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Full Length Research Paper

RAPD analysis of colchicine induced variation of the *Dendrobium* Serdang beauty

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Variation was detected in *Dendrobium* Serdang Beauty V (DSB V) plantlets regenerated from protocorm like bodies (plbs) induced by various concentration levels of colchicines in the Murashige and Skoog media (MS) supplemented with 1.5 mg/L IBA. RAPD analysis detected 6 - 26% variation in the regenerants from the mother plant. The highest variation was obtained in regenerates treated with 25 mg/L colchicine, which also exhibited reduced regeneration rates from plbs and mean plantlet fresh weight. RAPD analysis also showed high polymorphism between the mutated regenerant DSB V, and 13 species of the *Dendrobium* genera, and 13 orchids across generas. However, despite the 26% colchicine induced variation in the regenerants, all RAPD analysis revealed that DSB V was closely related to the mother plant. Thus, the RAPD technique is favourable for variation detection as it was sensitive enough to detect variations at species level and among somaclonal variants in this study.

Key words: Colchicine, orchid, RAPD, *Dendrobium*.

INTRODUCTION

The Orchidaceae is a large flowering family that is undoubtedly recognized as an economically important commodity in the international floriculture industry, both as cut flowers and potted plants (Arditi, 1992; Kuehnle, 2007). Among various orchid categories in the family, the *Dendrobium* orchid have become increasingly popular due to its floriferous flower sprays, wide range of colours, sizes and shapes, year-round availability, and long flowering life of several weeks to months (Kuehnle, 2007).

In the US alone, about 90% of the 52 million imported orchid stems were *Dendrobium* orchids which valued a total of USD 3.6 million, in 2005. In the same year, potted *Dendrobium* orchids reached a production value of USD 5.8 million out of total potted orchids of USD 122 million (Jerardo, 2005). In Malaysia orchid exports value about RM 40 million annually, of which 11.7% comprise of the *Dendrobium* production. Due to growing domestic and export markets, Malaysia has recorded increasing floricultural products from RM 0.73 million in 1992 to RM 2.77 million in 1995. At the continued growth rate of 6% per annum, productions are expected to reach RM 36 billion by the year 2010 (Jong et al., 1997). However, increasing competition from established orchid producers such as the Phillippines, Thailand, Taiwan and Singapore constantly overshadow that of Malaysia's (Kuehnle, 2007). Therefore, research and development efforts for the production of unique and improved orchid varieties are desired to create increasing interest and demand in the midst of a competitive international floriculture market.

Orchid flowers with polyploidy traits have shown to be generally fuller and of greater substance than diploid flowers, although some may possess shorter racemes and fewer flowers per raceme (Kuehnle, 2007). Induced genetic variation by the colchicine mutagenic agent is widely used for desired polyploidy inductions (Blakeslee and Avery, 1930). Colchicine effects mitosis at the anaphase stage of the cell by interfering with the formation of spindle fibers. Cell wall formation is subsequently inhibited and the multiplied chromosome remains as polyploid within the undivided cell (Eigsti et al., 1955; Dirk et al., 1956). Despite the desired development of unique

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and improved varieties in orchids, the *Dendrobium* genus in particular, being the largest genera, is the most problematic genera with regards to its intrageneric classification and relationships to other taxa in the family. This is due to the existence of over 1000 species, distributed in Japan, throughout the Indo-Malayan region, Indonesia to Australia, New Zealand and the Pacific Island. The wide range of environmental adaptation causes extreme morphological diversity of plant organs such as the swelling of stems, and terete or laterally flattened leaves (Kuehnle, 2007).

The genetic material has been increasingly used to identify genetic distances and relationships using molecular markers. One of the easiest and fastest molecular marker detection of variation is the random amplified polymorphism DNA (RAPD) technique (Koh et al., 1999) as no prior knowledge of the genome is necessary for successful application (Williams et al., 1990). RAPD markers have shown to genetically link traits of interest used for individual and pedigree identification, pathogenic diagnostics, and trait improvement in genetics and breeding programmers (Yoon and Kim, 2001). The RAPD technique have been used in classifications of various orchid genera such as *Phalaenopsis* (Goh et al., 2005; Chen et al., 1994), *Goodyera* (Wong and Sun, 1999); *Zeucine* (Sun and Wong, 2001). Recently, an RAPD analysis reported no genetic alterations on a clonally propagated *Dendrobium* hybrid, indicating the non-occurrence of somaclonal variation (Ferreira et al., 2006).

In this paper, the RAPD technique was used to analyze the phylogenetic relationship between various local *Dendrobium* species, orchids of various genera and colchicine mutated *Dendrobium* Serdang Beauty plants.

MATERIALS AND METHODS

Plant materials

The *Dendrobium* Serdang Beauty variety is a hybrid of *Dendrobium* Genting × *Dendrobium* Hybrid. The mutated plant of the *Dendrobium* Serdang Beauty were regenerated at University Putra Malaysia induced by various treatment levels of colchicines (Table 1) and analyzed by RAPD. Subsequently, various *Dendrobium* species (Table 2) and various orchid genera (Table 3) were then analyzed with the mutated sample and mother plant.

Protocorm like bodies (plbs) raised in our laboratory were separated into individual pieces of approximately 0.3 cm^2 and cultured on MS basal media (Murashige and Skoog, 1962) supplemented with 1.5 mg/L IBA and various colchicine treatment levels (0, 5, 10, 15, 20, and 25 mg/L). Each treatment was prepared in 20 replicates in a Randomized Complete Block Design (RCBD). The cultures were maintained for 16 weeks and subculture every 4 weeks to fresh media. Incubation conditions were maintained at 25 ± 1°C under cool fluorescent light at 40 µmol m^{-2} s^{-1} of light of 16 h photoperiod per day. At the end of the 16 weeks of culture, the percentage of plbs explants regenerating plantlets and mean fresh weight of the regenerated plantlets were recorded. Recorded data were analyzed using ANOVA and the comparison of treatment means were analyzed using Duncan New Multiple Range Test (DNMRT) at α = 5%.

Table 1. List of colchicine treated regenerants of the *Dendrobium* Serdang Beauty.

Sample number	Concentration of colchicines	Cluster number
DSB 1	5 mg/L	1
DSB 2	10 mg/L	1
DSB 3	15 mg/L	1
DSB 4	20 mg/L	1
DSB 5	25 mg/L	2
C	Mother plant	1

DSB = *Dendrobium* Serdang Beauty.C= Control.

DNA extraction

Genomic DNA was extracted from leaf samples using a modification of the cetyltrimethy lammonium bromide (CTAB) method (Doyle and Doyle, 1990). 200 mg of fresh young leaves were lyophilized with liquid nitrogen and 700 µL of CTAB buffer [2% (w/v) CTAB; 1.4 M NaCl; 0.5% (v/v) 2-mercaptoethanol; 20 mM EDTA; 100 mM Tris-Hcl, (pH 8.0); 4% (w/v) PVP] was added to the tissue powder. The mixture was preheated at 65°C for 20 min and 5 ml of SEVAG [chloroform-isoamylalcohol (24:1)] was added to mixture and centrifuged at 10000 rpm for 15 min at room temperature to obtain a clear supernatant containing the genomic DNA. The supernatant was transferred to new tube, DNA was precipitated with 2/3 volume of isopropanol, and the mixture was washed with 5 ml wash buffer [76% (v/v) ethanol, ammonium acetate 10 mM] and remained in the wash buffer for 20 min at room temperature. Subsequently, the mixture was centrifuged at 10000 rpm for 5 min, and the pellet was dissolved with 500 µl of TE [Tris-Hcl 10 mM, EDTA 1 mM] buffer. Then, 1 µL of RNAase (10 mg/ml) was added to the DNA solution and incubated for 30 min at 37°C to remove the RNA. After incubation, an equal volume of PCI (Phenol: Choloroform: Isoamyl alchol) (25:24:1) was added to DNA solution, centrifuged at 12000 rpm for 5 min at room temperature, and PCI extraction was repeated for the top aqueous layer. Then 3 M sodium acetate (pH 5.2) and equal volume of cold Isopropanol were added to the PCI extraction, centrifuged at 12000 rpm for 10 min at room temperature. The obtained DNA pellet was washed with 500 µl of 70% ethanol, centrifuged at 12000 rpm for 5 min at room temperature, and the pellet was dried. The pellet was dissolved in 500 µl TE and kept at -20°C.

Screening of primers

Twenty RAPD primers: OPA 4; OPAW 12-13, 17-18; OPB 2-3, 4-6, 11-14, 16-18; OPD 1; OPG 3,13-15; OPZ 4, 9-10, (Operon Technologies, Alameda, Califonia) were used for analysis among mutated samples (Table 4), the selected mutated sample with various *Dendrobium* species (Table 5) and orchid genera (Table 6). The chosen primers were screened from a total of 40 primers. Primers were chosen based on the production of better and scorable bands for analysis. The rest of the primers were discarded due to absence of polymorphism, no production or low number of bands.

RAPD amplification

The RAPD mixture contained 1 µl of sample DNA, 500 mM PCR buffer, 2.5 U *Taq* DNA polymerase, 2 µml of $MgCl_2$, 20 µm of each dNTP, 1.4 µml of each primer to a final volume of 25 µl. Amplifica-

Table 2. List of *Dendrobium* species and parentage according to Sanders (1991).

No	Name	Parentage
1	*D.* Thongchai	Spellbound × Theodore Takiguch
2	*D.* Sonia	Caser × Tomie Darke
3	*D. crumenatum*	Species
4	*D. nobile*	Species
5	*D.* Shavin white	Walter × Queen
6	*D.* Tun Ku Imran	Theodore Takiguchi × Elaine
7	*D.* Bobby Mesina	Imelda Romualdez × Jaquelyn Thomas
8	*D.* Sharifah Fatimah	Lim Chong×May Neal
9	*D.* Burana Sombati	Queen Thiland×Thonglor Beauty
10	*D. stratiotes*	Species
11	*D. strabloceras*	Species
12	*D.* Serdang Beauty V	Mutated plant
13	*D.* Serdang Beauty	(*D.*Genting ×*D.*Hybrid)
14	*D.* Luck Lady	Angel Flower× Orion
15	*D.* Penang Beach	Circe × Jaquelyn Concert

Table 3. List of orchids of various generas and parentage according to Sanders (1991).

No	Name	Parentage
1	*Kasem* Delight Angie	Deight × Jennie Hashimoto
2	*Asco* Lek	Delight × Flambeau
3	*Kasem* Dlight Udom	Delight × Princess Blue
4	*Asco* Lena Chai	Honwichia × Lenavat
5	*Philonopsis* Hope	Iwaga × Lady weihe
6	*Oncidium* Siskdono	Letty Lim×Lancenum
7	*Vanda* Rena Pingthong	Bangkok × Jennie Hshimoto
8	*Vanda sanderana*	Species
9	*Vanda* Pink Cloud	Manila × Teres
10	*D.* Thongchai	Spellbound × Theodore Takiguch
11	*D.* Burana Sombati	Queen of Thailand×Thonglor Beuaty
12	*D.* Serdang beauty	(*D.*Genting ×*D.*Hybrid)
13	*D.* Serdang Beauty V	Mutated plant
14	*Oncidium* Genting Firy	Microchilum×Nanum
15	*Vanda* Taveesuksa	Pimsi × Bhimayothin

tion was conducted in a thermal cycler (Biometra thermal cycler, Whatman, USA) programmed for 1 cycle of 94°C for 5 min, 94°C for 3 s, and 42°C for 1 min, followed by a program run through

RAPD data analysis

Bands were viewed under UV light and photographed. Bands on photos were scored using GENE TOOLS of SYN GEN package (Gene Genius, Bio Imaging, USA). Clear RAPD bands were scored '1' if they were present and '0' for absent each DNA sample. The binary data obtained was analyzed with the program NTSYS-PC version 2.0 (Rohlf, 1993). The SIMQUAL module was used to generate a similarity matrix using the Jaccard coefficient of similarity (Jaccard, 1908). The measure of similarity was converted to genetic distances using the formula *Sij*= a/ a+b+c where *Sij* is the measure of genetic similarity between two individuals, i and *j*. 'a' is defined as the number of bands present in both *i* and *j*, 'b' is defined as the number of bands present in *i* and absent *j*, and 'c' is defined as the number of bands present in *j* and absent *i*. The distance matrix was then used to cluster analysis, and the Sequential Agglomerative, Hierachial and Nested clustering (SHAN) (Sneath and Sokal, 1973) module was used to produce a dendrogram with the unweighted pair-group method with arithmetic mean (UPGMA) clustering strategy. The experiments were carried out in the Laboratory of Agrobiotechnology, Faculty of Agriculture, Universiti Putra Malaysia.

RESULTS AND DISCUSSION

Plant regeneration on colchicine treatments

Plbs explants successfully regenerated plantlets on all

Table 4. List of random primers used for RAPD on colchicine regenerants (DSB I-V) and mother plant of the *Dendrobium* Serdang Beauty.

Name of primers	Sequence (5' – 3')	GC content (%)	Tm (°C)
OPAW18	GGCGCAACTG	70	43.6
OPAW17	TGCTGCTGCC	70	43.6
OPB02	TGATCCCTGG	60	39.5
OPB03	CATCCCCCTG	70	43.6
OPB06	TGCTCTGCCC	70	43.6
OPB16	TTTGCCCGGA	60	39.5
OPD01	ACCGCGAAGG	70	43.6
OPG15	ACTGGGACTC	60	39.5
OPZ09	CACCCCAGTC	70	43.6
OPZ10	CCGACAAACC	60	39.5

Table 5. List of random primers used for RAPD in the DSB V with various *Dendrobium* species.

Name of primers	Sequence (5' – 3')	GC content (%)	Tm (°C)
OPB 05	TGCGCCCTTC	70	43.6
OPB 12	CCTTGACGCA	60	39.5
OPB 13	TCCCCCCGCT	70	43.6
OPB 17	AGGGAACGAG	60	39.5
OPB 18	CCACAGCAGT	60	39.5
OPD 01	ACCGCGAGGG	70	43.6
OPG 03	GAGCCCTCCA	70	43.6
OPG15	ACTGGGACTC	60	39.5
OPAW 13	CTACGATGCC	60	39.5
OPAW 17	TGCTGCTGCC	70	43.6

Table 6. List of random primers used for RAPD in DSB V, mother plant and orchids of generas.

Name of primers	Sequence (5' – 3')	GC content (%)	Tm (°C)
OPA 04	AATCGGGCTG	60	39.5
OPB 05	TGCGCCCTTC	70	43.6
OPB 08	GTCCACACGG	70	43.6
OPB 11	GTAGACCCGT	60	39.5
OPB 13	TTCCCCCGCT	70	43.6
OPB 14	TCCGCTCTGG	70	43.6
OPG 13	CTCTCCGCCA	70	43.6
OPG 14	GGATGAGACC	60	39.5
OPZ 04	AGGCTGTGCT	70	39.5
OPZ 10	CCGACAAACC	60	39.5

colchicine treatments levels (Figure 3). Greenish nodular structures began to form on the surface after six weeks of culture, which regenerated plantlets (Figures 1 and 2). Significantly high percentage of explants regenerated plantlets on colchicine treatment levels at 5, 10 and 15 mg/l, compared to levels 20 and 25 mg/L. Furthermore, these treatments were not significantly different from the control (without colchicine). However, increased colchicine treatment levels to 20 and 25 mg/L significantly decrease plantlet regeneration of the explants.

In contrast to the plantlet regeneration data, the mean fresh weight of the regenerated plants on colchicine treatment level at 5 mg/L was significantly the highest, while plantlets on 10 mg/L were not significantly different

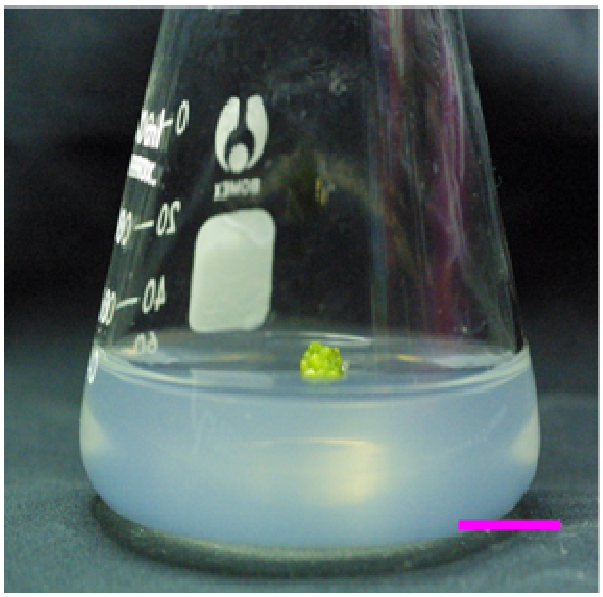

Figure 1. Callus forming greenish nodular structures on the surface after six weeks after culture (Bar = 1 cm).

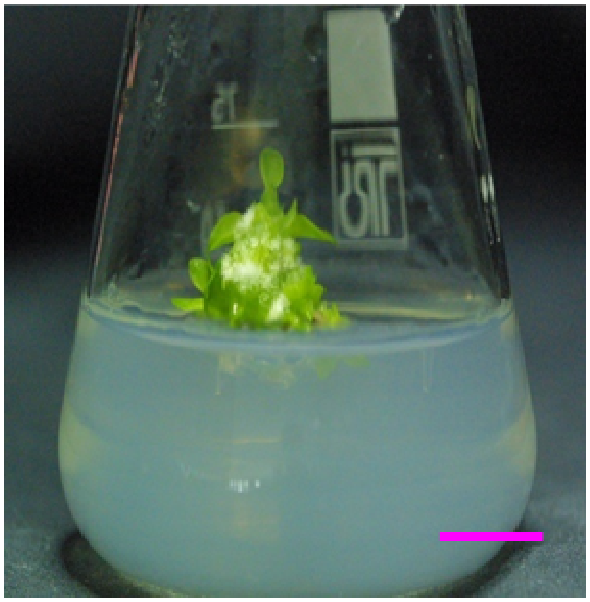

Figure 2. Plantlets regenerated from callus after the 16th week of culture (Bar = 1 cm).

from the control (Figure 4). Higher colchicine treatment levels at 15, 20 and 25 mg/L regenerated plantlets with low mean fresh weights.

Generally the low regeneration or growth response of the cultures may be attributed to the growth inhibiting effect of the colchicine, which is associated with the genetic variation induced within the explants (Wei et al., 2007; Duren et al., 1996; Wan et al., 1989; Van Tuyl et al., 1992). Nonetheless, it was important to determine the

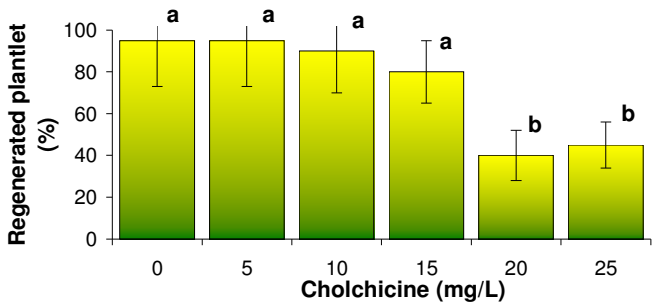

Figure 3. The effect of mutagenic agent (cholchicine) on the percentage of callus derived plbs explants regenerated to plantlet (%) after 16 weeks of culture. Bars followed by the same letter in the same trend line are not significantly different at p = 0.05.

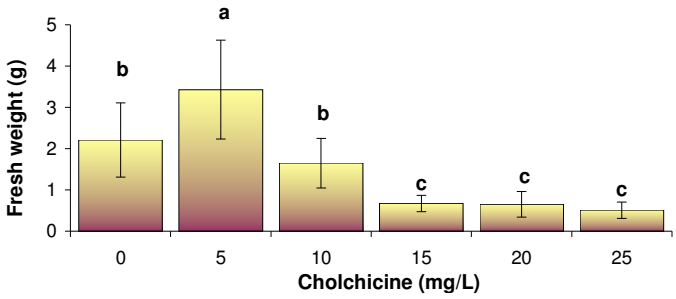

Figure 4. The effect of different concentrations of mutagenic agent (colchicines) on fresh weight of regenerated plant after 16 weeks of culture. Bars followed by the same letter in the same trend line are not significantly different at p = 0.05.

degree of variation incurred within the regenerated plantlets of all the colchicine treatment levels using RAPD.

RAPD analysis of colchicine treated regenerants with mother plant

Variation between the 5 colchicine treated regenerants was analyzed with the control plant. A total of 23 polymorphic bands were generated by the 10 primers used (Table 7) on the samples, with an average of 2 bands per primer. No monomorphic bands were obtained. The amplified products varied between 2154.76 – 409.03 bp. Two unique bands were produced by primers OPD01 and OPG15 with the length were 1342.45 bp and 1076.05 bp, respectively (Figure 5).

The coefficient of similarity analysis showed that colchicine treatment induced at least 6% dissimilarity in the regenerates from that of the mother plant, while the highest is 26% (Table 8). Cluster analysis divided the accessions into two major groups, I1 and I2 (Figure 6). The DSB V accession, which was a regenerant from the highest colchicine treatment level at 25 mg/L, also showed the highest dissimilarity from the mother plant, and

Table 7. Distribution of RAPD in colchicine treated regenerants and mother plant.

No.	Primer	No. Band	Ranges		Polymorphic Band	Polymorphic Band (%)
			High	Low		
1	OPAW18	4	1428.32	623.57	0	0
2	OPAW17	2	1406.25	1158.95	2	100
3	OPB02	8	1377.63	447.21	8	100
4	OPB03	2	1413.78	1065.6	1	50
5	OPB06	11	1500	491.49	1	9.09
6	OPB16	3	1496.31	1124.19	1	33.33
7	OPD01	6	1485.29	409.03	6	100
8	OPG15	8	2154.76	635.71	2	25
9	OPZ09	9	1481.63	495.86	3	33.33
10	OPZ10	2	1222.81	684.75	0	0
Total		53			23	

Table 8. Similarity coefficient of the colchicine regenerants and mother plant.

Samples	DSB I	DSB II	DSB III	DSB IV	DSB V	C
DSB I	1.000000					
DSB II	0.8400000	1.000000				
DSB III	0.8490566	0.8800000	1.000000			
DSB IV	0.7592593	0.7843137	0.8653846	1.000000		
DSB V	0.6037736	0.6200000	0.7058824	0.7872340	1.000000	
C	0.7818182	0.8431373	0.9230769	0.9400000	0.7400000	1.000000

DSB = Dendrobium Serdang Beauty
C = control

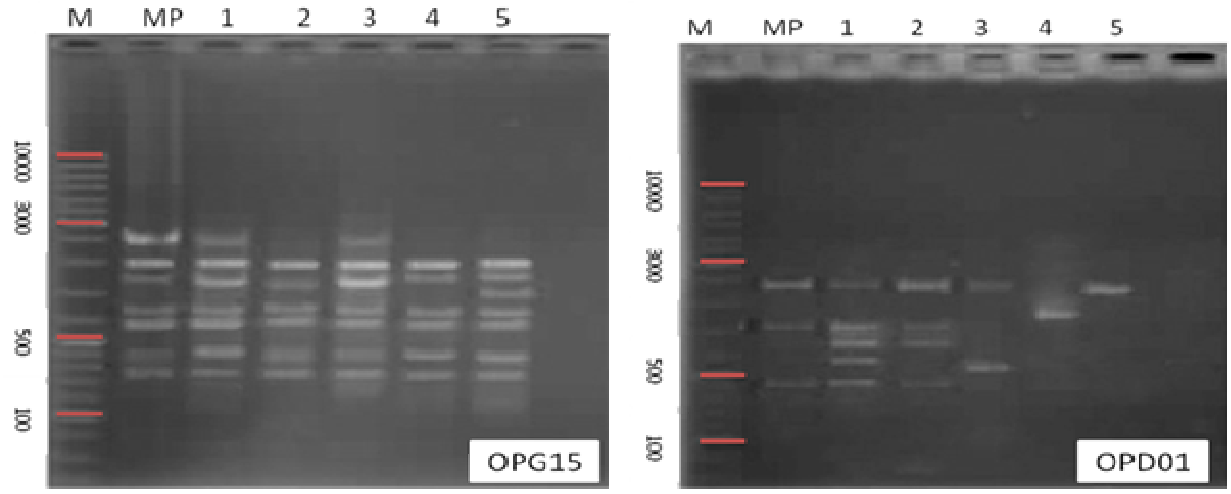

Figure 5. Unique bands amplified in colchicine regenerants and mother plant using OPD01 and OPG15 primers.

was isolated into the major group I2. The remaining colchicine regenerated accessions were clustered together with the mother plant into major group I1. However, within the major group I1, it was observed that subclusters separated the accessions into 2; lower colchicine treated plants of 5 and 10 mg/L (DSB I and DSB II respectively) were clustered into I11; and higher colchicine treated plants of 15 and 20 mg/L (DSB III and DSB IV) clustered into I12. DSB IV showed the highest similarity to the mother plant, with coefficient similarity of

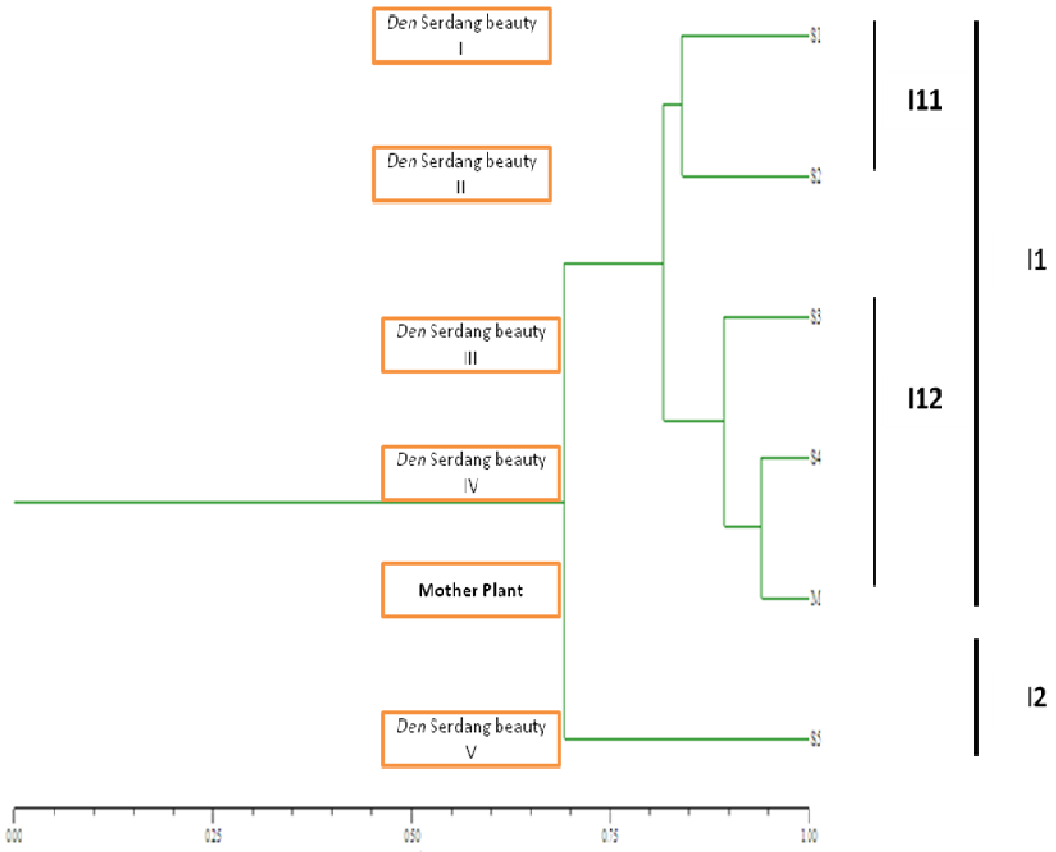

Figure 6. Dendrogram of colchicine treated regenerants and mother plant resuting from a UPGMA based on genetic distances from 10 RAPD primers. Numbers beside the bars indicate the cluster number

0.940. This indicated that the colchicine treatment levels used were capable of inducing variations of different degrees. Colchicine induced mutations have been reported to incur 23 to 29% variation in banana (Duren et al., 1996), and have been reported to incur up to 44.4% variation in *Lespedeza Formosa* (Wei et al., 2007).

RAPD analysis of the DSB V with mother plant and various *Dendrobium* species

In the subsequent study, the colchicine treated regenerant with the highest variation, DSB V, was used for RAPD analysis with the mother plant and 13 various *Dendrobium* species (Figure 7). A total of 147 polymorphic bands were produced by 10 primers on the DSB V, mother plant and 13 *Dendrobium* species, giving an average of 14 bands per primer (Table 9). No monomerphic bands were obtained. The amplified products varied between 8150.16 - 156.88 bp. All primers used produced 23 unique bands, with an average of 2 unique bands per primer.

Cluster analysis divided the accessions into three major groups, I, II and III (Table 10). *Dendrobium* Sonia and *Dendrobium curmenatum* were isolated into clusters II and III respectively (Figure 8). The DSB V accession and mother plant were clustered together in the major cluster I with 11 other *Dendrobium* varieties, and varied from these accessions in subcluster I1-2, with a similarity value of 0.393. The closest similarity to the DSB V and mother plant was *D. stratiotes*, with similarity value of 0.386, while *D.* Sonia and *D. curmenatum* showed the most distant similarity of 0.230 and 0.225 respectively.

Among the accessions, 5 *Dendrobium* species were clustered together (subcluster I1-1-2), namely the *Dendrobium nobile*, *Dendrobium* Shavin White, *Dendrobium* Bobby Mesina, *Dendrobium* Sharifanh Fatimah and *Dendrobium* Tun Imran (Table 10). *D.* Burana Sombati and *Dendrobium* Penang were clustered together (subcluster I1-1-3).

Although a reported RAPD study on the Vallina orchid genera, detection low intraspecific variations (Besses et al., 2004), our study revealed 100% variation between *Dendrobium* species. In addition, SSR markers also reflected high genetic diversity between the *Dendrobium* species (Yue et al., 2006). Nonetheless, RAPD analysis of various species in the *Dendrobium* genera showed that colchicine induced variation in the DSB V remained closely related to the mother plant.

RAPD analysis of the colchicine treated regenerant

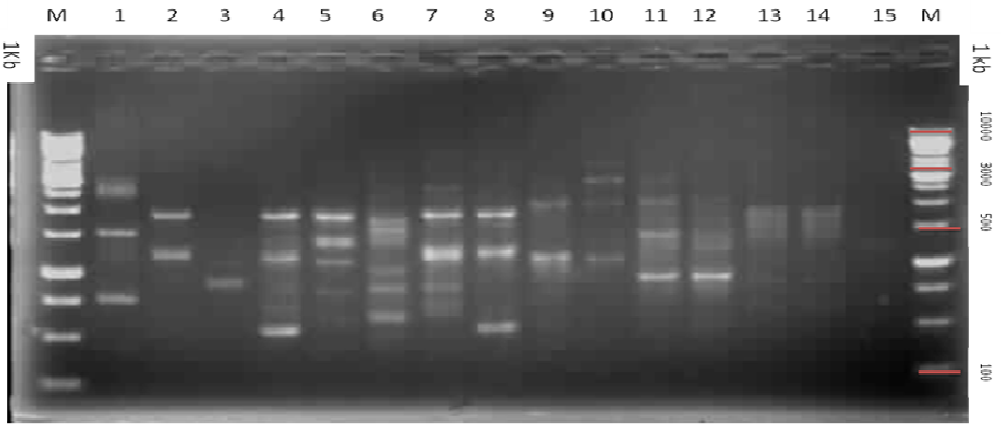

Figure 7. Polymorphic DNA amplification on *Dendrobium* species using RAPD primer OPG15.

Table 9. Distribution of RAPD in DSB V, mother plant and 13 *Dendrobium* species.

No.	Primer	No. Band	Ranges		Polymorphic Band	Polymorphic Band (%)
			Low	High		
1	OPB05	13	1030.88	5477.22	13	100
2	OPB12	19	376.99	2294.39	19	100
3	OPB13	12	967.35	8150.16	12	100
4	OPB17	13	428.63	3000	13	100
5	OPB18	12	649.502	2584.86	12	100
6	OPD01	14	378.93	3700	14	100
7	OPG03	16	561.49	2910.21	16	100
8	OPG15	16	1567.88	6000	16	100
9	OPAW13	15	519.69	3240.37	15	100
10	OPAW17	17	396.85	3825.86	17	100
Total		147				

Table 10. Cluster distribution of DSB V, mother plant and 13 *Dendrobium* Species.

No	Name	Cluster number	
		Cluster	Sub-cluster
1	*D.* Thongchai	I	I111
2	*D.* Sonia	II	I112
3	*D. curmenatum*	III	*
4	*D. nobile*	I	*
5	*D.* Shavin white	I	I112
6	*D.* Tun Ku Imran	I	I112
7	*D.* Bobby Mesina	I	I112
8	*D.* Sharifah Fatimah	I	I112
9	*D.* Burana Sombati	I	I113
10	*D. stratiotes*	I	*
11	*D. strabloceras*	I	*
12	*D.* Serdang beauty V	I	I12
13	*D.* Serdang beauty	I	I12
14	*D.* Luck Lady	I	I2
15	*D.* Penang	I	I113

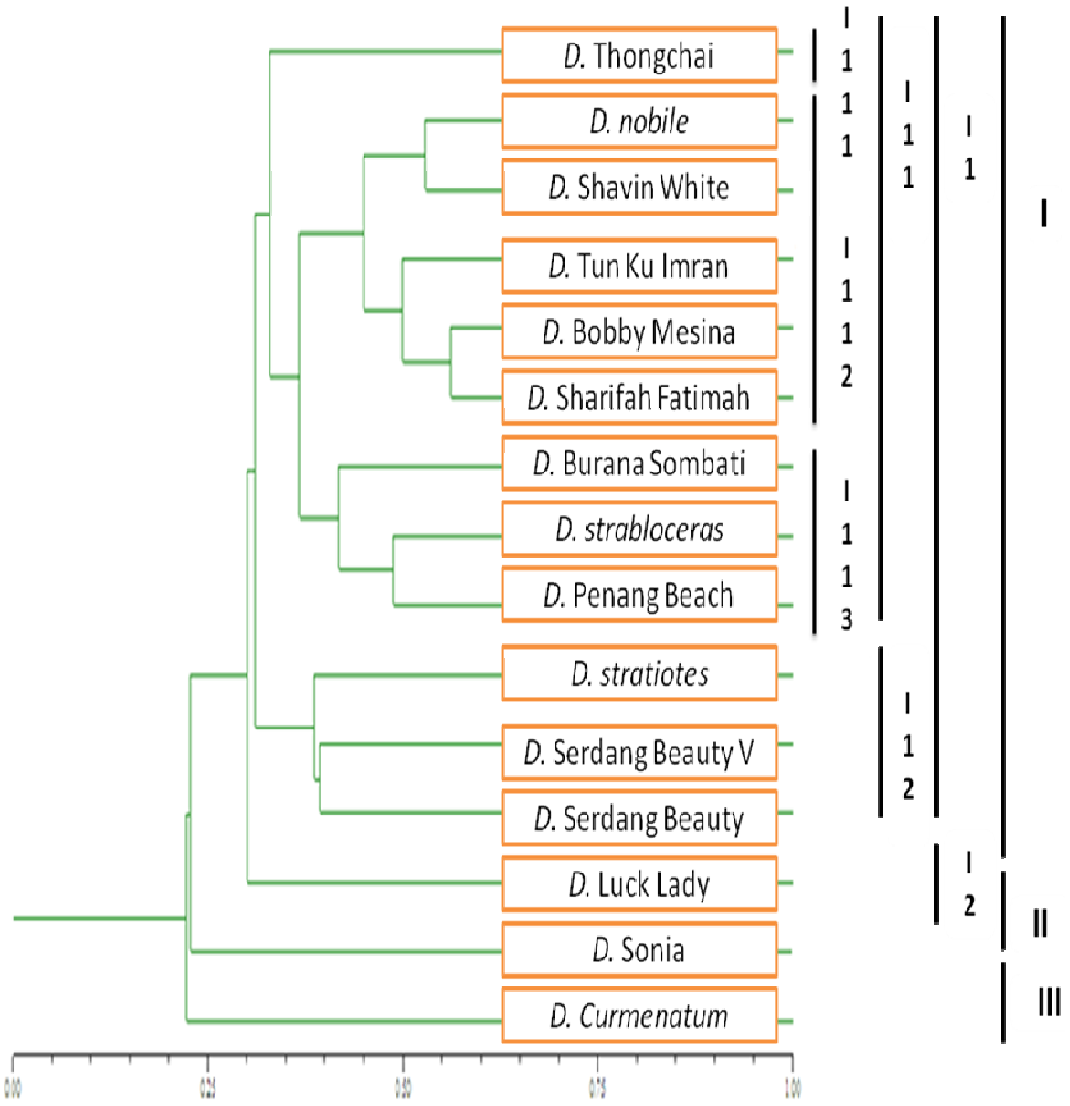

Figure 8. *Dendrogram* on UPGMA clustering analysis on mutated plant *D.* Serdang Beauty V (mutated), mother plant and 13 *Dendrobium* species and hybrids.

DSB V with mother plant and orchids of various genera For the characterization of across generas, two closely related accessions namely, the DSB V and the mother plant were analyzed with 13 orchids of various generas by RAPD. A total of 112 polymorphic bands were produced by ten primers in the DSB V, mother plant and orchids of various generas, which is an average production of 8. bands per primer (Table 11). No monomorphic bands were obtained. The amplified products varied between 228.49 – 1500 bp. OPB13 and OPG13 primers produced 2 unique bands, while primer OPB05, OPG14, and OPZ10 produced one unique band from the accessions. The accessions were generally grouped according to their various generas (Figure 8); Vanda orchids were clustered into group I; *Oncidium* orchids were clustered into group II; and *Dendrobiums* were clustered into group III (Figure 9). The DSB V accession and mother plant remained clustered together.

The RAPD comparison of accession DSB V with orchids of various generas, showed that the DSB V closely reflected the mother plant, which indicated that the degree of variation induced by colchicine treatment was controlled.

Conclusion

The colchicine mutating agent produced *Dendrobium* Serdang Beauty regenerants that varied from the mother plant by 6 - 26%. Regenerants on 25 mg/L colchicines resulted with the highest degree of variation, DSB V (26%). However, colchicine exhibited growth inhibiting

Table 11. RAPD distribution in DSB V, mother plant and 13 orchids of various genera.

No.	Primer	No. Band	Ranges		Polymorphic Band	Polymorphic Band (%)
			Low	High		
1	OPA04	15	330	1500	15	100
2	OPB05	15	628.14	1500	15	100
3	OPB08	9	1168.03	1500	9	100
4	OPB11	15	228.49	1440.34	15	100
5	OPB13	9	669.84	1500	9	100
6	OPB14	7	700	1500	7	100
7	OPG13	15	525.49	1500	15	100
8	OPG14	12	668.37	1468.83	12	100
9	OPZ04	7	742.6	1382.81	7	100
10	OPZ10	8	710.5	1491.74	8	100
Total		112				

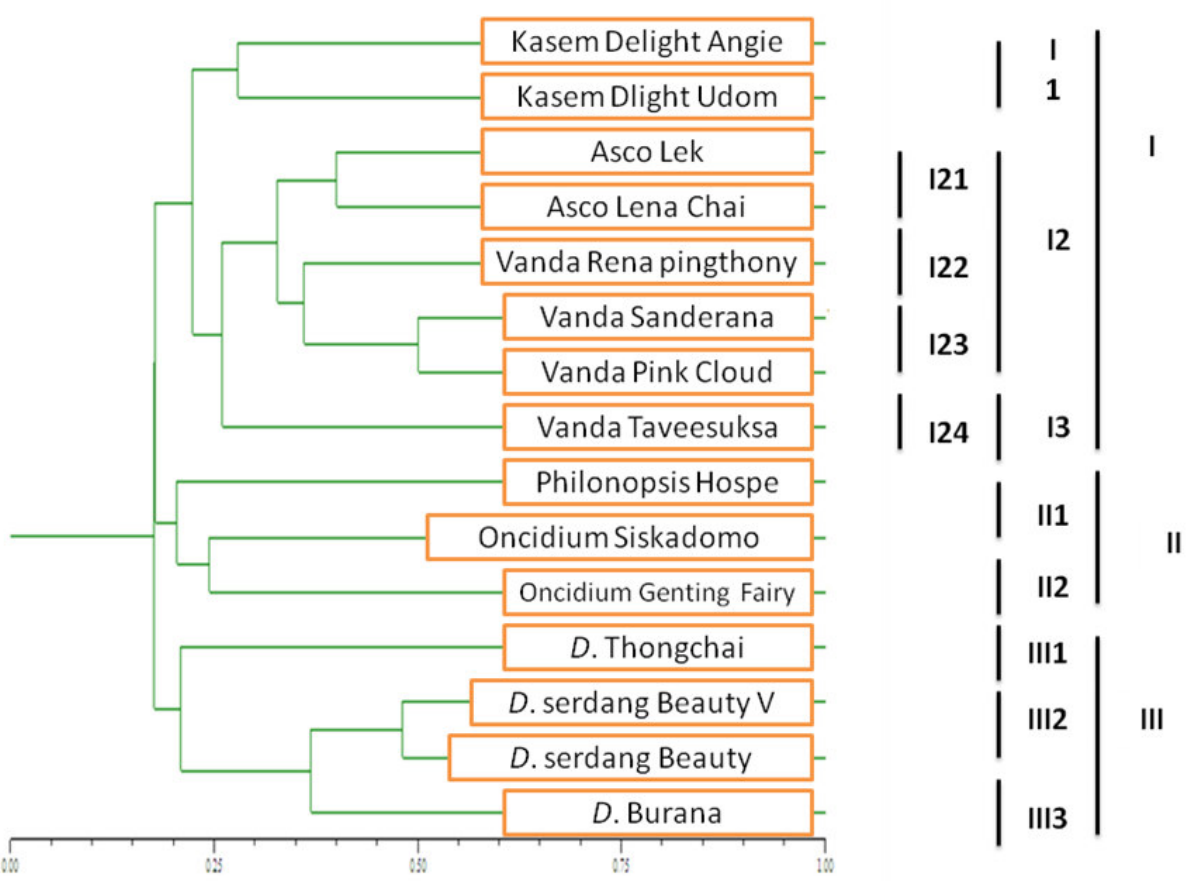

Figure 9. Dendrogram of 13 orchids of various generas with DSB V and mother plant resuting from a UPGMA based on genetic distances from 10 RAPD primers. Numbers beside the bars indicate the cluster number.

effects with higher treatment levels on the regenerating frequency of the plbs (20 and 25 mg/L) and the fresh weight of the regenerants (15, 20 and 25%). The RAPD protocol used was highly sensitive as it detected high polymorphism among mutated DSB regenerants from the mother plant and very high polymorphism among species in the *Dendrobium* genera and species across generas. Thus, the RAPD is a suitable marker for variation detection at species level and also among somaclonal variants.

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