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Full Length Research Paper

Assessment of genetic relationships among Spring *Dendrobium* cultivars and varietal materials using amplified fragment length polymorphism (AFLP) analysis

Zheng Quan[1], Zheng Yongping[2*], Guo Weiming[3], Lin Weijun[1] and Wang Guangdong[3]

[1]Zhejiang College of Construction, Hangzhou 311231, People's Republic of China.
[2]SenheSeed Company Limited, Hangzhou, Zhejiang 310020, People's Republic of China.
[3]College of Horticulture, Nanjing Agricultural University, Nanjing 210095, People's Republic of China.

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Spring *Dendrobiums* have been largely produced as one of the top grade ornamental pot plants due to their various flower colors, multitudinous flowers and graceful flower shape as well as their long period of florescence. Genetic relationships among Spring *Dendrobium* cultivars, however, have not been documented. This study analyzed the genetic relatedness of 30 commonly grown cultivars or varietal materials using amplified fragment length polymorphism (AFLP) markers with near-infrared fluorescence-labeled primers. Eight EcoRI + 3 bases/MseI + 3 bases primer set combinations were used in this investigation. Each selected primer set generated 113 to 158 scorable fragments. A total of 1102 AFLP fragments were detected, of which 778 were polymorphic (70.6%). An unweighted pair-group method of the arithmetic averages (UPGMA), principal coordinate analysis (PCOA), and bootstrap analysis were used to analyze the genetic relationships. The 30 cultivars were separated into five clusters. Cluster I contains 6 cultivars that are either from Senlan No.1 or Senlan No. 6 with Jaccard's similarity coefficients ranging from 0.70 to 0.80. All of these 6 cultivars came from Taiwan, and were derived from somaclonal variants or sports. Just 3 cultivars were positioned in cluster II ranging from 0.71 to 0.76, and also originated from Taiwan. Cluster III included 13 cultivars, Jaccard's similarity coefficients varied from 0.69 to 0.84. Seven cultivars from Senlan No. 15 or 'Snowboy Romance' were situated in cluster IV with Jaccard's similarity coefficients ranging from 0.69 to 0.82. Only 'Santana Canary' was positioned in cluster Ⅴ with Jaccard's similarity coefficient at 0.62. This study established the genetic relationships of these commonly cultivated Spring *Dendrobiums*, and raised a concern over genetic vulnerability of cultivars in this study because of their close genetic similarities.

Key words: Spring *Dendrobium*, amplified fragment length polymorphism (AFLP), genetic relationship, cultivars and varietal materials.

INTRODUCTION

Spring *Dendrobium* is the group of top grade pot flowers of *Dendrobium* representing blooming in spring, and is one of the top grade pot flowers which are popular in Japan, Britain, America and so on. It is provided with various flower colors, long period of florescence, multitudinous flowers and graceful flower shape, which makes it possess the skyscraping appreciation value (Hu, 2002). It is easily planted and conserved, adapting to the daily familial decoration. Nowadays, it is enthusiastically adored by Chinese citizens and salable in the flower markets

*Corresponding author. E-mail: zhengquan69@yahoo.com.cn.

in China.

The wild *Dendrobium* distributes mainly in the tropic and subtropic regins in Asia. More than 70 species of *Dendrobium* originated in China, they distribute chiefly in the provinces to the south of Qinling Mountains, and the most concentrative distributing region is Yunnan province. Modern pot flower Spring *Dendrobium* cultivars are mainly created by the multiple hybridizations of *D. nobile* etc., which resulting in the numerous flower colors (Hu, 2002).

Amplified fragment length polymorphisms (AFLP) emerged at the beginning of 1990s. Vos et al. (1995) showed that AFLP is a novel polymerase chain reaction (PCR) based assay for plant DNA fingerprinting that reveals significant levels of DNA polymorphism. The advantages of this technique included reproducibility, high levels of polymorphism detection, genome-wide distribution of markers, and no required prior knowledge of the genome being studied (Mueller et al., 1999). AFLP has been used to study genetic relationships and diversity of a wide range of species, such as *Calathea* (Chao et al., 2005), *Gladiolus* (Ranjan et al., 2010), *Eriobotrya* (Yang et al., 2009), *Ginkgo biloba* (Wang et al., 2006), *Pistacia* (Karimi et al., 2009), *Kobresia* (Zhang et al., 2009), *Boesenbergia* (Techaprasan et al., 2008), *Typha* (Na et al., 2010), *Rosa damascene* (Baydar et al., 2004), Sri Lankan *Oryza sativa* (Raajkumar et al., 2010), *Cherry salmon* (Gwo et al., 2008), Chinese eared pheasant (Li et al., 2010). It also has been widely applied in cultivars genetic analysis (Vega et al., 2006; Polanco and Ruiz, 2002; Baraket et al., 2009; Kong and Li, 2007; Devenand et al., 2004), genetic structures analysis (John et al., 2004), the establishment of the genetic catenation maps (Xiao et al., 2007; Shen et al., 2007), the orientation and cloning of genes (Xiao et al., 2009), species differentiation (Torriani et al., 2001; Schmidt et al., 2004) and so on because of its high automatization, good polymorphism and strong stability (Rajapakse et al., 1995; Dehaan et al., 2003) and proven to be extremely sensitive to distinguishing closely related cultivars (Chao et al., 2005).

For the first time, this study was intended to determine genetic relatedness of popular pot ornamental Spring *Dendrobium* cultivars planted in Zhejiang Senhe Seed Company Limited using AFLP markers with near-infrared fluorescence-labeled primers, providing a reference for the parent selection in the breeding of Spring *Dendrobium*.

MATERIALS AND METHODS

Thirty cultivars or varietal materials of Spring *Dendrobium* were selected from the warehouse of Zhejiang Senhe Seed Company Limited (Table 1). Some of them were introduced into China from Japan, Thailand and Taiwan. Whereas, another varietal material (Still have not been registered) were cultivated by Zhejiang Senhe Seed Company Limited in somaclonal variant hybrid and other ways. These varietal materials, for commercial reasons, in this paper were named as Senlan No.1, 2, 3 and so on.

Tender leaves were collected randomly, immediately frozen in liquid nitrogen, and quickly ground into powder in a white porcelain pestle after adding approximate 15 mL liquid nitrogen and frozen in -80°C.

Preparation of genomic deoxyribonucleic acid (DNA)

Genomic DNA was extracted from young leaves as Zeng et al. (2002), have some modified (Zheng et al., 2010).

Fluorescent- amplified fragment length polymorphism (AFLP) analysis

AFLP analysis was performed based on the study of Chen et al. (2004a), and was conducted using the AFLP System I (DingGuo Inc. China) and visualized with the ABI 377 automated sequencer (ABI Inc, USA). Total DNA (125 ng) from all samples was digested with 1 ml of mixture of EcoRI/MseI (1.25 units/ ml) at 37.8 °C overnight and ligated to EcoRI/MseI adapters with 1.5 ml (1 unit / ml) of T4 DNA ligase at 25.8 °C for at least 6 h. Pre-amplification reactions were performed on a Gene Amp PCR System 9600 (Perkin Elmer, USA). The pre-amplified PCR product was quantified in the fluorometer, the amount of template for subsequent PCR was diluted to 125 ng/ml for selective amplification. The selective-amplification PCRs were performed by another touchdown program as described by Chen et al. (2004a). Both pre- and selective-amplification conditions were modified according to the study of Myburg et al. (2000). The products from the selective amplification were electrophoresed on 25 cm to 0.25 mm 8% denaturing polyacrylamide Long Ranger1 Gel Solution (BMA, Rockland, ME, USA) in 0.8 % TBE buffer using ABI 377 automated sequencer (ABI, USA). Initially, a total of 64 AFLP primers were screened, from which eight *EcoR* I + 3 bases/*Mse* I + 3 bases primer sets (E-AAG/M-CAG, E-AAG/M-CTG, E-ACA/M-CTA, E-ACA/M-CTC, E-ACG/M-CTC, E-AGG/M-CAG, E-AGG/ M-CTA and E-AGG/ M-CTG) that showed clear scorable and highly polymorphic fragments (Table 2) were selected for fluorescent-AFLP reactions with the samples of the 30 Spring *Dendrobium* cultivars listed in Table 1.

Data analysis

AFLP fragments were visually scored as present (1) or absent (0) to create the binary data set. The data were entered into a binary data matrix as discrete variables. Jaccard's coefficient of similarity (Sneath and Sokal, 1973) was calculated for all pair-wise comparesons among the 30 cultivars. A dendrogram was generated by cluster analysis using the unweighted pair-group method of the arithmetic averages (UPGMA). Principal coordinate analysis (PCOA) was also carried out to show the broad multiple dimensional distributions of the cultivars in a scatter-plot (NTSYS-pc, version 2.1) (Rohlf, 2000). The consensus tree was drawn using the Tree-View software (Page et al., 1996).

RESULTS

Amplified fragment length polymorphism (AFLP) profiles and analysis

Clear-cut AFLP profiles for 30 Spring *Dendrobium* cultivars or varietal materials were generated by the eight primer sets. An example of fluorescent-AFLP profiles for the 30 samples using primer E-AAG/M-CAG is shown in

Table 1. List of 30 Spring *Dendrobium* cultivars used in this study.

S/N	Cultivar	Origin	Cultivar property
1	Senlan No. 1	Taiwan	White flower, pink flower corner, pink flower eye
2	Senlan No. 2	Taiwan	Strong pink flower, yellow flower center, pink flower eye
3	Senlan No. 3	Taiwan	White flower, pink flower corner, white flower center, pink flower eye
4	Senlan No. 4	Taiwan	Light pink flower, green flower center
5	Senlan No. 5	Taiwan	Pink flower, white flower center, pink flower eye
6	Senlan No. 6	Taiwan	White flower, pink flower corner, yellow flower center, orange red flower eye
7	Senlan No. 7	Taiwan	Light pink flower, white flower center, purple flower eye
8	Senlan No. 8	Taiwan	Light pink flower, light yellow flower center
9	Pittero Gold Grace'	Japan	Light yellow flower, green flower center
10	Senlan No. 9	Unknown	White flower, pink laciness, green flower center
11	Senlan No.10	Unknown	Pink flower, yellow flower center
12	'Pink Dell Elegame'	Japan	Pinkish white flower, yellow flower center
13	Senlan No. 11	Unknown	White flower, pink laciness, yellow flower center
14	Senlan No. 12	Unknown	Light yellow flower, faint pink flower corner
15	Senlan No. 13	Unknown	Light yellow flower
16	Senlan No. 14	Unknown	Pink flower, pink laciness, yellow flower center
17	Senlan No. 15	Unknown	Light yellow flower, faint red flower center
18	Senlan No. 16	Unknown	Light yellow flower, pink laciness
19	Senlan No. 17	Unknown	Pinkish white flower, pink laciness, yellow flower center
20	'Sanya'	Unknown	Pinkish white flower, green flower center
21	'China Doll'	Unknown	White flower, faint pink flower corner
22	'SnowBoy Romance'	Thailand	Light pink flower, green flower center
23	Senlan No. 18	Unknown	Yellow flower, red flower center
24	'Hamana Lake Maki'	Thailand	Purple flower, red flower center
25	'White Rabbit Sakura'	Thailand	White flower, yellow flower center
26	'Sakura Hime'	Thailand	Pinkish white flower, green flower center, green flower center, mini plant shape
27	'Santana Canary'	Thailand	Yellow flower
28	'Lai's Pearl Queen'	Thailand	White flower, comparatively small plant shape
29	'Snowflake Otome'	Japan	Pink flower, white flower center
30	'Snowflake Red Star'	Japan	Pink flower, purple flower center

Table 2. Amplified results of 30 Spring *Dendrobium* cultivars with the selected AFLP primer combinations

Primer	Total number of bands	Number of polymorphic bands	% of polymorphism bands
E-AAG/ M-CAG	126	91	72.2
E-AAG/ M-CTG	158	112	70.9
E-ACA/ M-CTA	137	91	66.4
E- ACA/ M-CTC	149	121	81.2
E- ACG/M- CTC	142	101	71.1
E- AGG/M-CAG	142	93	65.5
E- AGG/ M-CTA	135	95	70.4
E- AGG/ M-CTG	113	72	637
Total	1102	776	
Average	137.6	97	70.6

Figure. 1. The AFLP fragments ranged from 100 to 1020 bp, with the majority of them being distributed between 400 and 900 bp. Each primer set produced 113 to 158 scorable fragments, and the primer pair with the maximum amplification sites, namely 158, is the E-AAG/M-CTG. A total of 1102 AFLP fragments were detected, of which 776 (70.6%) were polymorphic (Table 2). Therefore, each primer set on an average, generated 137.6 fragments with 97 being polymorphic. The 8 pairs of primers differentiated the 30 Spring *Dendrobium* cultivars or varietal materials completely. The primer combination E-ACA/M-CTC produced the strong bands with good consistency and distribution. Comparatively, the primer combination E-AGG/M-CTG produced the

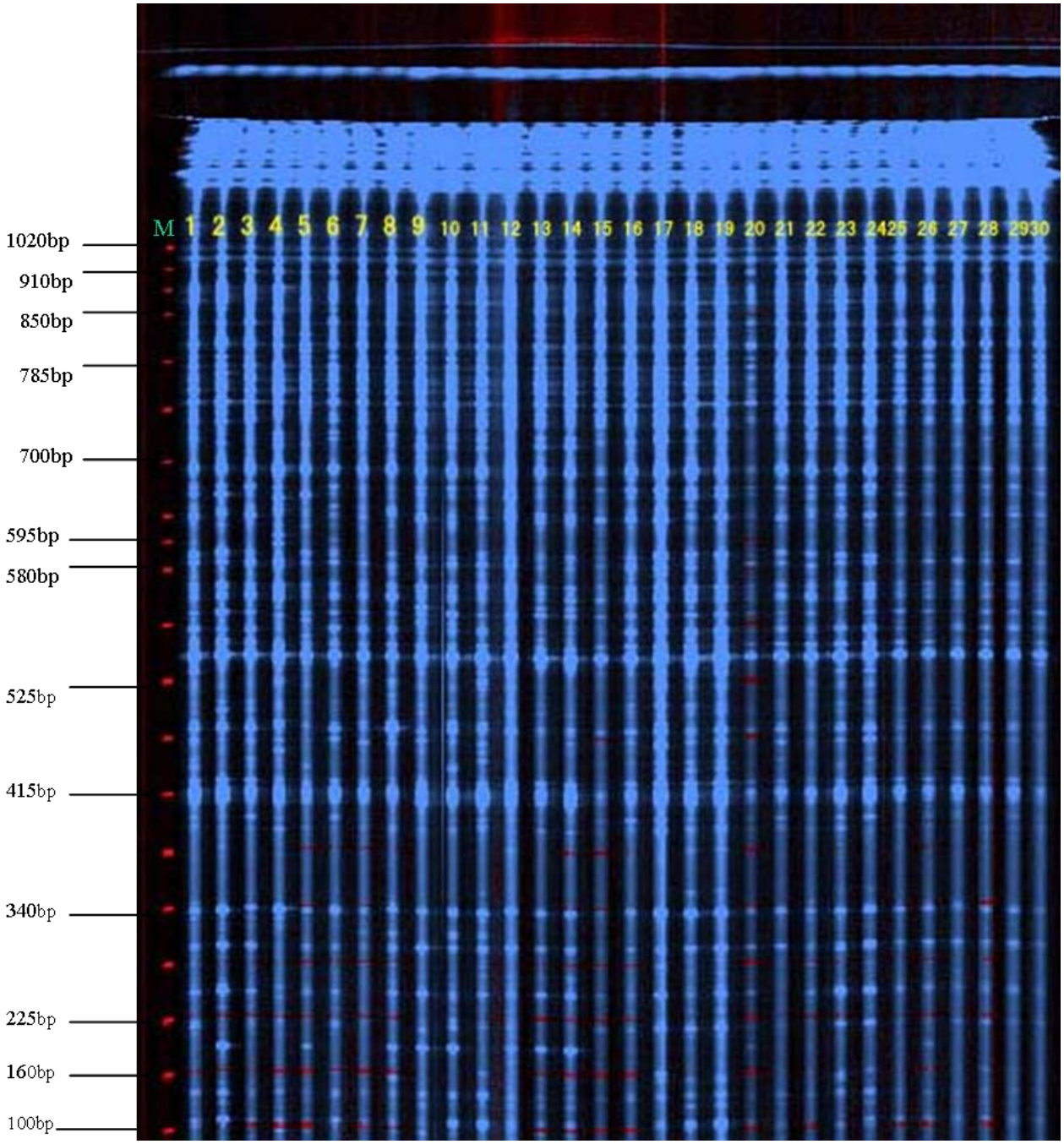

Figure 1. Near-infrared fluorescence-labeled AFLP fingerprinting diagram amplified by the primer combination E-AAG/M-CAG of the 30 Spring *Dendrobium* cultivars. From left to right, M: Near-infrared fluorescence-labeled Mark; lanes 1-30 electrophoresis channels: 30 Spring *Dendrobium* cultivars are designed according to Table 1. 1: Senlan No. 1; 2: Senlan No. 2; 3: Senlan No. 3; 4: Senlan No. 4; 5: Senlan No. 5; 6: Senlan No. 6; 7: Senlan No. 7; 8: Senlan No. 8; 9: 'Pittero Gold Grace'; 10: Senlan No. 9; 11: Senlan No. 10; 12: 'Pink Dell Elegame Elegame'; 13: Senlan No. 11; 14: Senlan No. 12; 15: Senlan No. 13; 16: Senlan No. 14; 17: Senlan No. 15; 18: Senlan No. 16; 19: Senlan No. 17; 20: 'Sanya'; 21: 'China Doll'; 22: 'SnowBoy Romance'; 23: Senlan No. 18; 24: 'Hamana Lake Maki'; 25: 'White Rabbit Sakura'; 26: 'Sakura Hime'; 27: 'Santana Canary'; 28: 'Lai's Pearl Queen'; 29: 'Snowflake Otome'; 30: 'Snowflake Red Star'.

bands with different strength intensity and even distribution. The resolution of the AFLP in this study was similar to the other studies of ornamental foliage plants. Chen et al. (2004a, b) reported 69% polymorphism in *Aglaonema* Schott and 71% polymorphism in *Dieffenbachia* Schott. Devanand et al. (2004) identified 64% fragments being polymorphic in cultivated ornamental *Philodendron* Schott. Some possible reasons for

these similar levels of polymorphism were that all of them were out-crossing genera, and these studies included several dozens of cultivars across multiple species.

Genetic diversity analysis

According to Figure 1, 30 Spring *Dendrobium* cultivars or varietal materials were generated by primer combinations E-AAG/M-CAG, a total of 126 AFLP fragments were detected, of which 91 (72.2%) were polymorphic. And using the other seven primer combinations achieved on average 70.6% of polymorphic. Figure 1 also showed that the more close genetic relationship among cultivars, the less differences in bands. For example, 'Pink Dell Elegame' and Senlan Number 11, using this primer combination only had 3 polymorphic loci, using other primer combinations in the DNA fingerprints had very small difference too, which indicated that these two cultivars had very close genetic relationship (Jaccard's similarity coefficients 0.84). However, genetic distance of 'Santana anary' was the farthest with other cultivars in this study, and its polymorphic band was also the most one among the cultivars using this primer combination, on the fingerprint of a total of 23 polymorphic loci. Which could be inferred AFLP technology was effective for relationship analysis of Spring *Dendrobium* cultivars.

Genetic relationship among cultivars

A dendrogram for the Spring *Dendrobium* cultivars or varietal materials was constructed on the basis of the UPGMA analysis (Figure 2). The Jaccard's coefficient of similarity matrix for all 30 accessions was presented in Table 3. A scatter plot of the PCOA was shown in Figure 3. Based on the UPGMA analysis and PCOA, 30 Spring *Dendrobium* cultivars or varietal materials were divided into five clusters. Clusters I, II, III, IV and V had 6,3,13, 7 and 1 cultivar, respectively.

Among the cultivars or varietal materials in cluster Ⅰ, all six of them originated in Taiwan, which were flowering about 20 days. According to the information, they were multi-generation hybrids, or clones mutant material of Taiwanese species 'Moniliforme' and 'Zuiko'. The similarity of them was high, between 0.70 to 0.80. Both Senlan No.1 and Senlan No.6 had white flower, pink flower corner, respectively. The Jaccard's similarity coefficient between the two was 0.74. Relationship of Senlan No.2 and Senlan No.4 was the closest in this cluster. The similarity coefficient between them was 0.8.

Clusters Ⅱ contained 3 cultivars or varietal materials. The similarity coefficient ranged from 0.71 to 0.76. Among them, 'Pittero Gold Grace' originated from Japan. Its resistance was strong and easily to be cultivated and managed. It had light yellow flowers with green flower center. The other two varietal materials were Senlan No.9 and Senlan No.14. Their parents originated from Taiwan. Senlan No.9 had white flowers, pink edge, and green flower center. Senlan No.14 had Pink flowers with red pink flower margin. Although flower colour of Senlan No.9 different from 'Pittero Gold Grace', but the similarity between them had reached 0.76, which was higher than its similarity with Senlan No.14 (0.72). It's estimated relationship between Senlan No.9 and 'Pittero Gold Grace' was close.

Clusters III was the largest group in this study, including 13 cultivars or varietal materials, Jaccard's similarity coefficients varied from 0.69 to 0.84, which origin was very complicated. Senlan No.11 was similar with 'Pink Dell Elegame' in genetic distance, and their similarity coefficient 0.84 was the highest in this study, even if they had some differences in flower color. The origin of Senlan No.11 was unknown. It was estimated to be a sport of the Japanese variety 'Pink Dell Elegame'. Both Senlan No.7 and Senlan No.8 had pink flower. The similarity coefficient of them was 0.77, Senlan No.7 originated from Taiwan while background of Senlan No.8 was unknown. Flower color of Senlan No.10 was white, and its flowering period was short (only 16d). Its genetic distance was close to Senlan No.12 and Senlan No.13 (similarity coefficient 0.72 and 0.75). All above three varietal materials were introduced from Taiwan. 'Sanya' was one of the most important potted cultivars of Spring *Dendrobium* because of its light pink flowers and great ornamental value. It came from Thailand, and had the closest genetic distance with the same originated cultivar 'Hamana Lake Maki' (similarity coefficient 0.75). Another three Thailand cultivars 'White Rabbit Sakura', 'Lai's Pearl Queen', 'China Doll' and a Japanese cultivar 'Snowflake Red Star' were far away from the above cultivars (similarity coefficient 0.71, 0.71, 0.70 and 0.69), in them, 'Snowflake Red Star 'had the farthest genetic distance with the others.

Seven cultivars from Senlan No. 15 or 'Snowboy Romance' are situated in Clusters IV with Jaccard's similarity coefficients ranging from 0.69 to 0.82. Among them, Senlan No.15 and Senlan No.16 maybe selected from somaclonal variants of the same parents, and the genetic distance between them was very close (0.82). Senlan No.17, Senlan No.18 and a Japanese cultivar 'Snowflake Otome' had close genetic relationship as 0.73 and 0.66, respectively. Hybrid parents of Senlan No.17 came from Japan, while the background of Senlan No.18 was unknown, and it was presumably origin from Japan. In this clusters, 'Sakura Hime' and 'Lai's Pearl Queen' were far away from another varieties in genetic distance (0.63, 0.66, respectively), and these two species were relatively small, especially 'Sakura Hime', it's pure mini, which height was only 11 cm, far below the average height of the other cultivars (25 to 30 cm). Both of them were introduced from Thailand.

Clusters V only included one cultivar 'Santana Canary'. It came from Hawaii, USA, and it had the farthest genetic

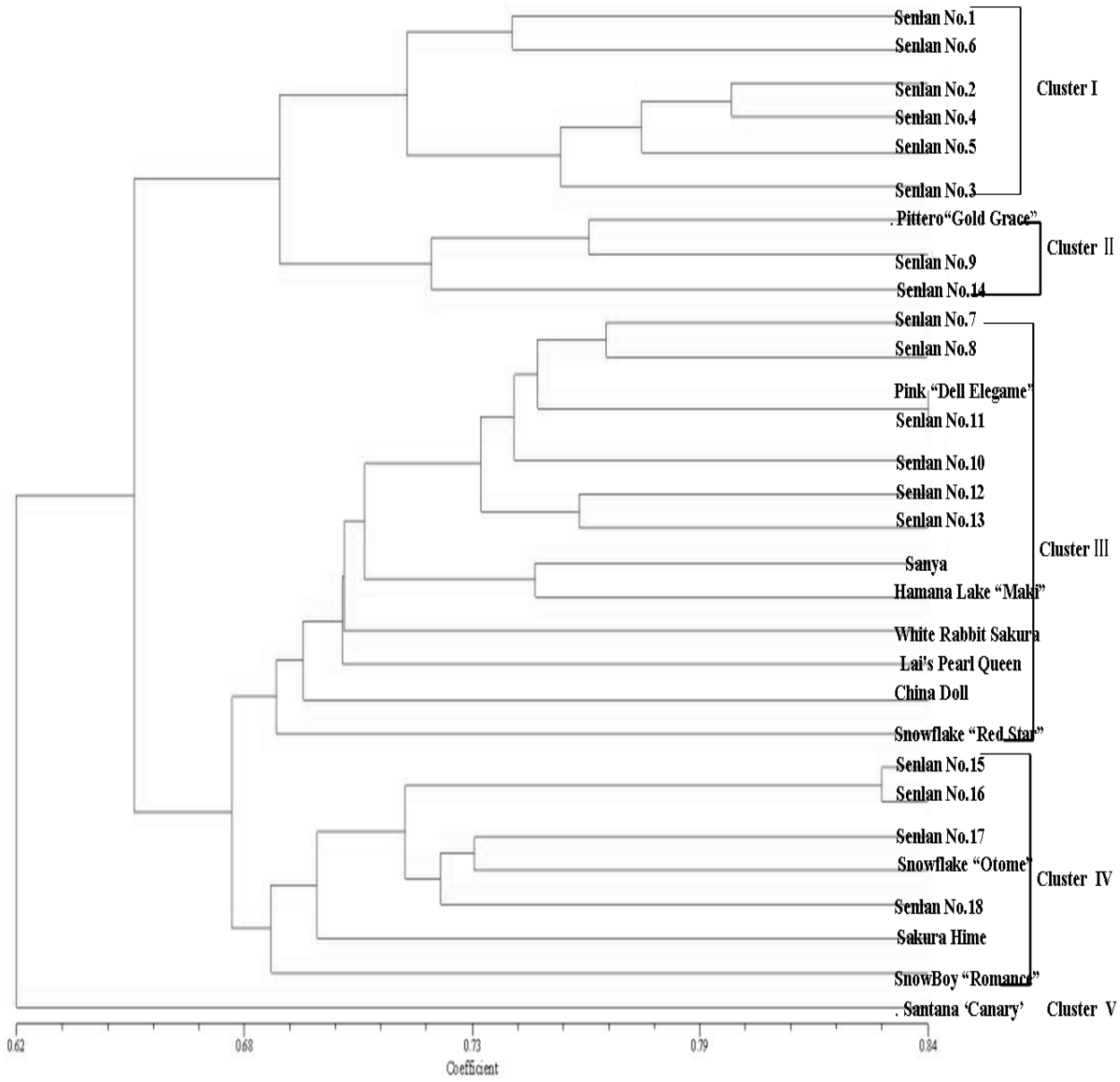

Figure 2. Dendrogram of 30 Spring *Dendrobium* cultivars resulting from the UPGMA cluster analysis based on Jaccard's similarity coefficients obtained from 1102 AFLP fragments.

distance (0.62) with another cultivars or varietal materials in this work. Its flower color was beautiful orange, without flower eye, flower center and flower corner. It was very different from another Spring *Dendrobium* species of this study in flower color and shape. And, its flower period was also longer than the other cultivars, reaching to 35 days.

In summary, cultivar 'Santana Canary' of Hawaii had the farthest genetic distance with another, which was at the bottom of this dendrogram. Cultivars or varietal materials mainly from Thailand were also located in lower. Cultivars from Taiwan had the farthest relationship with 'Santana Canary' of Hawaii, thus they occupied the top, as well as the cultivars from Japan were close to that from Taiwan, and more focused in the central of the cluster.

DISCUSSION

Characters analysis of cultivars with their genetic distance

From the analysis of the Spring *Dendrobium* cultivars or varietal materials in the listed 5 clusters, the follo-wing points could be inferred: Firstly, height and size of

Table 3. Jaccard's coefficient of similarity matrix for 30 Spring *Dendrobium* cultivars used in the study.

S/N	Cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	Senlan No. 1	1.00																													
2	Senlan No. 2	0.70	1.00																												
3	Senlan No. 3	0.71	0.74	1.00																											
4	Senlan No. 4	0.66	0.80	0.74	1.00																										
5	Senlan No. 5	0.71	0.77	0.77	0.77	1.00																									
6	Senlan No. 6	0.74	0.75	0.74	0.70	0.75	1.00																								
7	Senlan No. 7	0.62	0.67	0.65	0.65	0.70	0.66	1.00																							
8	Senlan No. 8	0.62	0.67	0.64	0.65	0.68	0.66	0.77	1.00																						
9	'Pittero Gold Grace'	0.70	0.68	0.67	0.65	0.67	0.73	0.65	0.71	1.00																					
10	Senlan No. 9	0.70	0.71	0.70	0.69	0.71	0.74	0.65	0.68	0.76	1.00																				
11	Senlan No. 10	0.60	0.64	0.64	0.62	0.68	0.66	0.73	0.75	0.68	0.65	1.00																			
12	'Pink Dell Elegame'	0.63	0.70	0.66	0.65	0.70	0.67	0.74	0.75	0.66	0.65	0.73	1.00																		
13	Senlan No. 11	0.57	0.63	0.61	0.60	0.65	0.63	0.74	0.76	0.65	0.63	0.77	0.84	1.00																	
14	Senlan No. 12	0.60	0.64	0.62	0.61	0.67	0.64	0.76	0.74	0.69	0.67	0.75	0.73	0.77	1.00																
15	Senlan No. 13	0.66	0.69	0.66	0.69	0.70	0.69	0.73	0.74	0.72	0.73	0.72	0.69	0.71	0.76	1.00															
16	Senlan No. 14	0.69	0.66	0.67	0.64	0.65	0.68	0.57	0.59	0.70	0.74	0.57	0.58	0.53	0.57	0.64	1.00														
17	Senlan No. 15	0.61	0.62	0.64	0.62	0.66	0.68	0.65	0.65	0.67	0.64	0.67	0.65	0.66	0.65	0.67	0.58	1.00													
18	Senlan No. 16	0.64	0.65	0.66	0.63	0.69	0.70	0.66	0.66	0.70	0.68	0.67	0.66	0.66	0.67	0.69	0.59	0.83	1.00												
19	Senlan No. 17	0.66	0.67	0.66	0.65	0.71	0.68	0.71	0.69	0.68	0.68	0.71	0.70	0.71	0.73	0.73	0.60	0.71	0.74	1.00											
20	'Sanya'	0.59	0.64	0.66	0.63	0.66	0.66	0.71	0.70	0.69	0.67	0.71	0.71	0.73	0.74	0.71	0.62	0.69	0.68	0.70	1.00										
21	'China Doll'	0.62	0.67	0.64	0.63	0.68	0.69	0.70	0.73	0.70	0.68	0.72	0.71	0.71	0.72	0.72	0.60	0.72	0.72	0.73	0.73	1.00									
22	'SnowBoy Romance'	0.63	0.65	0.62	0.63	0.65	0.70	0.62	0.64	0.70	0.66	0.63	0.65	0.64	0.62	0.65	0.58	0.70	0.71	0.67	0.67	0.67	1.00								
23	Senlan No. 18	0.59	0.63	0.62	0.61	0.63	0.64	0.68	0.71	0.73	0.66	0.71	0.66	0.69	0.72	0.72	0.58	0.66	0.67	0.68	0.69	0.70	0.65	1.00							
24	'Hamana Lake Maki'	0.66	0.69	0.67	0.67	0.69	0.72	0.69	0.70	0.71	0.72	0.69	0.71	0.68	0.70	0.72	0.66	0.67	0.69	0.71	0.75	0.71	0.68	0.69	1.00						
25	'White Rabbit Sakura'	0.63	0.68	0.66	0.67	0.71	0.68	0.69	0.72	0.67	0.69	0.71	0.69	0.67	0.70	0.71	0.62	0.66	0.65	0.68	0.71	0.71	0.63	0.67	0.69	1.00					
26	'Sakura Hime'	0.64	0.65	0.63	0.62	0.65	0.66	0.63	0.65	0.67	0.65	0.64	0.64	0.64	0.66	0.65	0.60	0.70	0.72	0.69	0.66	0.68	0.68	0.66	0.69	0.64	1.00				
27	'Santana Canary'	0.54	0.57	0.57	0.56	0.59	0.59	0.65	0.66	0.63	0.61	0.66	0.63	0.67	0.66	0.64	0.51	0.62	0.64	0.64	0.65	0.66	0.62	0.66	0.61	0.61	0.64	1.00			
28	'Lai's Pearl Queen'	0.60	0.68	0.67	0.65	0.67	0.67	0.71	0.70	0.67	0.69	0.67	0.69	0.69	0.72	0.75	0.56	0.63	0.67	0.67	0.70	0.69	0.63	0.67	0.68	0.70	0.62	0.62	1.00		
29	'Snowflake Otome'	0.60	0.63	0.63	0.59	0.66	0.66	0.70	0.71	0.67	0.66	0.71	0.73	0.74	0.73	0.67	0.57	0.70	0.70	0.73	0.69	0.72	0.67	0.66	0.69	0.65	0.68	0.65	0.66	1.00	
30	'Snowflake Red Star'	0.62	0.66	0.64	0.64	0.65	0.65	0.72	0.70	0.65	0.65	0.66	0.70	0.68	0.71	0.71	0.60	0.64	0.63	0.68	0.67	0.66	0.61	0.64	0.68	0.68	0.61	0.62	0.66	0.65	1.00

cultivars were less relevant with their genetic relationship such as 'Sakura Hime', even if its height and size differed greatly with other species, but their similarity coefficient still reached to 0.70 at least. Secondly, flower color characteristics of varieties have certain relevance with their genetic relationship. For example, 'Santana Canary' had the biggest difference in color with the other cultivars in this study, also had the furthest genetic distance (similarity coefficient 0.62). However, color characteristics were not entirely consistent to the genetic distance between species, such as clusters Ⅲ, in which not only has white or pink flowers varieties, but also has yellow color cultivars. Thirdly, the origin of

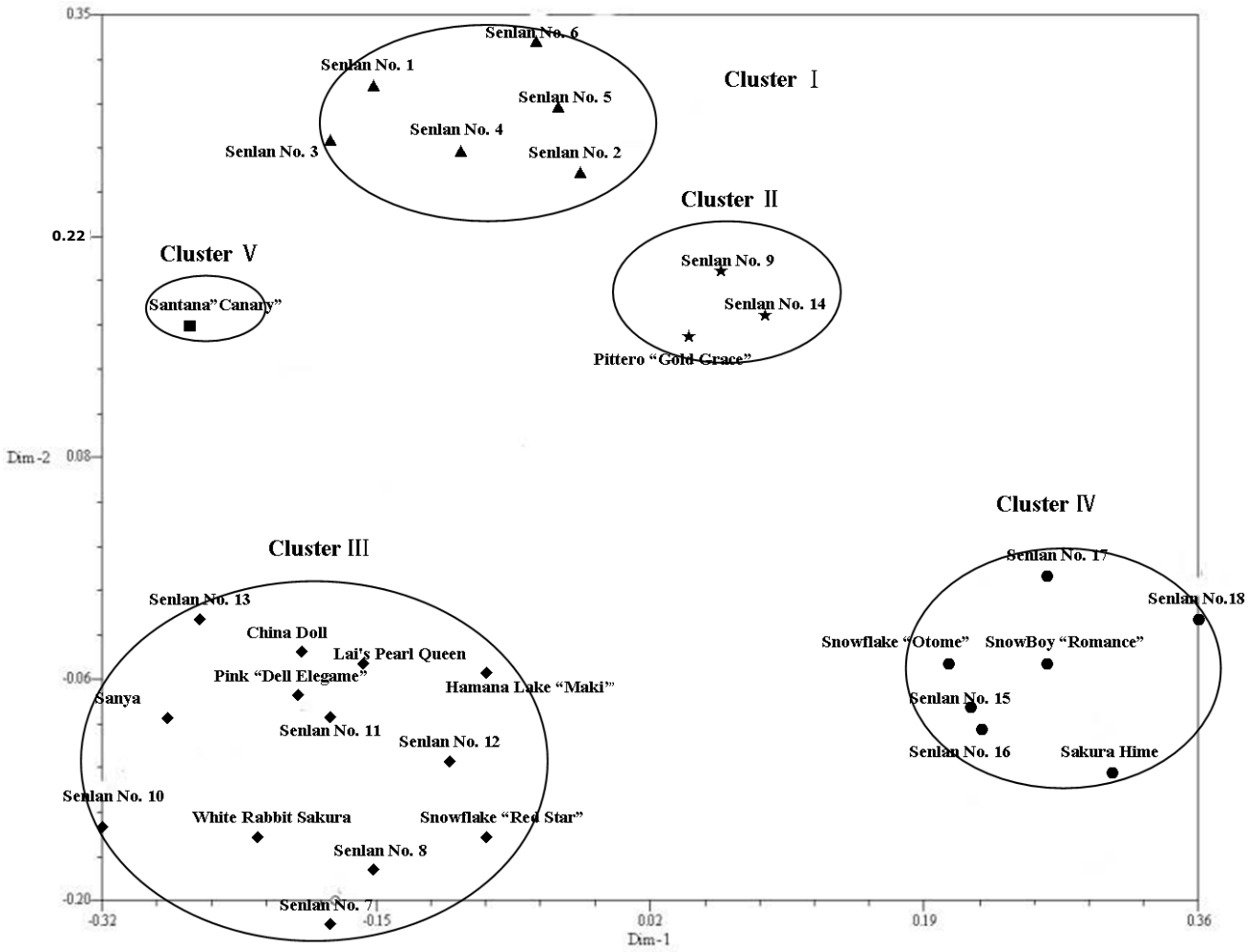

Figure 3. Principal coordinate analysis plot of 30 Spring *Dendrobium* cultivars for the first and second principal coordinates estimates based on 1102 AFLP fragments derived from 8 primer sets using Jaccard's similarity matrix.

cultivars was closely related to their genetic distance. Most cultivars or varietal materials in clusters Ⅰ and clusters Ⅱ originated from Taiwan area. While, cultivars or varietal materials in clusters Ⅲ not only came from Taiwan area and Japan, but also origined from Thailand. This may be related with the extensive exchange of breeding materials today.

Implication in spring dendrobium breeding

According to the Dendrogram of 30 Spring *Dendrobium* cultivars in Figure 2, their genetic relationship were close, had the lowest similarity coefficient of 0.62, which reflected the parents of currently cultivars used for horticulturists were relatively single, and relationships between the parents were close. All these scenarios raised a serious concern over the genetic vulnerability of the cultivars to potentially new diseases and pests in production. Therefore, we needed to broaden the genetic diversity of cultivated Spring *Dendrobium*. Strategies for increasing genetic diversity included the introduction of new species, development of hybrids using distant related plants as parents, and identification of somaclonal variants or sports whose parents were interspecific hybrids. The current study provided genetic relationships among cultivated Spring *Dendrobium*, which used genetically distant cultivars as parents for hybridization and will result in progenies with increased genetic diversity. In all 30 Spring *Dendrobium* cultivars, 'Santana Canary', because of its furthest genetic distance (similarity coefficient 0.62) with other cultivars, high ornamental value colors, and longer flowering time, was considered as an ideal cross-breeding parent materials. Although the characteristics of 'Sanya' and other cultivar are acceptable, due to the shorter flowering (20 d) and lack of the ability of anti-ethylene (Zhou, 2006), they will be needed to be improved. 'Santana Canary' could be considered to be selected as hybridization male parent to improve their flowering characteristics.

This is the first molecular fingerprint study of the genetic relationships of cultivated Spring *Dendrobium*, the 30

cultivars were clearly distinguished by AFLP analysis. The AFLP profiles and the clusters established could be used as bases for comparison with other *Dendrobium* species or Spring *Dendrobium* cultivars. The genetic similarity among cultivars established could help future Spring *Dendrobium* germplasm identification, presservation, and new cultivar development.

Abbreviations

AFLP, Amplified fragment length polymorphism; **PCOA,** principal coordinate analysis; **PCR,** polymerase chain reaction; **UPGMA,** unweighted pair-group method of the arithmetic averages.

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