Genetic Relationships among Native Species and Hybrid Cultivars of Asian *Dendrobium* (Orchidaceae) Using Amplified Fragment Length Polymorphism Markers

Gen-Fa Zhu¹ and Dong-Mei Li

Guangdong Key Laboratory of Ornamental Plant Germplasm Innovation and Utilization, Floricultural Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China

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Abstract. This study addresses the phylogenetic relationships among native species and hybrid cultivars of Asian *Dendrobium* by amplified fragment length polymorphism (AFLP). The plant materials of this study are composed of 37 accessions belonging to native species in China and 63 accessions proposed to be hybrid cultivars originating from Japan and Korea. Eight AFLP primer combinations produced a total of 1658 fragments with an average of 207 fragments per primer pair, of which 1655 bands were polymorphic. Specific AFLP markers were identified in 29 of 100 tested *Dendrobium* accessions. Unweighted pair group method based on arithmetic average (UPGMA) analysis was performed on Dice's similarity coefficient matrix and also average similarity of each species and cultivar. The tested 100 Asian *Dendrobium* accessions were grouped into seven clusters with the similarity coefficient of 0.49. A first cluster consisted of 63 hybrid cultivars, 17 species of section *Dendrobium*, one species of section *Formosae*, and one species of section *Callista*. A second, fourth, and seventh cluster included five, three, and two species of section *Dendrobium*, respectively. A third group comprised five species of section *Formosae*. A fifth and sixth cluster contained three and two species of section *Callista*, respectively. These results indicated that the genetic relationships among tested Asian *Dendrobium* accessions were related to their origins, morphological classification, flower color, and pedigree, to some extent.

The genus *Dendrobium* is one of the largest genera in Orchidaceae family, comprising ≈1200 species (http://en.wikipedia.org/wiki/list_of_Dendrobium_species) of *Dendrobium* distributed in tropical and subtropical Asia and Oceania. They have been used not only in traditional Chinese medicine in Asian countries (Bulpitt et al., 2007), but also in commercial cut flowers and potted plants (Chen and Tsi, 2000). For commercial production of cut flowers and potted plants with new floral characteristics and other beneficial traits, a large number of novel cultivars of *Dendrobium* have been produced through interspecific hybridization in Japan and Korea. In China, there are 74 native species and two varieties of *Dendrobium* classified into 12 sections by traditional characteristics, including *Grastidium* (four species), *Callista*

(six species), *Dendrobium* (36 species), *Distichophyllum* (one species), *Breviflores* (two species), *Stuposa* (one species), *Pedilonum* (three species), *Formosae* (seven species), *Stachyobium* (five species), *Crumenatae* (four species), *Aporum* (three species), and *Strongyle* (two species) (Tsi et al., 1999). Therefore, these species of *Dendrobium* should be exploited for breeding.

It is important for breeding to understand the genetic relationships and molecular phylogeny of the genus *Dendrobium*. In recent years, genetic relationships and molecular identification of *Dendrobium* species have been measured by different molecular tools, including chloroplast DNA (Yukawa et al., 1993, 1996), internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA and plastid *mat K* (Burke et al., 2008; Lau et al., 2001; Tsai et al., 2004; Wongsawad et al., 2001; Yuan et al., 2009; Yukawa, 2001), simple sequence repeat (SSR) marker (Gu et al., 2007; Yue et al., 2006), and intersimple sequence repeat (ISSR) marker (Wang et al., 2009). However, much information related to taxonomy and phylogenesis of the *Dendrobium* genus still needs to be solved. Hence, there is a need to study the genus *Dendrobium* using more abundant and reliable characters such as AFLP markers. The AFLP analysis

provides a powerful DNA fingerprinting technique for DNAs of any origin or complexity, needs no cloning and sequencing, and produces a large number of recordable fragments, which enhance its power to detect polymorphism (Vos et al., 1995). The AFLP technique had been successfully used in the estimation of genetic relationships and differentiation among individuals, populations, and species of a wide range of plant families (e.g., Depypere et al., 2009; Karimi et al., 2009; Pamidiamarri et al., 2009; Seehalak et al., 2006). In particular, the genetic diversity and population structure of *D. officinale* was identified by AFLP (Li et al., 2008).

In the present study, we collected 37 native species of *Dendrobium* in China and 63 hybrid cultivars of *Dendrobium* originated from Japan and Korea. The pedigree of some hybrid cultivars, which was obtained by searching the web (<http://apps.rhs.org.uk/horticulturaldatabase/orchidregister/orchidregister.asp>), was displayed in Supplemental Figure 1. The main objectives of this research were: 1) to test the AFLP technique for molecular identification of collected Asian *Dendrobium*; 2) to assess phylogenetic relationships among tested native species and hybrid cultivars of Asian *Dendrobium*; and 3) to supply molecular evidence for classification of Asian *Dendrobium*.

Materials and Methods

Plant materials. One hundred *Dendrobium* accessions were selected (Table 1). Thirty-seven of them originated from floricultural research institute, Guangdong Academy of Agricultural Sciences, China; the others came from Korea (three) and Japan (60). According to horticultural characters, they included 63 hybrid cultivars (c1 to c63), six species of section *Callista* (c67, c71, c81, c82, c89, c94), six species of section *Formosae* (c72, c74, c76, c79, c88, c95), and 25 species of section *Dendrobium* (c64 to c66, c68 to c70, c73, c75, c77, c78, c80, c83 to c87, c90 to c93, c96 to c100). Fresh leaf samples were collected from three individuals of each cultivar or species for genomic DNA extraction.

Genomic DNA extraction and amplified fragment length polymorphism reaction. Genomic DNA was extracted from leaf samples using a modified CTAB protocol as previously described (Li et al., 2007). AFLP assays and resolution of amplification products were performed according to the method of Zhu et al. (2007). Briefly, the restriction reaction was carried out with 200 ng genomic DNA digested with *EcoR* I and *Mse* I. *EcoR* I and *Mse* I adapters were ligated to the fragments. The adapter sequences, preselective amplification primers, and selective primers are listed in Table 2. Of these, eight primer pairs that generated well-defined patterns were chosen for use in this study (Table 3).

Data analysis. Generated AFLP bands were individually scored and statistically analyzed by following the assumption that fragment size as a locus was considered as either present (1) or absent (0) and finally

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¹To whom reprint requests should be addressed; e-mail genfazhu@163.com.

constructed a binary matrix. Only fragments that had a molecular weight greater than 50 bps and high intensity were considered for data analysis. The Dice's similarity coefficient matrix was calculated using NTSYSpc package (Version 2.11) (Rohlf, 2004), and the dendrogram was produced using the UPGMA. The percent polymorphism was calculated as the ratio of total number of polymorphic bands compared with total number of bands.

Results

Amplified fragment length polymorphism profile and analysis. Eight selected primer combinations used in this study generated 1658 recordable bands with an average of 207 bands per primer pair, of which 1655 bands were polymorphic. The number of polymorphic bands varied from 185 (E_{ACA}/M_{CAG}) to 215 (E_{ACT}/M_{CAA} or E_{ACG}/M_{CTG}). The number of polymorphic markers and percent polymorphism for each pair of primers are shown in Table 3. These data suggest that polymorphism of genomic DNA exists among native species and hybrid cultivars of Asian *Dendrobium*.

Amplified fragment length polymorphism markers for native species and hybrid cultivar diagnosis. Each of the tested *Dendrobium* accessions showed a unique AFLP genotyping profile based on measurement of 1655 AFLP polymorphic bands. Among eight AFLP primer pair combinations, they revealed amplified DNA fragments that were unique to 29 *Dendrobium* accessions (Table 1). These AFLP primers amplifying specific DNA fragments provide molecular tools for identification of tested native species and hybrids of Asian *Dendrobium*. For example, 5, 4, 3, 3, 3 and 3 AFLP markers were specific for *D. sulcatum* (c94), *D. utopia* 'Messenger' (c9), *D. jenkinsii* (c82), *D. brymerianum* (c85), *D. aphyllum* (c87), and *D. densiflorum* (c89), respectively. These data indicated that the AFLP technique was a useful molecular method for the identification of native species and hybrid cultivars of Asian *Dendrobium* at their genomic DNA levels.

Genetic relationships among native species and hybrids of Asian *Dendrobium*. The AFLP-generated data in this study were analyzed using Dice's similarity coefficient. According to the similarity matrix and UPGMA clustering, these native species and hybrid cultivars were grouped into seven clusters (I to VII) with a similarity level of 0.49 (Fig. 1). Cluster I consisted of 82 accessions and was further divided into five subclusters with a similarity level of 0.53 (Fig. 1): Subcluster Ia with 63 hybrid cultivars (c1 to c63) originated from Japan and Korea and three native species (c65, c66, and c98) belonging to section *Dendrobium*; Subcluster Ib with 12 native species (c68, c70, c74, c80, c85, c90, c91, c94, c96, c97, c99, and c100) belonging to section *Dendrobium* except two native species (c74 and c94); c74 and c94 belonging to sections *Formosae* and *Callista*, respectively; and Subclusters Ic, Id,

Table 1. List of plant materials used in the phylogenetic analysis of *Dendrobium* by amplified fragment length polymorphism (AFLP) markers.

Sample no.	Species or cultivar	Section	Flower color	Origin	Specific (delete) bands	AFLP cluster
c1	<i>D. Fairyflake</i> 'Carmen'	Hybrid	Modena	Japan	0	Ia
c2	<i>D. Spot Right</i>	Hybrid	Modena	Japan	0	Ia
c3	<i>D. Kazuki</i>	Hybrid	Modena	Japan	0	Ia
c4	<i>D. Yohkou</i> 'Peach Boy'	Hybrid	Modena	Japan	1	Ia
c5	<i>D. Yohkou</i> 'Cat's Eyes'	Hybrid	Modena	Japan	0	Ia
c6	<i>D. Full Bloom</i> 'Hanahime'	Hybrid	Lilac	Japan	0	Ia
c7	<i>D. Comet King</i> 'Akatsuki'	Hybrid	Modena	Japan	0	Ia
c8	<i>D. Pink Beauty</i> 'Queen'	Hybrid	Modena	Japan	0	Ia
c9	<i>D. Utopia</i> 'Messenger'	Hybrid	Modena	Japan	4	Ia
c10	<i>D. Tomoflake</i> 'Naporí'	Hybrid	Modena	Japan	0	Ia
c11	<i>D. Maloflake</i> 'Yumedono'	Hybrid	Modena	Japan	0	Ia
c12	<i>D. Shinonome</i> 'Karen'	Hybrid	Lilac	Japan	0	Ia
c13	<i>D. 'Bizen Akebano'</i>	Hybrid	Modena	Japan	1	Ia
c14	<i>D. Casiflake</i>	Hybrid	Modena	Japan	0	Ia
c15	<i>D. Emur</i>	Hybrid	Modena	Japan	0	Ia
c16	<i>D. Tubuyaki</i>	Hybrid	White	Japan	0	Ia
c17	<i>D. Moon Harmaid</i>	Hybrid	White	Japan	0	Ia
c18	<i>D. Spring Color</i> 'Hohoemi'	Hybrid	White	Japan	0	Ia
c19	<i>D. Yaotome</i>	Hybrid	Modena	Japan	0	Ia
c20	<i>D. Red Arce</i>	Hybrid	Modena	Japan	1	Ia
c21	<i>D. Yukidaruma</i> 'Queen'	Hybrid	White	Japan	0	Ia
c22	<i>D. Love Story</i>	Hybrid	White	Japan	0	Ia
c23	<i>D. Payruroyzu</i>	Hybrid	Lilac	Japan	0	Ia
c24	<i>D. Sailor Boy</i> 'Hatsuyuki'	Hybrid	White	Japan	0	Ia
c25	<i>D. Second Love</i> 'Tokimeki'	Hybrid	White	Japan	0	Ia
c26	<i>D. 'Benikujaku'</i>	Hybrid	Amaranth	Japan	1	Ia
c27	<i>D. Oriental Sprit</i> 'Rudoruph'	Hybrid	Garnet	Japan	0	Ia
c28	<i>D. Sekand Rave</i> 'Yakigi Pon'	Hybrid	White	Japan	0	Ia
c29	<i>D. Shirasagi</i>	Hybrid	White	Japan	0	Ia
c30	<i>D. Marchen Color</i>	Hybrid	White	Japan	0	Ia
c31	<i>D. Rainbow</i> 'Saira'	Hybrid	White	Japan	0	Ia
c32	<i>D. Snowflake</i> 'Otome'	Hybrid	White	Japan	0	Ia
c33	<i>D. Himezakura</i> 'Fujikka'	Hybrid	White	Japan	0	Ia
c34	<i>D. Sping Color</i> 'Green Sleep'	Hybrid	White	Japan	0	Ia
c35	<i>D. Harugasumi</i>	Hybrid	White	Japan	0	Ia
c36	<i>D. Sailor Boy</i> 'Elite'	Hybrid	White	Japan	0	Ia
c37	<i>D. Pink Doll</i> 'Elegance'	Hybrid	Pink	Japan	0	Ia
c38	<i>D. Pittero Gold</i> 'Princess'	Hybrid	Yellow-green	Japan	1	Ia
c39	<i>D. Nice Dream</i> 'Lady'	Hybrid	Unknown	Japan	0	Ia
c40	<i>D. Yellow Magic</i> 'Carnival'	Hybrid	Orange	Japan	0	Ia
c41	<i>D. White Rabbit</i> 'Sakurahime'	Hybrid	White	Japan	0	Ia
c42	<i>D. Sunny Gold</i> 'Harp'	Hybrid	Unknown	Japan	0	Ia
c43	<i>D. Lucky Gal</i> 'Emito'	Hybrid	Reseda	Japan	0	Ia
c44	<i>D. Spring Zewel</i> 'Miui'	Hybrid	Reseda	Japan	0	Ia
c45	<i>D. Yourpin Red</i>	Hybrid	Purple	Japan	0	Ia
c46	<i>D. Okayama Gold</i> 'Harmony'	Hybrid	Yellow	Japan	0	Ia
c47	<i>D. Spring Dream</i> 'Kumiko'	Hybrid	White	Japan	0	Ia
c48	<i>D. Sea Mary</i> 'Snow King'	Hybrid	White	Japan	0	Ia
c51	<i>D. Yellow Song</i> 'Rhythm'	Hybrid	Yellow	Japan	0	Ia
c52	<i>D. Marohuiku</i>	Hybrid	Modena	Japan	0	Ia
c53	<i>D. Yohkou</i> 'Cat's Eyes'	Hybrid	Garnet	Japan	0	Ia
c54	<i>D. Lady Smile</i>	Hybrid	Modena	Japan	0	Ia
c55	<i>D. Stardust</i> 'Fire Bird'	Hybrid	Orange	Japan	0	Ia
c56	<i>D. Red Star</i>	Hybrid	Purple	Japan	0	Ia
c57	<i>D. Hamanal Lake</i> 'Dream'	Hybrid	Purple	Japan	0	Ia
c58	<i>D. Spring Jewel</i> 'Miki'	Hybrid	White	Japan	0	Ia
c59	<i>D. Yume Ji</i> 'No.2'	Hybrid	Yellow	Japan	0	Ia
c60	<i>D. 'Jin Shan'</i>	Hybrid	White	Korea	0	Ia
c61	<i>D. 'Yun Xue'</i>	Hybrid	White	Korea	0	Ia
c62	<i>D. 'Xu Yun'</i>	Hybrid	White	Korea	0	Ia
c63	<i>D. hybrid</i>	Hybrid	White	Japan	0	Ia
c64	<i>D. parishii</i>	<i>Dendrobium</i>	Purple	China	1	II
c65	<i>D. nobile</i>	<i>Dendrobium</i>	White	China	0	Ia
c66	<i>D. moniliforme</i> variation	<i>Dendrobium</i>	Kelly	China	0	Ia
c67	<i>D. thyrsoflorum</i>	<i>Callista</i>	White	China	1	VI
c68	<i>D. chrysanthum</i>	<i>Dendrobium</i>	Yellow	China	1	Ib
c69	Unknown	<i>Dendrobium</i>	Unknown	China	2	VII
c70	<i>D. fimbriatum</i>	<i>Dendrobium</i>	Orange	China	0	Ib
c71	<i>D. chrysotoxum</i>	<i>Callista</i>	Golden	China	2	V
c72	<i>D. williamsonii</i>	<i>Formosae</i>	Primrose	China	0	III
c73	<i>D. hancockii</i>	<i>Dendrobium</i>	Yellow	China	0	II

(Continued on next page)

Table 1. (Continued) List of plant materials used in the phylogenetic analysis of *Dendrobium* by amplified fragment length polymorphism (AFLP) markers.

Sample no.	Species or cultivar	Section	Flower color	Origin	Specific (delete) bands	AFLP cluster
c74	<i>D. trigonopus</i>	<i>Formosae</i>	Golden	China	0	Ib
c75	<i>D. guangxiense</i>	<i>Dendrobium</i>	Primrose	China	0	VII
c76	<i>D. cariniferum</i>	<i>Formosae</i>	White	China	0	III
c77	<i>D. pendulum</i>	<i>Dendrobium</i>	White	China	1	IV
c78	<i>D. capillipes</i>	<i>Dendrobium</i>	Yellow	China	1	Ie
c79	<i>D. christyanum</i>	<i>Formosae</i>	White	China	0	III
c80	<i>D. falconeri</i>	<i>Dendrobium</i>	White	China	0	Ib
c81	<i>D. lindleyi</i>	<i>Callista</i>	Primrose	China	1	V
c82	<i>D. jenkinsii</i>	<i>Callista</i>	Yellow	China	3	V
c83	<i>D. chryseum</i>	<i>Dendrobium</i>	Yellow	China	1	Ic
c84	<i>D. loddigesii</i>	<i>Dendrobium</i>	Rosy	China	1 (1)	II
c85	<i>D. brymerianum</i>	<i>Dendrobium</i>	Yellow	China	3	Ib
c86	<i>D. wardianum</i>	<i>Dendrobium</i>	White	China	2	Id
c87	<i>D. aphyllum</i>	<i>Dendrobium</i>	Lilac	China	3	II
c88	<i>D. bellatulum</i>	<i>Formosae</i>	White	China	0	III
c89	<i>D. densiflorum</i>	<i>Callista</i>	Golden	China	2	VI
c90	<i>D. devonianum</i>	<i>Dendrobium</i>	White	China	0	Ib
c91	<i>D. gibsonii</i>	<i>Dendrobium</i>	Orange	China	1	Ib
c92	<i>D. gratiosissimum</i>	<i>Dendrobium</i>	Pink	China	1	Id
c93	<i>D. primulinum</i>	<i>Dendrobium</i>	Pink	China	0	II
c94	<i>D. sulcatum</i>	<i>Callista</i>	Yellow	China	5	Ib
c95	<i>D. infundibulum</i>	<i>Formosae</i>	White	China	1	III
c96	<i>D. heterocarpum</i>	<i>Dendrobium</i>	Yellow	China	1	Ib
c97	<i>D. harveyanum</i>	<i>Dendrobium</i>	Golden	China	2	Ib
c98	<i>D. henryi</i>	<i>Dendrobium</i>	Golden	China	2	Ia
c99	<i>D. crystallinum</i>	<i>Dendrobium</i>	White	China	3	Ib
c100	<i>D. moniliforme</i>	<i>Dendrobium</i>	Primrose	China	0	Ib

Table 2. Sequences of oligonucleotide adaptor and primers used in phylogenetic analysis of 100 Asian *Dendrobium* accessions by amplified fragment length polymorphism markers.

Adaptor/primer	Code	Sequence (5' → 3')
Adaptors		
<i>Eco</i> R I adaptor		CTC GTA GAC TGC GTA CC AAT TGG TAC GCA GTC TAC
<i>Mse</i> I adaptor		GAC GAT GAG TCC TGA G TAC TCA GGA CTC AT
Preselective amplification primers		
<i>Eco</i> R I primer + A		GAC TGC GTA CCA ATT CA
<i>Mse</i> I primer + C		GAT GAG TCC TGA GTA AC
Selective amplification primers		
<i>Eco</i> R I + AAC	E _{AAC}	GAC TGC GTA CCA ATT C AAC
<i>Eco</i> R I + ACA	E _{ACA}	GAC TGC GTA CCA ATT C ACA
<i>Eco</i> R I + ACT	E _{ACT}	GAC TGC GTA CCA ATT C ACT
<i>Eco</i> R I + ACC	E _{ACC}	GAC TGC GTA CCA ATT C ACC
<i>Eco</i> R I + ACG	E _{ACG}	GAC TGC GTA CCA ATT C ACG
<i>Mse</i> I + CTG	M _{CTG}	GAT GAG TCC TGA GTA A CTG
<i>Mse</i> I + CAG	M _{CAG}	GAT GAG TCC TGA GTA A CAG
<i>Mse</i> I + CAA	M _{CAA}	GAT GAG TCC TGA GTA A CAA
<i>Mse</i> I + CTT	M _{CTT}	GAT GAG TCC TGA GTA A CTT
<i>Mse</i> I + CTC	M _{CTC}	GAT GAG TCC TGA GTA A CTC

Table 3. Banding pattern information in DNA polymorphism of 100 *Dendrobium* accessions with amplified fragment length polymorphism markers.

Primer combination	No. of total bands	No. of polymorphic bands	Percent of polymorphism
E _{AAC} /M _{CTG}	215	213	99.1
E _{ACA} /M _{CAG}	185	185	100
E _{ACT} /M _{CAA}	215	215	100
E _{ACT} /M _{CTT}	211	211	100
E _{ACC} /M _{CTG}	197	197	100
E _{ACC} /M _{CTT}	208	208	100
E _{ACG} /M _{CTC}	211	211	100
E _{ACG} /M _{CTG}	216	215	99.5
Total	1658	1655	99.8
Mean	207	206	—

and 1e with one (c83), two (c86 and c92), and one (c78) native species, respectively, all of them belonging to section *Dendrobium*. Cluster II contained five native species (c72, c76, c79, c88, and c95), all of which belonged to section *Dendrobium*. Cluster III also included five native species (c72, c76, c79, c88, and c95) belonging to section *Formosae*. Clusters IV and VII contained one (c77) and two (c69 and c75) native species, respectively, all belonging to section *Dendrobium*. Clusters V and VI included three (c71, c81, and c82) and two (c67 and c89) native species, respectively, all belonging to section *Callista*.

Discussion

For breeding, it is important to evaluate the phylogenetic relationships among native species and hybrid cultivars of Asian *Dendrobium*. However, despite different molecular tools being used, the available information of genetic relationships among native species and hybrid cultivars of Asian *Dendrobium* is still very limited (Burke et al., 2008; Gu et al., 2007; Lau et al., 2001; Tsai et al., 2004; Wang et al., 2009; Wongsawad et al., 2001; Yuan et al., 2009; Yue et al., 2006; Yukawa, 2001; Yukawa et al., 1993, 1996). In the current study, AFLP markers were used to analyze genetic relationships and molecular phylogeny among 63 hybrid cultivars and 37 native species of Asian *Dendrobium*. Eight primer combinations generated a total of 1658 bands with 99.8% polymorphism. In genus *Pistacia*, AFLP markers were used for the determination of phylogenetic relationships among 50 genotypes, including 44 accessions belonging to subspecies of *P. atlantica* (*atlantica*, *mutica*, *kurdica*, and *cabulica*), three unknown genotypes, and three accessions proposed to be hybrid from *P. eurycarpa* × *P. atlantica* (Karimi et al., 2009). Moreover, AFLP markers were successfully used to genetically classify cultivars or varieties of a bamboo species (Lin et al., 2009). Our results revealed that AFLP technique was a powerful tool for analyses of genetic relationships among native species and hybrid cultivars of Asian *Dendrobium*.

According to traditional morphological classification, 74 *Dendrobium* species and two varieties were classified into 12 sections (Tsi et al., 1999). In our AFLP clusters, Cluster II contained five species, all belonging to section *Dendrobium*, and Cluster III also included five species, all belonging to section *Formosae* (Fig. 1). These AFLP clusters were consistent with traditional classifications as described by Tsi et al. (1999). These results further indicated that morphological classifications are credible, to some extent. Meanwhile, our AFLP data were sometimes inconsistent with the morphological classifications. For example, all native species in Subcluster Ib belonged to section *Dendrobium* except c74 and c94. These results revealed that the two native species (c74 and c94) had a close relationship with section *Dendrobium* in Subcluster Ib. These discrepancies were useful supplements for the morphological classifications.

Fig. 1. Dendrogram constructed for 100 accessions based on unweighted pair group method based on unweighted pair group arithmetic average method (the same code numbers as listed in Table 1).

The AFLP classifications were to some extent related to flower colors of tested Asian *Dendrobium* accessions. For example, Cluster Ib contained 12 native species of Asian *Dendrobium*, all of which were yellow flowers or white flowers with yellow lips. These results were consistent with our analyses on genetic relationships among *Cymbidium* hybrids using random amplified polymorphic DNA markers (Li et al., 2007).

In this study, AFLP results indicated that the genetic relationships among tested Asian *Dendrobium* accessions were related to their origins and pedigree. On one hand, most hybrid cultivars of *Dendrobium* coming from Japan displayed close relationships, which were attributed to AFLP Cluster Ia (Fig. 1). On the other hand, three native species (c65, c66, and c98) were also classified into the same Cluster Ia (Fig. 1). These results revealed that these tested hybrid cultivars may be derived from the three native species (c65, c66, and c98). Among the hybrid cultivars, c10, c11, c14, c19, and c27 possessed the kin of *D. snowflake* 'Otome' (c32) (see Supplemental Fig. 1). However, these five hybrid cultivars and *D. snowflake* 'Otome' were classified into different subclusters in Ia (Fig. 1). The reason may be that these five tested hybrids not only gained the genetic background from *D. snowflake* 'Otome', but also acquired different genetic background from their the other parents. *D. moniliforme* variation (c66) and three hybrids (c60, c61, and c62) originating from Korea were classified into the same subcluster in Ia (Fig. 1). This result indicated that these three tested hybrids may be derived from a *D. moniliforme* variation.

We further found that 25 native species of section *Dendrobium* comprised at least four clusters (Fig. 1). Fifteen of these 25 *Dendrobium* species were the same species as used in ITS and *matK* classifications (Wongsawad et al., 2001). Our AFLP analysis was mostly consistent with molecular classifications by ITS and *matK* sequences (Wongsawad et al., 2001). For example, *D. parishii*, *D. primulinum*, *D. aphyllum*, and *D. lodigesii* were classified in the same cluster by both ITS and *matK* sequences and AFLP markers. However, considerable discrepancies were observed between these molecular results and ISSR classifications among 31 *Dendrobium* species (Wang et al., 2009). Therefore, multiple DNA molecular markers will show more accurate phylogenetic relationships among native species of *Dendrobium*.

In the present study, specific DNA markers were found in 29 of 100 tested *Dendrobium* accessions (Table 1). These were comparable with the species-specific ISSR markers discovered in nine of 31 investigated *Dendrobium* species (Wang et al., 2009). Transferring of these specific AFLP markers into sequence-characterized amplified region markers would be very useful for molecular diagnosis of *Dendrobium* species and hybrid cultivars.

In conclusion, our study indicated that the AFLP tool was very effective in evaluating genetic relationships among native species and hybrid cultivars of Asian *Dendrobium*.

Our AFLP cluster analysis clearly identified native species and hybrid cultivars of Asian *Dendrobium* and provided a molecular diagnosis tool for them. The AFLP markers reported in this study will accelerate understanding genetic structure, genetic diversity, and phylogenetic relationships of native species and hybrid cultivars of Asian *Dendrobium*.

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