Genetic Relatedness among *Dendrobium* (Orchidaceae) Species and Hybrids Using Morphological and AFLP Markers

Lydia E. Wahba

School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 Bangi, Malaysia

Nor Hazlina, MS, and A. Fadelah

Horticulture Research Centre, MARDI Headquarters, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia

Wickneswari Ratnam¹

School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 Bangi, Malaysia

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Abstract. *Dendrobium* is one of the largest genera in the Orchidaceae family. Information on the genetic diversity and relationships among species and hybrids is important for breeding purposes and species conservation. The objectives of this study were to assess genetic relatedness and to determine whether morphological, molecular, or combined analysis can discriminate among *Dendrobium* species, commercial hybrids, and interspecific hybrids. A total of 81 *Dendrobium* accessions were characterized with 12 amplified fragment length polymorphism (AFLP) primer pairs and 21 morphological characters. Mean genetic relatedness for morphological characters, AFLP analysis, and combined analysis were 0.61, 0.37, and 0.43, respectively. Dendrograms were generated using an unweighted pair group method with arithmetic averages (UPGMA); the analysis was performed on a Jaccard similarity coefficient matrix. The data from morphological characters revealed that the Jaccard similarity coefficient ranged from 0.20 to 1.0, where the tested 81 *Dendrobium* accessions could be grouped into four clusters. For the AFLP analysis, the number of polymorphic fragments for each primer varied from 80 to 284 with 78% average percentage of polymorphic loci and the similarity coefficient ranging from 0.125 to 1.0 with *Dendrobium* accessions grouped into three clusters. The similarity coefficients estimated through a combined analysis of morphological and AFLP data ranged from 0.21 to 1.0 and the *Dendrobium* accessions appeared clustered into two groups. The results revealed some similarities among the three data sets. The combined data set was the most useful in discriminating *Dendrobium* accessions based on species sections and relationship among species and their hybrids. The correlation between the AFLP data and the combined data was highly significant ($r = 0.98$, $P > 0.001$), indicating the usefulness of AFLP data for species discrimination and hybrid identity in the absence of floral morphological characters.

Orchids are commercially important in Malaysia. Export value is expected to increase further because of a wider product range, longer shelf life, and ability to export flowers year-round. The orchid family is probably one of the most important plant families from a horticultural point of view. Orchidaceae is one of the largest families among angiosperms. The family includes 800 genera and 25,000 species (Stewart and Griffiths, 1995). Orchids are distributed in all regions of the world except Antarctica and are found growing in

many different habitats and elevation gradients (Pridgeon, 2000). They are well known for their economic importance and widely cultivated for ornamental purposes and cosmopolitan in distribution (Bechtel et al., 1992).

The genus *Dendrobium* is the third largest in the family Orchidaceae, comprising ≈ 1184 species worldwide (Leitch et al., 2009). *Dendrobium* plants are among the most prevalent orchids for commercial production of cut flowers and potted plants (Chen and Tsi, 2000) and the most popular genus in horticultural industries. The genetic diversity of the *Dendrobium* genus is not well known (Wang et al., 2009).

Orchid classification has been poorly understood because of the lack of fossil records,

the large number of species, and a historical emphasis on characters related to floral morphology. Circumscription of genera, subtribes, tribes, and subfamilies and the relationships among them were unclear because of homoplasy in morphological and anatomical features (Chase, 2006). There has been no explicit morphological cladistic analysis of Orchidaceae (Freudenstein and Rasmussen, 1999). Phylogenetic research using DNA sequences is now unraveling the complex picture at every taxonomic level. Assessment of genetic variability is important for the use of genetic resources and for determining the uniqueness of genotypes to exploit heterosis (Franco et al., 2001). Genetic diversity can be inferred from genetic similarity or distance estimates determined using phenotypic variation and/or molecular markers.

Molecular markers are not subject to environmental influence, and because of that, they are considered superior to morphological markers (Máric et al., 2004). Several types of molecular markers are available and have been used in assessment of orchid genetic diversity; ribosomal DNA internal transcribed spacer (ITS) region-based analysis was used to ascertain the phylogenetic relationship among 20 *Dendrobium* species (Chiang et al., 2012), whereas a phylogenetic tree from ribosomal-ITS2 sequences was constructed for 18 species of the genus *Dendrobium* (Chaudhary et al., 2012; Wang et al., 2009) used the intersimple sequence repeat technique to evaluate genetic diversity and phylogenetic relationship among 31 *Dendrobium* species and Shengnan et al. (2011) determined the genetic diversity among 31 species of *Dendrobium* species using random amplified polymorphic DNA markers.

AFLP markers are dominant markers. The AFLP technique can generate hundreds of highly replicable markers, allowing for high-resolution genotyping (Mueller and Wolfenbarger, 1999). Approximately 90% of the AFLP polymorphisms reflect point mutations and most AFLP fragments are independent loci (Buntjer et al., 2002). The loss of a restriction site removes the fragment from the profile rather than changing its size (Albertson et al., 1999), which means there is no need for prior DNA sequence information. When compared with other DNA-based markers (Vos et al., 1995), many markers can be analyzed in a short time, and only a small amount of DNA is needed. AFLP markers are highly reproducible and reliable (Bagley et al., 2001; Jones et al., 1997), high-throughput, cost-effective, and have wide genome coverage (Hansen et al., 1999; Prashanth et al., 2002). AFLP has proven to be an extremely effective tool for distinguishing closely related genotypes (Xiang et al., 2003).

AFLP markers have been used in the assessment of genetic relationships in many plant species such as rice (Zhu et al., 1998), *Arabidopsis thaliana* (L.) (Breyne et al., 1999), bluegrass (Renganayaki et al., 2001), *Carica papaya* (Kim et al., 2002), *Hibiscus tiliaceus* (Tang et al., 2003), and potato (Esfahani et al., 2009).

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¹To whom reprint requests should be addressed; e-mail wicki@ukm.my.

Molecular and morphological data matrices are very informative tools for the estimation of genetic distances (Vieira et al., 2007). Few orchid studies have analyzed at the same time both molecular data and morphological characters for the same set of taxa, but these have demonstrated that both types of characters are complementary and the structural characters often mark clades recovered in the combined analyses (Albert, 1994; Figueroa et al., 2008). Different studies found that the distribution of morphological character states provides additional support to the groupings recovered in molecular phylogenetic trees (Figueroa et al., 2008; Salazar, 2009; Salazar et al., 2009).

The objectives of this study were to 1) determine genetic relatedness among *Dendrobium* accessions from different species and hybrids; and 2) determine whether morphological, molecular, or combined analysis can discriminate *Dendrobium* species, *Dendrobium* commercial hybrids, and *Dendrobium* interspecific hybrids.

Materials and Methods

Plant materials. Eighty-one *Dendrobium* accessions were used in this study from 13 *Dendrobium* species, eight commercial hybrids, and 17 interspecific hybrids. All plant materials were ≈ 2 years old. *Dendrobium* species were propagated by seeds or cuttings, whereas the commercial hybrids and interspecific hybrids were vegetatively propagated. Leaf samples of accessions were collected from the Malaysian Agricultural Research and Development Institute orchid greenhouse. A list of the *Dendrobium* accessions, their corresponding accession numbers, and classification is provided in Table 1. The geographical origin of the *Dendrobium* species in Malaysia is given in Nor Hazlina et al. (2013).

Morphological data analysis. All plants were assessed for 21 morphological traits, all of which were referred by Sheehan and Sheehan (1994): plant height, root type, leaf type, leaf height, leaf width, pseudobulb habit, pseudobulb character, pseudobulb structure of cross-section, pseudobulb inter node, flower arrangement, flower structure, sepal structure, petal structure, flower color arrangement, hairy flowers, sepal color, sepal with more than one color, petal color, petal with more than one color, labellum color (upper side), and labellum color (inner side). All measurements were made using a digital caliper and flower color was standardized using the Royal Horticultural Society Color Chart.

Average values were applied for each quantitative character. All morphological data were standardized using the Transform option in Past Version 1.92 (Hammer et al., 2001) to reduce the influence of different scales. Morphological character data were used to estimate similarity coefficients, according to Jaccard (1908) and to construct dendrograms using the UPGMA in PAST Version 1.92 software (Hammer et al., 2001).

Amplified fragment length polymorphism analysis. Genomic DNA was extracted from

Table 1. List of *Dendrobium* accessions, their corresponding accession numbers, and classification.

Accessions No.	Species or cultivar	Section
Aloi1	<i>Dendrobium aloifolium</i> Rchb.f.	Aporum
Aloi2	<i>Dendrobium aloifolium</i> Rchb.f.	Aporum
Aloi3	<i>Dendrobium aloifolium</i> Rchb.f.	Aporum
Aloi4	<i>Dendrobium aloifolium</i> Rchb.f.	Aporum
Grand1	<i>Dendrobium grande</i> Hook.f.	Aporum
Grand2	<i>Dendrobium grande</i> Hook.f.	Aporum
Grand3	<i>Dendrobium grande</i> Hook.f.	Aporum
Croc1	<i>Dendrobium crocatum</i> Hook.f.	Pedilonum
Croc2	<i>Dendrobium crocatum</i> Hook.f.	Pedilonum
Croc3	<i>Dendrobium crocatum</i> Hook.f.	Pedilonum
Croc4	<i>Dendrobium crocatum</i> Hook.f.	Pedilonum
Lamel1	<i>Dendrobium lamellatum</i> Lindl.	Pedilonum
Lamel2	<i>Dendrobium lamellatum</i> Lindl.	Pedilonum
Lamel3	<i>Dendrobium lamellatum</i> Lindl.	Pedilonum
Hend1	<i>Dendrobium hendersonii</i> A.D. Hawkes & A.H. Heller	Rhopalanthe
Hend2	<i>Dendrobium hendersonii</i> A.D. Hawkes & A.H. Heller	Rhopalanthe
Hend3	<i>Dendrobium hendersonii</i> A.D. Hawkes & A.H. Heller	Rhopalanthe
Hend4	<i>Dendrobium hendersonii</i> A.D. Hawkes & A.H. Heller	Rhopalanthe
Crum1	<i>Dendrobium crumenatum</i> Sw.	Rhopalanthe
Crum2	<i>Dendrobium crumenatum</i> Sw.	Rhopalanthe
Crum3	<i>Dendrobium crumenatum</i> Sw.	Rhopalanthe
Seti1	<i>Dendrobium setifolium</i> Ridley	Rhopalanthe
Seti2	<i>Dendrobium setifolium</i> Ridley	Rhopalanthe
Seti3	<i>Dendrobium setifolium</i> Ridley	Rhopalanthe
Seti4	<i>Dendrobium setifolium</i> Ridley	Rhopalanthe
Seti5	<i>Dendrobium setifolium</i> Ridley	Rhopalanthe
Leon1	<i>Dendrobium leonis</i> Reichb.f.	Aporum
Leon2	<i>Dendrobium leonis</i> Reichb.f.	Aporum
Leon3	<i>Dendrobium leonis</i> Reichb.f.	Aporum
Leon4	<i>Dendrobium leonis</i> Reichb.f.	Aporum
Leon5	<i>Dendrobium leonis</i> Reichb.f.	Aporum
Fram1	<i>Dendrobium farmerii</i> Paxt.	Callista
Fram2	<i>Dendrobium farmerii</i> Paxt.	Callista
Fram3	<i>Dendrobium farmerii</i> Paxt.	Callista
Fram4	<i>Dendrobium farmerii</i> Paxt.	Callista
Fram5	<i>Dendrobium farmerii</i> Paxt.	Callista
Anos1	<i>Dendrobium anosmum</i> Lindl.	Dendrobium
Anos2	<i>Dendrobium anosmum</i> Lindl.	Dendrobium
Anos3	<i>Dendrobium anosmum</i> Lindl.	Dendrobium
Anos4	<i>Dendrobium anosmum</i> Lindl.	Dendrobium
Anos5	<i>Dendrobium anosmum</i> Lindl.	Dendrobium
Anos6	<i>Dendrobium anosmum</i> Lindl.	Dendrobium
Aggr1	<i>Dendrobium aggregatum</i> Roxb.	Callista
Aggr2	<i>Dendrobium aggregatum</i> Roxb.	Callista
Aggr3	<i>Dendrobium aggregatum</i> Roxb.	Callista
Aggr4	<i>Dendrobium aggregatum</i> Roxb.	Callista
Pier1	<i>Dendrobium pierardii</i> Roxb. Ex W.J. Hooker	Dendrobium
Pier2	<i>Dendrobium pierardii</i> Roxb. Ex W.J. Hooker	Dendrobium
Pier3	<i>Dendrobium pierardii</i> Roxb. Ex W.J. Hooker	Dendrobium
Pier4	<i>Dendrobium pierardii</i> Roxb. Ex W.J. Hooker	Dendrobium
Pier5	<i>Dendrobium pierardii</i> Roxb. Ex W.J. Hooker	Dendrobium
Bigi1	<i>Dendrobium bigibbum</i> Lindl.	Phalaenanth
Bigi2	<i>Dendrobium bigibbum</i> Lindl.	Phalaenanth
Bigi3	<i>Dendrobium bigibbum</i> Lindl.	Phalaenanth
Bigi4	<i>Dendrobium bigibbum</i> Lindl.	Phalaenanth
Bigi5	<i>Dendrobium bigibbum</i> Lindl.	Phalaenanth
Soni1	<i>Dendrobium Sonia</i>	<i>D. Caesar Giant</i> $\times$ <i>D. Tomie Drake</i> Commercial hybrid
Soni2	<i>Dendrobium Sonia</i>	<i>D. Caesar Giant</i> $\times$ <i>D. Tomie Drake</i> Commercial hybrid
Caes1	<i>Dendrobium Caesar</i> 'Giant'	<i>D. phalaenanth</i> $\times$ <i>D. stratiotes</i> Commercial hybrid
Caes2	<i>Dendrobium Caesar</i> 'Giant'	<i>D. phalaenanth</i> $\times$ <i>D. stratiotes</i> Commercial hybrid

(Continued on next page)

Table 1. (Continued) List of *Dendrobium* accessions, their corresponding accession numbers, and classification.

Accessions No.	Species or cultivar	Section
Caes3	<i>Dendrobium</i> Caesar 'Giant'	<i>D. phalaenanthæ</i> × <i>D. stratiotes</i> Commercial hybrid
Chao1	<i>Dendrobium</i> Chao Praya Gem	<i>D. Madame Thong-In</i> × <i>D. Pinky</i> Commercial hybrid
Chao2	<i>Dendrobium</i> Chao Praya Gem	<i>D. Madame Thong-In</i> × <i>D. Pinky</i> Commercial hybrid
Chao3	<i>Dendrobium</i> Chao Praya Gem	<i>D. Madame Thong-In</i> × <i>D. Pinky</i> Commercial hybrid
TN1	<i>Dendrobium</i> Tuanku Najihah	<i>D. Caesar Giant</i> × <i>D. Sonia</i> Interspecific hybrid
TN2	<i>Dendrobium</i> Tuanku Najihah	<i>D. Caesar Giant</i> × <i>D. Sonia</i> Interspecific hybrid
TN3	<i>Dendrobium</i> Tuanku Najihah	<i>D. Caesar Giant</i> × <i>D. Sonia</i> Interspecific hybrid
TN4	<i>Dendrobium</i> Tuanku Najihah	<i>D. Caesar Giant</i> × <i>D. Sonia</i> Interspecific hybrid
TN5	<i>Dendrobium</i> Tuanku Najihah	<i>D. Caesar Giant</i> × <i>D. Sonia</i> Interspecific hybrid
DS1	<i>Dendrobium</i> Doctor Sharif	<i>D. bigibbum</i> × <i>D. Chao Praya Gem</i> Interspecific hybrid
DS2	<i>Dendrobium</i> Doctor Sharif	<i>D. bigibbum</i> × <i>D. Chao Praya Gem</i> Interspecific hybrid
DS3	<i>Dendrobium</i> Doctor Sharif	<i>D. bigibbum</i> × <i>D. Chao Praya Gem</i> Interspecific hybrid
DS4	<i>Dendrobium</i> Doctor Sharif	<i>D. bigibbum</i> × <i>D. Chao Praya Gem</i> Interspecific hybrid
DS5	<i>Dendrobium</i> Doctor Sharif	<i>D. bigibbum</i> × <i>D. Chao Praya Gem</i> Interspecific hybrid
TF1	<i>Dendrobium</i> Tuanku Fauziah	<i>D. peeweewas</i> × <i>D. Chao Praya Gem</i> Interspecific hybrid
TF2	<i>Dendrobium</i> Tuanku Fauziah	<i>D. peeweewas</i> × <i>D. Chao Praya Gem</i> Interspecific hybrid
TF3	<i>Dendrobium</i> Tuanku Fauziah	<i>D. peeweewas</i> × <i>D. Chao Praya Gem</i> Interspecific hybrid
TF4	<i>Dendrobium</i> Tuanku Fauziah	<i>D. peeweewas</i> × <i>D. Chao Praya Gem</i> Interspecific hybrid
TF5	<i>Dendrobium</i> Tuanku Fauziah	<i>D. peeweewas</i> × <i>D. Chao Praya Gem</i> Interspecific hybrid
TF6	<i>Dendrobium</i> Tuanku Fauziah	<i>D. peeweewas</i> × <i>D. Chao Praya Gem</i> Interspecific hybrid
TF7	<i>Dendrobium</i> Tuanku Fauziah	<i>D. peeweewas</i> × <i>D. Chao Praya Gem</i> Interspecific hybrid

Table 2. Total number of amplified fragment length polymorphism loci examined using 12 primer combinations, percentage of polymorphic loci per primer combination among 81 *Dendrobium* accessions, and hybrids.

Primer combination <i>EcoRI/MseI</i>	No. of amplified bands	No. of polymorphic bands	Polymorphism ratio (%)
E-AAC/M-CAA (NED)	246	210	85
E-ACA/M-CAA (FAM)	226	103	46
E-ACC/M-CAA (NED)	251	229	91
E-ACT/M-CAA (FAM)	248	189	76
E-AGC/M-CAA (NED)	284	284	100
E-AAC/M-CAC (FAM)	196	196	100
E-ACA/M-CAC (NED)	176	119	68
E-ACC/M-CAG (NED)	207	178	86
E-AGC/M-CAT (NED)	288	80	28
E-ACT/M-CTA (FAM)	187	156	83
E-AGC/M-CTC (NED)	284	284	100
E-ACT/M-CTA (NED)	108	87	81
Total	2701	2115	78

fresh leaf tissues using the modified 2× cetyltrimethyl ammonium bromide protocol based on Doyle and Doyle (1990). Some samples had very limited leaf tissues; in this case, a DNeasy Plant Mini Kit (QIAGEN, Germany) was used.

AFLP analysis was carried out according to Vos et al. (1995). Briefly, 250 ng of genomic

DNA was digested with restriction enzymes *EcoRI* and *MseI*. The digested DNA fragments were ligated to *EcoRI* and *MseI* adaptors with T₄ DNA ligase. Sixty-four primer combinations were used for the selective amplification. The products were separated on 5% denaturing polyacrylamide gels, and the DNA bands were visualized using silver

staining (Fritz et al., 1999). Of these, 12 primer pairs that generated well-defined patterns were chosen for this study (Table 2).

For the selected 12 primer combinations, the genomic DNA of 81 *Dendrobium* accessions were further amplified using selected *EcoRI* and *MseI* primers. The *EcoRI*-based primer was labeled with fluorescent dye (FAM and NED) at the 5' end. After selective amplification, amplified fragments were separated on polyacrylamide gel with an ABI Genetic Analyser 3100 (Applied Biosystems, USA) after being mixed with DNA size standards labeled with fluorescent dye ROX (red). Data of fragments were processed with GeneMapper 4.0 (Applied Biosystems, USA) for size and intensity of each band.

Only clear and unambiguous DNA bands were included in the analysis. DNA bands were scored for presence (1) and absence (0). A pairwise comparisons of accessions, based on the presence of unique and shared polymorphic products, were used to estimate similarity coefficients, according to Jaccard (1908), and while constructing dendrograms using the UPGMA, the Mantel test was performed to test the correlation between the morphological data and AFLP data with PAST Version 1.92 software (Hammer et al., 2001).

Combined data analysis. All 81 *Dendrobium* accessions with morphological and molecular data were included in a third data set to compute the similarity coefficient of Jaccard (1908). This data set was used as input for cluster analysis using the UPGMA to generate a dendrogram using PAST Version 1.92 software (Hammer et al., 2001). Correlation coefficients between the combined data with morphological data and between the combined data with AFLP data were inferred from Mantel tests (Hammer et al., 2001).

Results

Morphological characterization. The data from morphological characters (Supplemental Table 1) revealed that the similarity coefficient, ranging from 0.20 to 1.0 with an 0.61 average (data not provided), imply a broad genetic base for the *Dendrobium* accessions investigated. Figure 1 represents the dendrogram for the *Dendrobium* accessions generated by UPGMA analysis of the 81 *Dendrobium* accessions with the 21 morphological characters. *Dendrobium* accessions were grouped into four well-defined clusters; the first cluster, which consisted of 51 accessions, was further divided to two subclusters: subcluster Ia contained 46 accessions (Fig. 1). In this subcluster (Ia), the coefficient similarity was 1 between (Bigi1, Bigi2, Bigi3, Bigi4, Bigi5) and (DS1, DS2, DS3), which is plausible because *Dendrobium bigibbum* is one of the parents for the interspecific hybrid DS (data not provided). On the other hand, the coefficient similarity was also 1 between (Bigi1, Bigi2, Bigi3, Bigi4, Bigi5) and (TF3, TF7), although *Dendrobium bigibbum* was not a parent for the interspecific hybrid TF (data

Fig. 1. Dendrogram for the *Dendrobium* accessions generated by unweighted pair group method with arithmetic averages (UPGMA) analysis of the 81 *Dendrobium* accessions with the 21 morphological characters.

not provided) and subcluster Ib with five hybrids (Soni1, Soni2, Caes1, Caes2, and Caes3). These findings imply that these hybrids are closely related because *Dendrobium* *Cesar* 'Giant' is an ancestor of *Dendrobium* *Sonia*. The second cluster consisted of 10 accessions and was further divided into two subclusters (Fig. 1): subcluster IIa with one species (Anos2, Anos3, Anos5, and Anos6) belonging to section *Dendrobium* and subcluster IIb, which contained three hybrids (Chao1, Chao2, and Chao3) belonging to *Dendrobium* *Chao Praya Gem* and three interspecific hybrids (TF1, TF2, and TF5)

belonging to *Dendrobium* *Tuanku Fauziah*. This result is acceptable because the interspecific hybrid TF is a progeny from *Dendrobium* *Chao Praya Gem*. The third cluster comprised eight accessions, all belonging to *Dendrobium* *crumenatum* and *Dendrobium* *setifolium*, which are in section *Rhopalanthé*. The fourth cluster comprised 12 accessions from *Dendrobium* *leonis*, *Dendrobium* *grande*, and *Dendrobium* *aloifolium*, all belonging to section *Aporum*.

AFLP analysis. A total of 2701 fragments ranging in size from 70 bp to 500 bp were scored using 12 primer combinations. The

number of polymorphic fragments for each primer varied from 80 [E-AGC/M-CAT (NED)] to 284 [E-AGC/M-CAA (NED), (E-AGC/M-CTC (NED)]. The average percentage of polymorphic loci (polymorphic loci/total loci) was 78%, ranging from 28% for primer combination E-AGC/M-CAT (NED) to 100% for primer combinations E-AGC/M-CAA (NED), E-AAC/M-CAC (FAM), and E-AGC/M-CTC (NED). The number of bands and the degree of polymorphism revealed by each primer combination is given in Table 2.

The similarity coefficient ranged from 0.125 to 1.0 with an average of 0.37 (data not provided), indicating wide genetic distance among the accessions. Figure 2 represents the dendrogram for the 81 *Dendrobium* accessions generated by a UPGMA analysis of the AFLP data. *Dendrobium* accessions appeared clustered into three well-defined clusters; the first cluster consisted of 42 accessions and was further divided into two subclusters: subcluster Ia contained all commercial hybrids, all interspecific hybrids, and two species (Bigi1, Bigi2, Bigi3, Bigi4, Bigi5) belonging to section *Phalaenthe* and (Croc1, Croc2, Croc3, and Croc4) belonging to section *Pedilonum*. Subcluster Ib had eight accessions (Lamel1, Lamel2, Lamel3) from section *Pedilonum* (Farm1, Farm2, Farm3, Farm4, and Farm5) from section *Callista*. The second cluster comprised 24 accessions and was divided into two subclusters: subcluster IIa contained 12 accessions, all belonging to section *Aporum*, and subcluster IIb contained 12 accessions all belonging to section *Rhopalanthé*. The third cluster comprised 15 accessions and was further divided into two subclusters: subcluster IIIa contained Anos1, Anos4, Anos2, Anos3, Anos5, and Anos6 from section *Dendrobium*; Aggr1, Aggr2, Aggr3, and Aggr4 from section *Callista*; and subcluster IIIb contained five accessions (Pier1, Pier2, Pier3, Pier4, and Pier5) from section *Dendrobium*.

The correlation between the morphological data and AFLP data was highly significant ($r = 0.55$) with $P > 0.001$.

Combined analysis. The similarity coefficient estimated through a combined analysis of morphological and AFLP data ranged from 0.21 to 1.0 with an average of 0.43 (data not provided), indicating broad genetic distance among the accessions. Figure 3 represents the dendrogram for 81 *Dendrobium* accessions generated by UPGMA analysis for the combined analysis of the AFLP and morphological data. *Dendrobium* accessions appeared clustered into two well-defined groups; the first group consisted of 39 accessions and was divided into four subclusters: subcluster Ia contained 11 accessions all belonging to section *Dendrobium*, and subcluster Ib contained four accessions (Aggr1, Aggr2, Aggr3, and Aggr4) and they belonged to section *Callista*, whereas subcluster Ic contained 12 accessions (Hend1, Hend2, Hend3, Hend4, Seti1, Seti2, Seti3, Seti4, Seti5, Crum1, Crum2, and Crum3); all 12 accessions belonged to the *Rhopalanthé* section.

Fig. 2. Dendrogram for the 81 *Dendrobium* accessions generated by a unweighted pair group method with arithmetic averages (UPGMA) analysis of the amplified fragment length polymorphism (AFLP) data.

Subcluster Id comprised 12 accessions (Leon1, Leon2, Leon3, Leon4, Leon5, Grand1, Grand2, Grand3, Alo1, Alo2, Alo3, and Alo4), all belonging to section *Aporum*. The second cluster consisted of 42 accessions and was further divided into two subclusters; subcluster IIa contained 12 accessions from *Callista* and *Pedilonum* sections, whereas subcluster IIb contained 30 accessions; five accessions (Bigi1, Bigi2, Bigi3, Bigi4, and Bigi5) belonged to the *Dendrobium bigibbum* species from section *Phalaenantha* and 25 accessions were all commercial and interspecific hybrids. The interspecific hybrids (TF1, TF2, TF3, TF4,

TF5, TF6, and TF7) were in the same cluster with (Chao1 and Chao2) and the similarity coefficient between them ranged from 0.81 to 0.86 because *Dendrobium Chao Praya Gem* is a parent for the interspecific hybrid *Dendrobium Tuanku Fauziah*. The interspecific hybrids (TN1, TN2, TN4, and TN5) were in the same cluster with (Caes1 and Caes2) with the similarity coefficient of 0.90 (data not provided) because *Dendrobium Caesar 'Giant'* is a parent for the interspecific hybrid *Dendrobium Tuanku Najihah*.

The correlation coefficients between both the morphological and AFLP matrices were

positive and significant ($r = 0.69^{**}$ and $r = 0.98^{**}$ respectively) with the combined analysis matrix.

Discussion

Genetic diversity in plants is essential for successful breeding and creation of new cultivars. Evaluation of the relationship of *Dendrobium* accessions (species, commercial hybrids, and interspecific hybrids) with morphological and AFLP data analysis can help breeders choose parental plants for breeding. However, phylogenetic relationships and the genetic diversity information of the *Dendrobium* genus are very limited (Burke et al., 2008; Gu et al., 2007; Wang et al., 2009; Yue et al., 2006).

The cluster analysis in this study for the 81 *Dendrobium* accessions based on 21 morphological characters revealed four clusters (Fig. 1). An earlier examination using 16 different morphological characters revealed only three clusters (Nor Hazlina et al., 2013). Wang et al. (2009) reported that using a Euclidian distance matrix for 31 *Dendrobium* species based on 28 morphological traits revealed six clusters belonging to six sections. Our results indicated that the morphological data used in the cluster analysis were efficient in differentiating among the hybrids from their parents and according to the species section with some inconsistencies as reported by Chen and Tsi (2000) and Tsi et al. (1999).

AFLP fingerprinting data have the potential to be an integral part of the new plant varieties protection system (Xiang et al., 2003). In this study, 78% of the AFLP loci were polymorphic among the 81 *Dendrobium* accessions. Our results are in agreement with other studies on *Dendrobium* species, where 70.6% of the AFLP loci were polymorphic among 30 commonly grown *Dendrobium* cultivars (Quan et al., 2012). Xiang et al. (2003) found that 83% of the AFLP loci were polymorphic among 43 *Dendrobium* hybrids. Other studies reported higher polymorphism; Yin et al. (2007) reported 99.7% polymorphism among 38 *Dendrobium* species using AFLP techniques with eight pairs of primer combinations, and Zhu and Li (2011) found 99.8% polymorphism among 63 hybrid cultivars and 37 native species of Asian *Dendrobium* with the use of eight primer AFLP combinations.

The moderate association ($r = 0.55$) between genetic relatedness using morphological and AFLP analysis may be the result of several reasons; molecular analysis provides a wider genome sampling than the morphological analysis, and molecular markers are not subject to either natural or artificial selection, whereas the morphological characters are subject to both natural and artificial selection (Vieira et al., 2007).

Our results strongly indicated that AFLP markers were effective in distinguishing between the *Dendrobium* accessions. Compared with morphological characters, DNA fingerprints reflect directly the variability for

Fig. 3. Dendrogram for 81 *Dendrobium* accessions generated by unweighted pair group method with arithmetic averages (UPGMA) analysis for the combined analysis of the amplified fragment length polymorphism (AFLP) and morphological data.

plants at the genetic level with reliable and tremendous data sets for reproducible estimation of genetic relatedness for investigations on plant systematics (Wang et al., 2009).

The analysis for the combined morphological and AFLP data revealed a dendrogram somewhat similar to the dendrograms generated using the morphological and molecular data. The combined analysis concluded in the subcluster Id with the *Aporum* section (Leon, Grand, and Aloï) (Fig. 3), which was similar to the results seen in the cluster analysis of the morphological data (Fig. 1) for the fourth

cluster, from the molecular data cluster analysis (Fig. 2) for subcluster IIa; also subcluster Ic in the combined analysis (Fig. 3), which was similar to subcluster IIB in the AFLP data analysis (Fig. 2); and the second cluster for the combined data analysis (Fig. 3) similar to the first cluster for the AFLP data analysis (Fig. 2) with respect for some differences in the similarity coefficient between the accessions.

The correlation coefficient for the AFLP data with the combined analysis ($r = 0.98$) was higher than the correlation coefficient between the morphological characters and

combined analysis ($r = 0.69$), which could be the result of the different number of data points for AFLP markers and morphological characters (Vieira et al., 2007). The cluster analysis of the combined data widely resembles that of the AFLP data but with reliable assignment of species to sections. Crosses between species, which are closely related (in the same cluster), are expected to yield a high seed set with good viability.

Conclusions

In this study we combined morphological and AFLP data to evaluate genetic relatedness among *Dendrobium* accessions. The combined data set coincides best to species sections and relationship between species and their hybrids. The integration of morphological and AFLP markers can be a powerful tool for distinguishing among closely related germplasm materials and can be used in recognizing unknown origins of germplasm materials, plant variety preservation, and/or cultivar identification.

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Supplemental Table 1. Morphological Character Data for 81 *Dendrobium* accessions based on 21 traits.

Traits	Accessions																							
	Alol1	Alol2	Alol3	Alol4	Grand1	Grand2	Grand3	Croc1	Croc2	Croc3	Croc4	Lamel1	Lamel2	Lamel3	Hend1	Hend2	Hend3	Hend4	Crum1	Crum2	Crum3	Set1	Set2	Set3
A	1	1	1	1	1	1	1	2	2	2	2	1	1	1	2	2	2	2	2	2	2	1	1	1
B	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	0	0	0	1	1	1
C	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
E	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1
F	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
G	0	0	0	0	0	0	0	1	1	1	1	3	3	3	2	2	2	2	2	2	2	5	5	5
H	0	0	0	0	0	0	0	0	0	0	0	4	4	4	3	3	3	3	1	1	1	1	1	1
I	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	0	0	0
J	0	0	0	0	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	0	0	0
K	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
L	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M	1	1	1	1	1	1	1	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1
N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	7	7	7	9	9	9	9	2	2	3	1	1	1	1	0	0	0	0	0	0
Q	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
R	0	0	0	0	7	7	7	9	9	9	9	1	1	2	1	1	1	1	0	0	0	0	0	0
S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T	0	0	0	0	0	0	0	9	9	9	9	2	2	2	1	1	1	1	0	0	0	0	0	0
U	0	0	0	0	6	6	6	9	9	9	9	9	9	9	1	1	1	1	0	0	0	7	7	7

Supplemental Table 1. (Continued) Morphological Character Data for 81 *Dendrobium* accessions based on 21 traits.

Seti4	Seti5	Leon1	Leon2	Leon3	Leon4	Leon5	Farm1	Farm2	Farm3	Farm4	Farm5	Anos1	Anos2	Anos3	Anos4	Anos5	Anos6	Aggr1	Aggr2	Aggr3	Aggr4	Pier1	Pier2	Pier3	Pier4	Pier5	Bigil
1	1	1	1	1	1	1	2	2	2	2	2	3	3	3	3	3	3	1	1	1	1	3	3	3	3	3	1
1	1	1	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	0	0	0	0	0	2
1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	0
2	2	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	2	2	2	2	2	2
1	1	1	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	3
5	5	0	0	0	0	0	4	4	4	4	4	1	1	1	1	1	1	4	4	4	4	1	1	1	1	1	3
1	1	1	0	0	0	0	5	5	5	5	5	0	0	0	0	0	0	2	2	2	2	0	0	0	0	0	0
0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
1	1	2	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	2	2	2	2	2	0
1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	3	3	3	3	3	0
0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	0
0	0	8	8	8	8	8	5	5	5	5	5	3	0	0	3	0	0	8	8	8	8	5	5	5	5	5	3
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	8	8	8	8	8	4	4	4	4	4	2	0	0	2	0	0	8	8	8	8	4	4	4	4	4	2
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	8	8	8	8	8	5	5	5	5	5	4	0	0	4	0	0	8	8	8	8	1	1	1	1	1	3

Supplemental Table 1. (Continued) Morphological Character Data for 81 *Dendrobium* accessions based on 21 traits.

Bigi2	Bigi3	Bigi4	Bigi5	Soni1	Soni2	Caes1	Caes2	Caes3	Chao1	Chao2	Chao3	TN1	TN2	TN3	TN4	TN5	DS1	DS2	DS3	DS4	DS5	TF1	TF2	TF3	TF4	TF5	TF6	TF7
1	1	1	1	2	2	2	2	2	1	1	1	2	2	2	2	2	1	1	1	1	1	1	1	1	1	1	1	
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
3	3	3	3	0	0	0	0	0	3	3	3	0	0	0	0	0	3	3	3	3	3	3	3	3	3	3	3	
3	3	3	3	1	1	1	1	1	3	3	3	1	1	1	1	1	3	3	3	3	3	3	3	3	3	3	3	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
2	2	2	2	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
1	1	1	1	1	1	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
0	0	0	0	3	3	1	1	1	0	0	0	3	3	3	3	3	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3	3	3	3	6	6	0	0	0	0	0	0	6	6	4	4	6	5	5	5	5	5	5	5	5	5	5	5	
0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	
2	2	2	2	6	6	5	5	5	0	0	0	5	5	3	3	5	2	2	2	2	2	0	0	1	0	0	1	
0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3	3	3	3	7	7	6	6	6	0	0	0	6	6	6	6	6	3	3	3	3	3	0	0	1	1	0	1	
5	5	5	5	0	0	0	0	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	

A = plant height, B = root type, C = leaf type, D = leaf height, E = leaf width, F = pseudobulb habit, G = pseudobulb character, H = pseudobulb structure of cross section, I = pseudobulb inter node, J = flower arrangement, K = flower structure, L = sepal structure, M = petal structure, N = flower colour arrangement, O = hairy flowers, P = sepal colour, Q = sepal with more than one colour, R = petal colour, S = petal with more than one colour, T = labellum colour (upper side), U = labellum colour (inner side).