Inter Simple Sequence Repeat markers for analysis of molecular diversity and genetic structure of eighteen *Dendrobium* cultivars in Sri Lanka

[1]T.T.D. Dharmarathna*, [1]H.M.D.A.K. Herath, [1]P.A. Weerasinghe, [2] H.M.V.G. Herath

Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka, Puliyankulama, Anuradhapura, Sri Lanka

[2]Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka

ABSTRACT

The genus Dendrobium is one of the largest genera in the family Orchidaceae having more than thousand species over the world with diverse morphological characters. Dendrobium is a popular ornamental plant with complex genetic background which emphasize on the species identification at molecular level. The present study was aimed to identify Inter-Simple Sequence Repeat (ISSR) markers capable of detecting genetic polymorphism to characterize 18 hybrid, commercially available Dendrobium cultivars. Genomic DNA of each cultivar was extracted using CTAB method. A total of 17 different ISSR primers were evaluated. Only the reproducible bands were scored and number of different alleles (Na), number of effective alleles (Ne), Shannon's Information Index (I), Expected heterozygosity (He), Unbiased expected heterozygosity (UHe), polymorphic percentage and polymorphic information content (PIC) of each primer were calculated. The highest Shannon's Information Index (0.537±0.08) was recorded by the primer UBC 826 while the highest polymorphic information content (PIC) was generated by primer UBC 807. The PIC values of the primers were ranged from 0.0068 to 0.451, indicating that primers are moderately informative. In total, 631 bands representing 120 loci were amplified showing 85.71% - 100% polymorphism. The genetic similarities between individuals were compiled in the Nei's genetic identity matrix in order to construct the UPGMA dendrogram. Principle component analysis (PCA) and clustering analysis were done to divide different cultivars into groups. The analysis revealed the presence of four major clusters and two minor clusters among the cultivars. The study suggested that the ISSR markers originated from eight primers 12, 155, UBC 807, UBC 812, UBC 826, UBC 835, UBC 841 and UBC 842 can be used in the detection of molecular variation among cultivars in the genus Dendrobium.

Keywords: *Dendrobium*, Genetic diversity, ISSR markers, Polymorphism

1. Introduction

The genus *Dendrobium*, belongs to family *Orchidaceae* which is the third largest group having more than thousand species over the world (Leitch *et al*, 2009). The orchid family is probably one of the most important plant families from a horticultural point of view. *Dendrobiums* are popular as a cut flower and a pot plant in commercial floriculture industry. The cultivar identifications currently based on morphological

characterization, is time consuming, observer dependent and difficult (Adams *et al*., 2006). Morphological parameters also rely on variability in species and plant growth conditions. Thus it makes more complicated to figure out the genetic diversity and individual genotypes of *Dendrobium* cultivars accurately.

Due to the increasing demand for cut flowers in the world market, a large number of novel *Dendrobium* cultivars are continually being imported for commercial cultivation, leading to a narrow genetic base for cultivar improvements under Sri Lankan conditions. Identification of all these different cultivars has become a major issue due to their similar plant morphologies. So far, cultivars have mainly been identified based on flower morphology, which is seldom available at the juvenile stage of plant development. The increasing number of *Dendrobium* hybrids and cultivars emphasize the need of molecular basis for the identification of genus *Dendrobium* (Burke *et al*., 2008). It is important for the sustainable conservation and increased use of plant genetic resources on novel hybrid production hence avoid the genetic erosion of the local varieties.

As a consequence, the development of new reliable and efficient tools based on molecular analysis has become popular (Primrose *et al*., 2010). These molecular biology techniques are powerful and good in species identification due to their consistency, independent from environmental parameters and developmental stage of the plant.

Among them PCR based techniques are the most popular due to their simplicity and requirement of little amount of DNA. The widely-using PCR based approaches are RAPDs, SSRs or microsatellites, ISSR and AFLPs. However, each and every markers have their own positive and negative remarks.

RAPD markers are very quick and easy to develop but have low reproducibility. AFLP markers have medium reproducibility but high operational cost, high developmental cost and high labour requirement. RAPD, ISSR and AFLP utilize arbitrary priming and do not require prior knowledge about genome sequence. The SSRs or Microsatellites are specific and highly polymorphic (Jones *et al*., 1997), but they are limited in use as they require prior knowledge of genomic sequence in primer designing. ISSR uses Simple Sequence Repeats (SSR) presence throughout the genome which are ubiquitous, abundant and highly polymorphic tandem repeat motifs composed of 1 to 7 nucleotides.

In 1994, Inter Simple Sequence Repeat (ISSR) was introduced (Zietkiewicz *et al*., 1994) which is carrying one primer complementary to a target microsatellite. They are best known as they are reproducible, highly polymorphic, highly informative, quick and easy to handle, but they have the reproducibility of SSR markers because of the longer length of their primers (Bornet *et al*., 2001).

The genetic variability within specific study mainly concerns non-coding regions of the genome that are characterized by the abundance of highly repetitive sequences within which the mutations are quite frequent. This variability has been studied by the technique of ISSR. ISSR marker is reliable having high sensitivity with low cost relative to other molecular markers. The choice of technique for any given application depends upon the material used and the nature of the question being addressed.

For the present study, a promising and effective genotyping marker like ISSR is absolutely crucial. These molecular studies bring useful information on the determination of genetic variation and the organization

of genetic diversity within the genus *Dendrobium*. Hence this study was aimed at screening and selecting compatible polymorphic ISSR markers with eighteen *Dendrobium* cultivars to obtain molecular identification. Ultimately, it was expected to develop a reliable genotyping method to assess molecular diversity and to determine the genetic structure of *Dendrobium* cultivars.

2. Materials and methods

2.1 Plant materials

A collection of eighteen *Dendrobium* hybrid cultivars were selected for the assay as mentioned in Table 2.1. The uppermost tender leaf of the plant was harvested from each and every individual of cultivars. The samples were washed under running tap water and wiped with 70% ethanol. Approximately, 0.25g of each sample was weighed with two replicates, cut into small pieces and kept at -20^{0}C for freezing.

2.2 DNA extraction

The frozen leaf samples were subjected to genomic DNA extraction using cetyltrimethyl ammonium bromide (CTAB) method. The DNA pellet was washed with 70% ice cold ethanol and dissolved in Tris EDTA buffer. Then the samples were verified for the presence of genomic DNA along with quantity and quality in 1% agarose gel buffered with TAE.

Table 2.1 List of *Dendrobium* cultivars used in the study

Code in the study	Common Name	Code in the study	Common Name
1	Burana Jade	**10**	Y Red Lip
2	Blue Pink	**11**	Banana Ancheng
3	Blue Amathis	**12**	Red
4	Orange	**13**	Hollywood
5	Ni – P Pink	**14**	TG Blue
6	Brown	**15**	Blue Planet
7	Aridang Green	**16**	Pink Candy
8	Brown Yellow	**17**	Burana Emerald
9	Saleya Candy	**18**	Liberty White

2.3 ISSR markers and PCR amplification

In this study, seventeen ISSR primers (Table 2.2) were evaluated among eighteen *Dendrobium* cultivars. PCR amplification was performed using 25 µl of PCR reaction mixture solution containing 10µl of Go Taq® Colorless Master Mix 2X [Go Taq® DNA polymerase is supplied in 2X Colorless Go Taq® Reaction buffer (pH 8.5), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP and 3 mM $MgCl_2$] 8 µl of ISSR primer, 5 µl of template DNA and nuclease free water up to total volume of 25 µl according to manufacturer's instructions.

Touch-down PCR was performed in MyCycler thermal cycler (BioRad and city) with initial denaturation at 94^{0}C for 5 minutes followed by 10 cycles of 94^{0}C for 60 seconds, 53^{0}C for 45 seconds (decreasing 0.5^{0}C

per cycle), and 72^{0}C for 90 seconds and 35 cycles of 94^{0}C for 60sec, 48^{0}C for 45 seconds, 72^{0}C for 90seconds and a final extension at 72^{0}C for 10 minutes. The PCR run was end up with the final holding at dwell time temperature of 4^{0}C.

For ISSR marker profiling, PCR products were subjected to electrophoresis on 2% agarose gels, followed by staining with Ethidium Bromide along with a 100 base pair DNA molecular weight marker Gel Pilot by Promega. The electrophoretic patterns of the PCR products were recorded digitally using a Gel-Doc Major Science Smartview Pro 1100 Imager System.

Table 2.2 Characteristics of ISSR primers used for the analysis of *Dendrobium*cultivars.

No	Primer Name	Primer	Primer sequence	GC%	Annealing temperature (^{0}C)	Total bands	Polymorphic percentage
1	12	$(AC)_8AT$	ACACACACACACACACAT	44.44	51.6	8	100
2	14	$(AC)_8AG$	ACACACACACACACACAG	50	53.9	5	100
3	15	$(AC)_8TG$	ACACACACACACACACTG	50	53.9	10	100
4	125	$(AC)_8CA$	ACACACACACACACACCA	50	53.9	9	100
5	134	$(AG)_8AA$	AGAGAGAGAGAGAGAGAA	44.44	51.6	6	100
6	144	$(AC)_8GA$	ACACACACACACACACGA	50	53.9	15	100
7	155	$(TG)_8GG$	TGTGTGTGTGTGTGTGGG	55.56	56.1	8	100
8	165	$(AG)_8CC$	AGAGAGAGAGAGAGAGCC	55.56	56.1	2	100
9	174	$(ACTG)_4$	ACTGACTGACTGACTG	50	48.2	6	100
10	*UBC 807	$(AG)_8T$	AGAGAGAGAGAGAGAGT	47.06	50.0	7	85.71
11	*UBC 812	$(GA)_8A$	GAGAGAGAGAGAGAGAA	47.06	50.0	7	85.71
12	*UBC 826	$(AC)_8C$	ACACACACACACACACC	52.94	52.4	7	100
13	*UBC 834	$(AG)_8CT$	AGAGAGAGAGAGAGAGCT	50	53.9	7	100
14	*UBC 835	$(AG)_8TC$	AGAGAGAGAGAGAGAGTC	50	53.9	3	100
15	*UBC 841	$(GA)_8TC$	GAGAGAGAGAGAGAGATC	50	53.9	8	100
16	*UBC 842	$(GA)_8CG$	GAGAGAGAGAGAGAGACG	55.56	56.1	7	100
17	*UBC 866	$(CTC)_6$	CTCCTCCTCCTCCTCCTC	66.67	60.7	5	100

2.4 Data analysis

An electrophoresis gel of PCR products by each and every cultivar were used to score the markers. The genetic distance analysis was performed considering a binary matrix that was compiled using software PyElph (Version 1.4) where '1' to indicate band present and '0' to indicate band absent (Liu *et al.,* 2006). All the ISSR assays were repeated twise to confirm the validity of results. Only the reproducible bands were considered during gel scoring (Lentner and Bishop, 1986). Both monomorphic and polymorphic bands were scored for genetic distance calculations. The software GeneAlEx (Version 6.501) was used in generating Nei's coefficient (Nei, 1972), Shannon's information index (Shannon & Weaver, 1949). For the analysis of genetic relationship, a similarity matrix of ISSR genotyping patterns was created using Nei's genetic identity for all pair-wise comparisons among the eighteen cultivars.

The resultant identity matrix was used to construct a dendrogram via unweighted pair-group method by arithmetic averages (UPGMA) program in the software package Mega (version 6.06). Simultaneously, the Principle Component Analysis (PCA) was performed to prove evidences by cluster analysis using software GeneAlEx (Version 6.501).

The total number of amplified loci, total number of polymorphic loci, rate of polymorphism, polymorphic information content (PIC) values were calculated separately for each and every marker (Bostein *et al.*, 1980).

$$PIC_i = 1 - \sum_{j=1}^{n} p_{ij}^2$$

Where p_{ij} is the frequency of the allele j in primer I and n is the number of alleles.

3. Results and Discussion

3.1 ISSR polymorphism

Amplification profiles from different primers revealed a significant difference in their ability to determine genetic variation among the cultivars. Some of the primers amplified several different alleles through the eighteen *Dendrobium* cultivars but others produced less allelic variation. Genotyping with these markers produced 631 of distinct bands having 85.71% to 100% polymorphism with all the tested samples. It might be due to sufficient amount of GC content (47.06%) of the primers used in the study. Fukuota *et al.* (1992) observed an increased number of bands with increasing percentage of GC content in the primers. The explanation for the correlation between GC content and the number of bands may be the stability of base complementation of A with T.

A total of 120 ISSR loci were revealed across eighteen *Dendrobium* cultivars allocating an average nearly 7 loci over single primer. The genotyping profiles of each and every ISSR primer had unique pattern of banding. Primer 144 for di-nucleotide AC repeats detected 15 ISSR loci, was the most informative among all tested primers. The identity of eighteen different *Dendrobium* cultivars was easily detected using the primer UBC 807 and UBC 812 (Figure a and b). When compared to other multi-loci and PCR- based methods, ISSR amplifications gave more markers and showed a higher level of polymorphism between

Dendrobium cultivars. These observations are in agreement with many studies showing the higher reproducibility and efficiency of ISSR markers (Galvan *et al*., 2003).

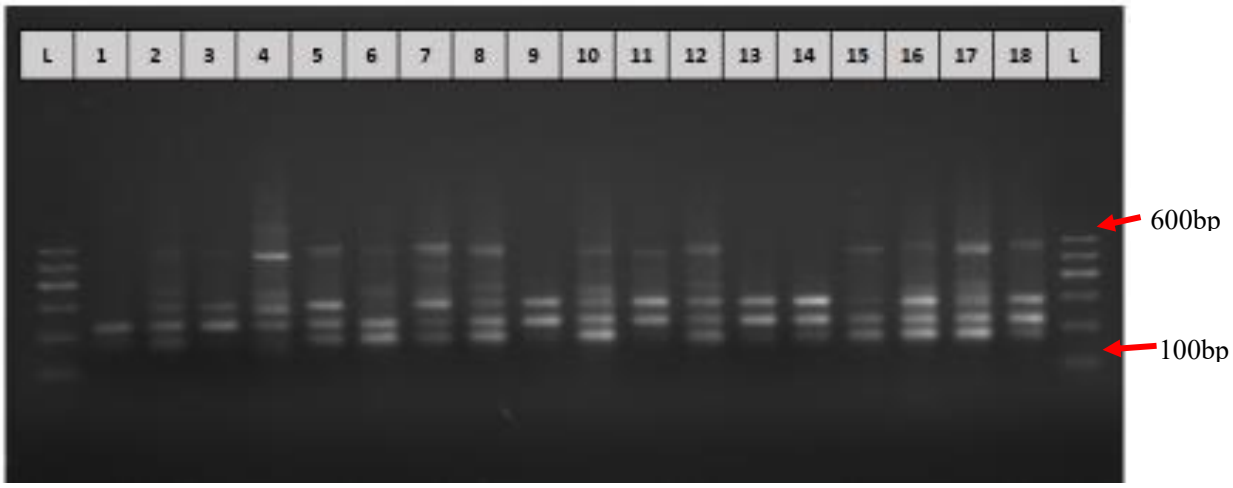

Figure a DNAfingerprinting analysis of eighteen *Dendrobium* cultivars by genotyping with ISSR marker UBC 807. The lane L represent the 100 bp molecular ladder. The codes of the different cultivars used in the figure are same as in the Table 2.1. (1) Burana Jade, (2) Blue Pink, (3) Blue Amathis, (4) Orange, (5) Ni-P Pink, (6) Brown, (7) Aridang Green, (8) Brown Yellow, (9) Saleya Candy, (10) Y Red Lip, (11) Banana Ancheng, (12) Red, (13) Hollywood, (14) TG Blue, (15) Blue Planet, (16) Pink Candy, (17) Burana Emerald, (18) Liberty White.

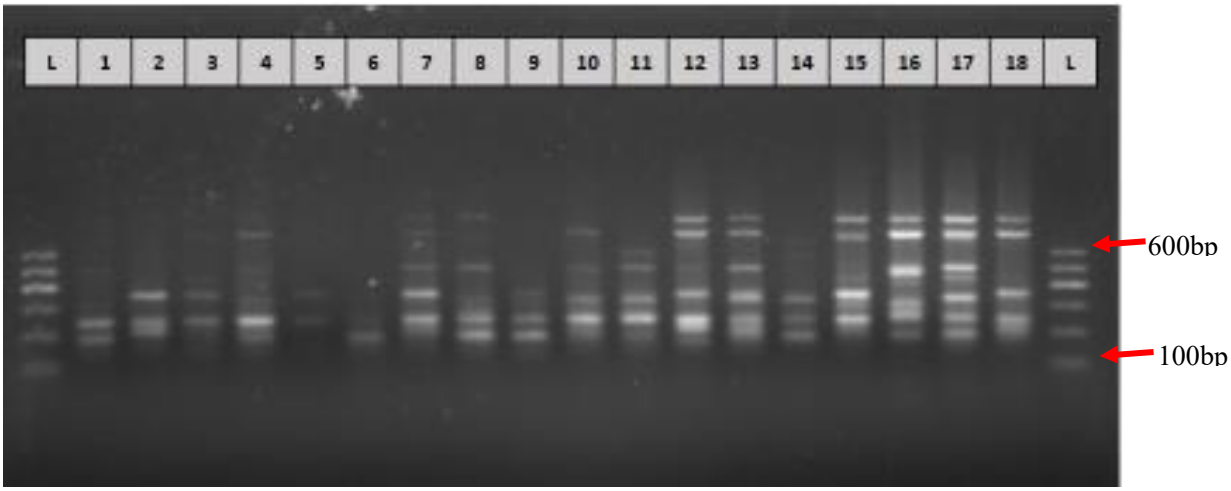

Figure b DNAfingerprinting analysis of eighteen *Dendrobium* cultivars by genotyping with ISSR marker UBC 812. The lane L represent the 100 bp molecular ladder. The codes of the different cultivars used in the figure are same as in the Table 2.1. (1) Burana Jade, (2) Blue Pink, (3) Blue Amathis, (4) Orange, (5) Ni-P Pink, (6) Brown, (7) Aridang Green, (8) Brown Yellow, (9) Saleya Candy, (10) Y Red Lip, (11) Banana Ancheng, (12) Red, (13) Hollywood, (14) TG Blue, (15) Blue Planet, (16) Pink Candy, (17) Burana Emerald, (18) Liberty White.

The seventeen markers were evaluated for their polymorphic level by calculating the polymorphic information content (PIC) which is an indicator of allele diversity and frequency among various *Dendrobium* cultivars. The values for PIC calculations varied widely from 0.0068 (primer 14) to 0.4511 (primer UBC 807) indicating that primers are moderately informative according to the studies by Bosten

et al. (1980). There are much evidences on use of moderately informative markers by Kesari *et al.* (2010) on *Pongamia pinnata* Pierre, Alikhani *et al*. (2014) on *Quercus brantii* Lindl and Aryanegad *et al.* (2013) on *Trifolium* spp.The most promising ISSR markers for proper identification of the genus *Dendrobium* are 12, 155, UBC 807, UBC 812, UBC 826, UBC 835, UBC 841 and UBC 842 Which were selected based on higher polymorphic information content (Blair *et al.,* 2003).

3.2 Genetic diversity analysis

The highest Shannon's information index was in primer UBC 826 and lowest in primer 14 (Table 4.2). Shannon Information Indices have been widely employed in ecology but largely overlooked in genetics. The presence of different values on Nei's genetic diversity and Shannon's information index (Table 4.2) revealed the variation in abundance of the complementary sequence to the primer on genomic DNA. Both parameters indicate higher values when the primers are synthesized with homo-dimer as the expected abundance of the homo-dimer is greater than homo-trimer and homo-tetramer. A survey report published by Toth *et al*. (2000) support the evidence on low availability of homo-tetramer sequences in embryophyta.

Table 3.1 Genetic diversity of eighteen *Dendrobium* cultivars identified by seventeen primers

No	Primer code	Na	Ne	I	He	UHe	PIC
01	12	2.000	1.408±0.110	0.416±0.066	0.262±0.053	0.269±0.054	0.1489
02	14	2.000	1.083±0.015	0.165±0.022	0.076±0.013	0.078±0.013	0.0068
03	15	2.000	1.171±0.046	0.250±0.043	0.135±0.030	0.139±0.031	0.0201
04	125	2.000	1.185±0.036	0.277±0.039	0.151±0.026	0.155±0.027	0.0278
05	134	2.000	1.214±0.109	0.257±0.085	0.148±0.062	0.152±0.064	0.0566
06	144	2.000	1.288±0.047	0.351±0.039	0.209±0.029	0.215±0.030	0.0333
07	155	2.000	1.535±0.130	0.471±0.079	0.312±0.063	0.321±0.065	0.2365
08	165	2.000	1.345±0.082	0.420±0.057	0.254±0.046	0.261±0.047	0.0802
09	174	2.000	1.159±0.038	0.250±0.044	0.132±0.029	0.136±0.030	0.0242
10	*UBC807	1.857±0.143	1.489±0.170	0.400±0.114	0.270±0.085	0.278±0.088	0.4511
11	*UBC812	1.857±0.143	1.579±0.150	0.470±0.107	0.323±0.078	0.332±0.080	0.4480
12	*UBC826	2.000	1.681±0.146	0.537±0.086	0.369±0.069	0.380±0.071	0.2932
13	*UBC834	2.000	1.226±0.041	0.317±0.041	0.178±0.029	0.183±0.029	0.0423
14	*UBC835	2.000	1.535±0.256	0.457±0.167	0.306±0.129	0.315±0.133	0.2984
15	*UBC841	2.000	1.365±0.121	0.368±0.079	0.230±0.061	0.236±0.063	0.1385
16	*UBC842	1.857±0.143	1.258±0.103	0.290±0.086	0.175±0.061	0.180±0.063	0.2138
17	*UBC866	2.000	1.242±0.074	0.324±0.057	0.185±0.043	0.190±0.045	0.0506

Na = Number of different alleles, Ne = Number of effective alleles, I = Shannon's Information Index, He = Expected heterozygosity, UHe = Unbiased expected heterozygosity and PIC = Polymorphic information content

3.3 Genetic distance

The different cultivars were resolved by their genetic distance during pairwise comparison, calculating Nei's genetic distance. The distance values ranged from 0.007 to 0.499 (Table 3.2). The highest distance value (0.499) was observed between cultivar Ni P Pink and Burana Emarald. This calculation of pairwise genetic distances for binary data follows the method of Huff *et al.*, in which any comparison with the same state yields a value of 0 (both 0 vs 0 comparisons and 1 vs 1 comparisons), while any comparison of different states (0 vs 1 or 1 vs 0) yields a value of 1.

3.4 Genetic similarity and cluster analysis

For the determination of genetic relatedness among different *Dendrobium* cultivars the Nei's genetic identity matrix was compiled during pair-wise comparison between individuals based on ISSR genotyping data. Figure 3.1 represents the Dendrogram for the *Dendrobium* cultivars generated by UPGMA analysis. The Nei and Li genetic distance (GD_{NL}) coefficient values of the Dendrogram ranged from 0.01 to 0.13, with a mean value of 0.07.

The low GD_{NL} values indicated that *Dendrobium* cultivars were closely related to each other and resulted in their close clustering in the dendrogram. The genotypes which were derived from the genetically similar ancestors were clustered together at the similarity coefficient value of 0. 07 which is close to the genetic similarity level of 0.06 used by Rajesh *et al*. (2002) to categorize both annual and perennial wild *Cicer* species using ISSR markers.

Table 3.2 Matrix of Genetic distances among pairs of Dendrobium cultivars based on ISSR fingerprinting analysis

Burana Jade	Blue Pink	Blue Amathis	Orange	Ni-P Pink	Brown	Aridang Green	Brown Yellow	Saleya Candy	Y Red Lip	Banana Ancheng	Red	Hollywood	TG Blue	Blue Planet	Pink Candy	Burana Emerald	Liberty white	
0.000																		Burana Jade
0.095	0.000																	Blue Pink
0.182	0.085	0.000																Blue Amathis
0.183	0.098	0.011	0.000															Orange
0.223	0.171	0.259	0.220	0.000														Ni-P Pink
0.183	0.103	0.015	0.007	0.226	0.000													Brown
0.332	0.273	0.377	0.332	0.085	0.338	0.000												Aridang Green
0.185	0.103	0.190	0.186	0.231	0.180	0.136	0.000											Brown Yellow
0.193	0.095	0.174	0.191	0.265	0.199	0.180	0.026	0.000										Saleya Candy
0.288	0.196	0.285	0.283	0.129	0.288	0.047	0.100	0.094	0.000									Y Red Lip
0.286	0.186	0.280	0.288	0.128	0.287	0.049	0.096	0.099	0.010	0.000								Banana Ancheng
0.310	0.188	0.289	0.316	0.398	0.305	0.299	0.110	0.114	0.219	0.190	0.000							Red
0.100	0.010	0.088	0.096	0.177	0.099	0.281	0.099	0.092	0.194	0.191	0.191	0.000						Hollywood
0.182	0.085	0.163	0.185	0.252	0.187	0.175	0.020	0.009	0.099	0.091	0.101	0.093	0.000					TG Blue
0.410	0.287	0.399	0.407	0.226	0.410	0.137	0.196	0.192	0.097	0.087	0.101	0.296	0.186	0.000				Blue Planet
0.420	0.285	0.393	0.421	0.283	0.406	0.193	0.196	0.198	0.115	0.097	0.085	0.287	0.184	0.015	0.000			Pink Candy
0.408	0.277	0.177	0.189	0.499	0.187	0.391	0.192	0.182	0.293	0.291	0.098	0.280	0.177	0.191	0.182	0.000		Burana Emerald
0.296	0.196	0.293	0.300	0.335	0.298	0.238	0.101	0.097	0.189	0.185	0.022	0.197	0.098	0.093	0.105	0.099	0.000	Liberty White

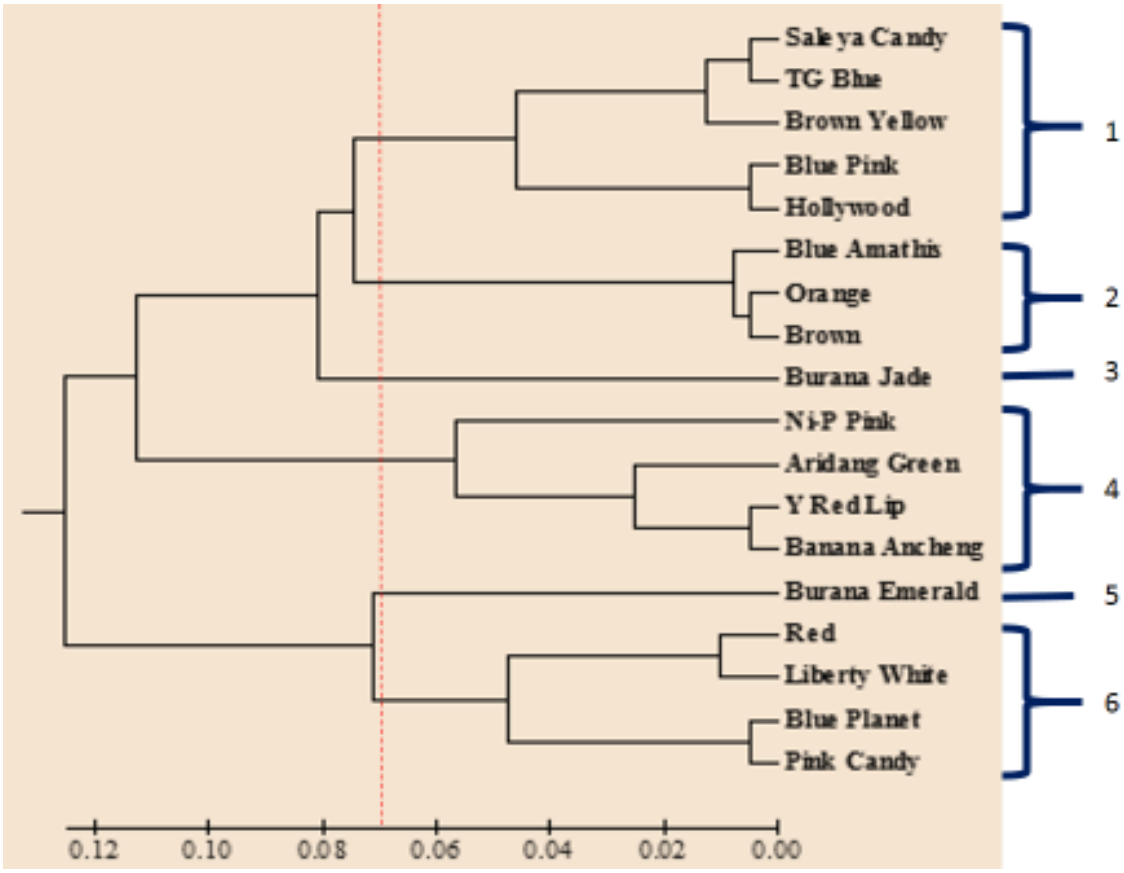

Figure3.1 Dendrogram of eighteen *Dendrobium* cultivars derived from UPGMA cluster analysis of

Nei's genetic identity based on seventeen ISSR markers

Cluster 1 grouped five cultivars, 'Saleya Candy', 'TG Blue', 'Brown Yellow', 'Blue Pink' and 'Hollywood'. Two cultivars including 'Blue Amathis' and 'Orange Brown' formed cluster 2 whereas four cultivars including 'Ni P Pink', 'Aridang green', 'Y Red Lip' and 'Banana Ancheng' formed the forth cluster. Finally, cluster six was formed by the 'Red', 'Liberty White', 'Blue Planet' and 'Pink Candy' cultivars. Both cluster 3 and 5 carried only single cultivars, 'Burana Jade' and 'Burana Emerald' respectively. Both cultivars had similar performance producing independent branches. In addition it was found that the highest genetic similarity value is between the *Dendrobium* cultivars Brown Yellow and Saleya Candy which are morphologically related in real. It was able to detect the different similarity level between different *Dendrobium* cultivars. But in the genetic identity matrix some cultivars have shared same values for similarity coefficient which emphasis the need of further study with more molecular markers to discriminate and characterize various *Dendrobium* cultivars.

Figure 3.2 Principle Component Analysis of eighteen *Dendrobium* cultivars UPGMA cluster analysis of Nei's genetic identity based on seventeen ISSR markers.

The principal component analysis based on genetic similarity matrices were used to visualize the genetic relationships among genotype which confirmed the results of cluster analysis. The contribution of the first and second principle component (PC1 and PC2) to the multivariate variation was 45.28% and 37.84% orderly, and the cumulative contribution was 83.12%. The results of the study showed that there is a relatively low level of genetic diversity between the studied cultivars. The procedure for PCA in GeneAlEx is based on an algorithm published by Orloci.

The genetic variability accessible in a gene pool is normally considered as being the major resource available to breeders (Ramanatha Rao and Hodgkin, 2002). The significantly low level of genetic variability detected among these *Dendrobium* cultivars highlighted the limited potential for cultivar improvement. Rapid replacement and elimination of numerous locally developed cultivars with commercially successful hybrids have resulted in a gradual genetic erosion of the local *Dendrobium* gene pool. Efficient handling of this low variability has thus become crucial for the success of the local *Dendrobium* industry.

5.0 Implication for the genetic improvement and conservation

Molecular based breeding techniques are one way to encourage the local breeders to improve the *Dendrobium* cultivars and thus to conserve the local germ plasm. It would be interesting to launch a multiplex ISSR genotyping platform based on current ISSR markers for imported and local *Dendrobium* cultivars identification in near future. The results from this study can be extended and further applied to tracing and monitoring of local and novel hybrid *Dendrobium* cultivars during breeding process.

6.0 Conclusions

The existence of low level of genetic diversity among the studied cultivars indicate that they are very closely related to each other. These cultivars can further be used as parental material for fixation of heterosis in *Dendrobium* improvement program. Knowledge on genetic diversity will help in the efficient management of *Dendrobium* germplasm by breeders.

In conclusion, ISSR markers have been successfully used to fingerprint and assess the extent of genetic variation among *Dendrobium* cultivars. They appeared to be a useful, quick and inexpensive molecular tool to solve the problems of morphological identification and cultivar characterization of genus *Dendrobium.* The study revealed that detection of best performing ISSR markers (12, 155, UBC 807, UBC 812, UBC 826, UBC 835, UBC 841, UBC 842) is a valuable milestone of sustainable conservation and future breeding programs on *Dendrobium*.

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