and selected after introduced from seed company
Sakuragawa	Cut flower	8902	pale purplish pink	Spring	+	Tall	Bred in Miyazaki Agri. Res. Inst.
Shikibu	Cut flower	0110	dark red	Spring	+	Tall	Bred in Miyazaki Agri. Res. Inst.
Silky Cherry	Cut flower	9203	purplish pink	Spring	+	Tall	Bred in Miyazaki Agri. Res. Inst.
Silky Peach	Cut flower	1315	pale brown	Winter	+	Tall	Bred in Miyazaki Agri. Res. Inst.
Spring Fair	Cut flower	0104	strong pink	Spring	+	Tall	Takachiho agricultural cooperatives
Stella	Cut flower	2503	pale yellow	Spring	+	Tall	Selfed and selected after introduced from seed company

Table 1. Continued.

Species and cultivar	Usage in Japan	Color of the standard ^z		Flowering habit ^y	Tendrils ^x	Plant type ^y	Origin
		Color code No.	Color name				
Super Lavender	Cut flower	8604	bright purple	Spring	+	Tall	Selfed and selected after introduced from seed company
Super Mid Blue	Cut flower	8310	strong bluish purple	Spring	+	Tall	Selfed and selected after introduced from seed company
Super Rose	Cut flower	9205	strong purplish pink	Spring	+	Tall	Selfed and selected after introduced from seed company
Super Scarlet	Cut flower	0406	bright red	Spring	+	Tall	Selfed and selected after introduced from seed company
Super Snoopea Pale Purple	Garden	8310	strong bluish purple	Summer	-	Tall	A mutant of 'Super Snoopea Purple'
Super Snoopea Pink	Garden	9205	strong purplish pink	Spring	-	Medium	Selfed and selected after introduced from seed company
Super Snoopea Purple	Garden	9218	dark grayish purple	Spring	-	Medium	Selfed and selected after introduced from seed company
Super Snoopea Red	Garden	0409	deep red	Spring	-	Medium	Selfed and selected after introduced from seed company
Super White	Cut flower	3302	yellowish white	Spring	+	Tall	Selfed and selected after introduced from seed company
Susie	Cut flower	0104	strong pink	Winter	+	Tall	Selfed and selected after introduced from seed company
Sweet Memory Blue	Garden	8310	strong bluish purple	Summer	+	Tall	Selfed and selected after introduced from seed company
Sweet Memory Purple	Garden	0410	dark red	Summer	+	Tall	Selfed and selected after introduced from seed company
Sweet Memory Rose	Garden	0106	bright red	Summer	+	Tall	Selfed and selected after introduced from seed company
Tendrill-less Honeymoon	Garden	8602	pale purple	Summer	-	Tall	A mutant of 'Honeymoon'
Utahime	Cut flower	0415	strong red	Winter	+	Tall	Bred in Miyazaki Agri. Res. Inst.
View Pink	Bedding	9704	strong purplish pink	Winter	+	Dwarf	Selfed and selected after introduced from seed company
View Purple	Bedding	8613	dull purple	Winter	+	Dwarf	Selfed and selected after introduced from seed company
View White	Bedding	3301	yellowish white	Winter	+	Dwarf	Selfed and selected after introduced from seed company
White Giant	Cut flower	3301	yellowish white	Winter	+	Tall	Selfed and selected after introduced from seed company
Winter Elegance Deep Rose	Cut flower	9707	vivid purplish red	Winter	+	Tall	Selfed and selected after introduced from seed company
Winter Elegance Lavender	Cut flower	8604	bright purple	Winter	+	Tall	Selfed and selected after introduced from seed company
Winter Elegance Pink Diana	Cut flower	9504	strong purplish pink	Winter	+	Tall	Selfed and selected after introduced from seed company
Winter Elegance Salmon	Cut flower	0105	strong pink	Winter	+	Tall	Selfed and selected after introduced from seed company
Winter Elegance Scarlet	Cut flower	0707	deep reddish brown	Winter	+	Tall	Selfed and selected after introduced from seed company
Winter Flowering Lavender	Cut flower	8604	bright purple	Winter	+	Tall	Selfed and selected after introduced from seed company
<i>L. belinensis</i> Maxted & Gloyder.	Garden	1308	deep brown	Summer	+	Tall	Tompson & Morgan Seed
<i>L. chloranthus</i> Boiss.	Garden	2911	strong greenish yellow	Summer	+	Medium	Dr.Inoue

^z Japan horticultural plant standard (JHS) color chart. 1984. (Japan. Color Res. Inst., Tokyo, Japan)^y Ito et al. (1991)^x +: presence of tendrils, -: absence of tendrils

Table 2. Nucleotide sequences of the primers and adapters used in AFLP analysis.

Primers/Adapters		Nucleotide sequences
<i>Mse</i> I	adapter 1	5'-gac gat gag tcc tga g-3'
<i>Mse</i> I	adapter 2	5'-tac tca gga ctc at-3'
<i>Xba</i> I	adapter 1	5'-ctc gta gac tgc gta cc-3'
<i>Xba</i> I	adapter 2	5'-cta ggg tac gca gtc tac-3'
<i>Mse</i> I	primer (+1)	5'-gat gag tcc tga gta ac-3'
<i>Xba</i> I	primer (+1)	5'-gac tgc gta ccc tag ac-3'
<i>Mse</i> I	primer (+3)	5'-gat gag tcc tga gta ac+aa(M1), ac(M2), ag(M3), at(M4), ca(M5), cc(M6)-3'
<i>Xba</i> I	primer (+3)	5'-gac tgc gta ccc tag ac+ac(X2), ag(X3), at(X4), cc(X6), cg(X7), ga(X9), gc(X10), gg(X11), gt(X12), ta(X13), tc(X14), tg(X15), tt(X16)-3'

Cluster analysis

A dendrogram was generated using the NJ method based on genetic distance. Eighty-seven sweetpea cultivars were grouped into two clusters (Fig. 1). Cluster I consisted of four cultivars, 'Cupani', 'Cupid', 'Painted Lady', and 'Pansy Lavender Flash'. Cluster II consisted of the remaining 83 sweetpea cultivars, and two other *Lathyrus* species belong to Cluster III as an out-group. It is significant that sweetpea cultivars were grouped into two clusters, I and II, since the genetic relationship

Table 3. AFLP primer pairs and the number of DNA fragments and polymorphic bands used for genetic analysis.

Primer	Number of DNA fragments	Number of polymorphic bands used for genetic analysis
<i>Mse</i> I- <i>caa</i> / <i>Xba</i> I-cgc	55	6
<i>Mse</i> I- <i>cac</i> / <i>Xba</i> I-ccg	46	8
<i>Mse</i> I- <i>cac</i> / <i>Xba</i> I-cgc	39	9
<i>Mse</i> I- <i>cag</i> / <i>Xba</i> I-cat	50	4
<i>Mse</i> I- <i>cag</i> / <i>Xba</i> I-cga	71	13
<i>Mse</i> I- <i>cag</i> / <i>Xba</i> I-cgt	42	7
<i>Mse</i> I- <i>cat</i> / <i>Xba</i> I-cgc	52	4
<i>Mse</i> I- <i>cat</i> / <i>Xba</i> I-cgg	49	16
<i>Mse</i> I- <i>cat</i> / <i>Xba</i> I-cgt	60	4
<i>Mse</i> I- <i>cat</i> / <i>Xba</i> I-cta	40	5
<i>Mse</i> I- <i>cca</i> / <i>Xba</i> I-cac	64	9
<i>Mse</i> I- <i>cca</i> / <i>Xba</i> I-cag	48	6
<i>Mse</i> I- <i>cca</i> / <i>Xba</i> I-ccg	54	6
<i>Mse</i> I- <i>cca</i> / <i>Xba</i> I-cgc	30	4
<i>Mse</i> I- <i>ccc</i> / <i>Xba</i> I-ccc	38	6
<i>Mse</i> I- <i>ccc</i> / <i>Xba</i> I-cgc	57	6
<i>Mse</i> I- <i>ccc</i> / <i>Xba</i> I-cgg	50	9
<i>Mse</i> I- <i>ccc</i> / <i>Xba</i> I-cgt	43	7
<i>Mse</i> I- <i>ccc</i> / <i>Xba</i> I-ctc	43	5
<i>Mse</i> I- <i>ccc</i> / <i>Xba</i> I-ctg	46	13
<i>Mse</i> I- <i>ccc</i> / <i>Xba</i> I-ctt	66	12
Total No.	1043	159
No./Primer pair	49.7	7.6

Fig. 1. Dendrogram of 87 sweetpea and 2 related species resulting from neighbor-joining cluster analysis based on simple-matching coefficient calculated from AFLP data. Numbers indicate bootstrap values greater than 50% for 1000 replicates.

among sweetpea cultivars was unclear based on morphological and ecological characteristics.

The history of sweetpea, *Lathyrus odoratus* L., cultivar development is well-documented. Sweetpeas are known to have been introduced from the island of Sicily into cultivation in the United Kingdom in 1699. Since then, many cultivars have been bred in the United Kingdom and the United States (Inoue, 2007; Inoue et al., 2000; Murray and Hammett, 1998; Rice, 2002); however, cultivars were established from spontaneous mutation or natural hybridization until Henry Eckford established the “Grandiflora type” using intraspecific hybridization in 1870s. The “Grandiflora type” has larger and more flowers than cultivars bred before the “Grandiflora type” was established. After the appearance of the “Grandiflora type”, many characteristics, such as flower color, flower size, flower form, and flowering habit varied significantly (Inoue, 2007; Inoue et al., 2000; Rice, 2002). Most of the present cultivars were bred after the “Grandiflora type” was established and are classified as “Acacia-leaved”, “Cuthbertson”, “Dwarf”, “Early or Winter-Flowering”, “Early Multiflora Giganteas”, “Early Spencer”, “Galaxy”, “Intermediate”, “Multiflora”, “Royals”, “Spencer”, and “Unwin type” (Rice, 2002). Most cultivars cultivated in Japan have been selected and fixed from imported market seeds (Inoue, 2007; Nakamura et al., 2008) of “Cuthbertson”, “Early Multiflora Giganteas”, “Early Spencer”, “Early or Winter-Flowering”, and “Royals”.

Cluster II was characterized by the 83 cultivars that were bred after the “Grandiflora type” was established. This cluster is considered to be the newer type cluster. No distinct subcluster shared common morphological or ecological characteristics. For example, regarding the tendril-less traits, a subcluster consisted of only seven tendril-less cultivars, ‘Super Snoopea Pink’, ‘Musica Rose’, ‘Super Snoopea Purple’, ‘Musica Purple’, ‘Ceremony Lavender’, ‘Ceremony Pink’, and ‘Ceremony Scarlet’. However, other tendril-less cultivars, such as ‘Tendril-less Honeymoon’, ‘Super Snoopea Pale Purple’, ‘Ceremony White’, and ‘Super Snoopea Red’ were grouped into other subclusters. Similarly, neither plant type nor flowering habit formed distinct subclusters. The results suggest that the same mutations occurred among phylogenically different cultivars and lines, and/or the selection and introduction of new characteristics have been repeated through the sweetpea breeding history. Thus, there may be little potential to obtain new useful characteristics within the cultivars of this cluster group except to discover and select new mutations and variations.

Cluster I was considered the cluster of old-type cultivars that were bred before the “Grandiflora type” was established. ‘Cupani’ and ‘Painted Lady’ are called “Originals” or “Old fashioned” (Rice, 2002). The reason why cluster I was distinguished from cluster II is considered to be because crossbreeding was not used

aggressively between them. ‘Cupani’, ‘Cupid’ and ‘Painted Lady’ were important cultivars in the history of sweetpea improvement. ‘Cupani’ was introduced from the wild in Sicily in the 1970s (Rice, 2002), and seems to be the same plant that was originally introduced from Sicily to the United Kingdom in 1699. ‘Painted Lady’ was the first cultivar registered in 1731 and is considered to originate from mutation or natural hybridization from the original sweetpea (Inoue, 2007; Inoue et al., 2000; Murray and Hammett, 1998). ‘Cupid’, a dwarf cultivar, was a mutant of ‘Blanche Ferry’ (Beal, 1912b; Rice, 2002) that was developed from ‘Painted Lady’ (Beal, 1912a, b; Inoue et al., 2000; Rice, 2002). These cultivars were used in the garden or as bedding plants and were not used for cut flowers since the flowers are few and small; however, these cultivars have a simple flower form and fragrance. Inoue et al. (2000) discovered flower color variations of wild individuals in Sicily. Assessment of the variations in these characteristics in old-type cultivars and wild individuals in Sicily would be an important point for breeding new cultivars.

Murray et al. (1992) reported no evidence of change in the chromosome structure and genome size throughout sweetpea development. In this study, sweetpea cultivars were neatly divided into two clusters based on whether they appeared before or after the “Grandiflora type” was established by intraspecific hybridization. We considered that the result reflects the sweetpea development history; that is, sweetpea breeding was promoted by the selection of mutants or natural hybridization in its early history, and then by crossbreeding after the “Grandiflora type” was established.

Cluster III consisted of two *Lathyrus* species, *L. belinensis* and *L. chloranthus*. These species have a yellow flower color that sweetpea does not have, and were reported to form interspecific hybrids with sweetpea (*L. odoratus*) (Hammett et al., 1994; Khawaja, 1988). Moreover, sweetpea has been reported to form interspecific hybrids with other *Lathyrus* species, *L. cassius* and *L. hirsutus* (Davies, 1957; Inoue, 2007; Murray and Hammett, 1998). It is thus expected that interspecific hybridization is an alternative way to promote the introduction of new useful characteristics to sweetpea.

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