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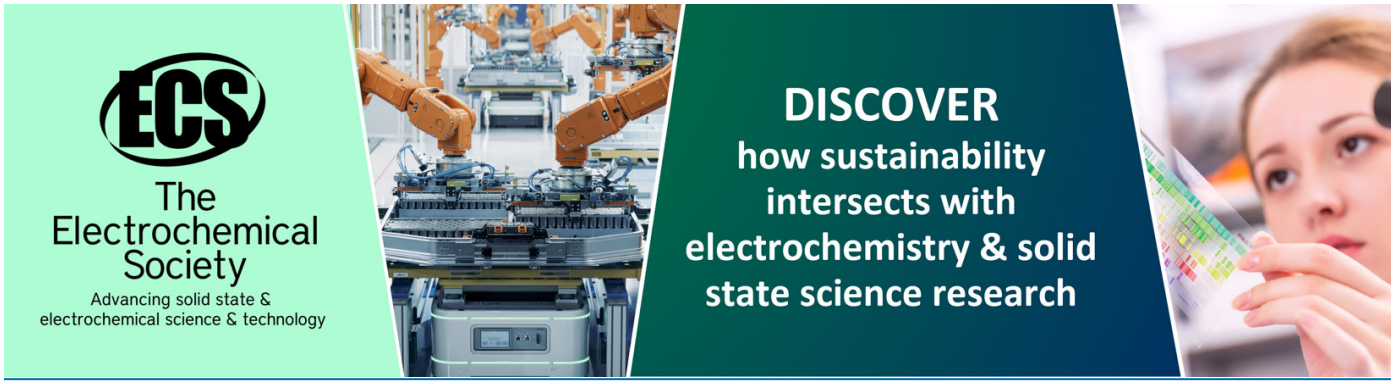

Clustering analysis on interspesific hybrid of *Paphiopedilum* Maudiae x *tonsum* crossing

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Abstract. *Paphiopedilum* (Slipper Orchid/Venus orchid) is a genus belongs to subfamily of *Cypripedioideae*. All *Paphiopedilum* species are covered by the Convention on International Trade in Endangered Species (CITES). Cluster analysis has been used to grouping of plant variation. The aim of the research was to estimate the relationship between parents (*Paph*. Maudiae "Black" & *Paph. tonsum*) and their 19 progenies. 23 morphological Qualitative traits and 13 morphometrical/quantitative traits were used in multivariate analysis. The morphological qualitative data was scored numerically as present (1) and absent (0), and then analyzed using NTSYS PC (Rohlf, 1998). Similarity between accessions was estimated using Dice coefficient. *Average taxonomic distance* was used to group the quantitative data using *Dist* coefficient. Result showed that hierarchical clustering of 21 genotipes indicated two main groups based on qualitative data as well of quantitative data. Group 1 had 5 accessions (PH1-01, PH1-16, PH1-51, Ph1-73, PH1-78, and group 2 had 10 accessions. Four accessions were separated and stand alone. Group 1 had morphological resemblance with *Paph*. Maudiae and group 2 had morphological resemblance with *Paph*. Maudiae and *Paph. tonsum*. Based on quantitative data, group 1 (11 accessions) had quantitative trait resemblance with *Paph. Tonsum*. Group 2 (7 accessions) had quantitative trait resemblance with *Paph*. Maudiae. Morphological trait of *Paph*. Maudiae was dominant inherited to their progenies.

1. Introduction

The orchids belongs to Orchidaceae family, had 25,000 to 30,000 species in some 700 to 800 genera. They exhibit almost innumerable hybrids and inexhaustible varieties. One of the exotic flower and its distinct floral morphology is *Paphiopedilum*. There are more than 75 species occurring in subtropical and tropical Asia (2) and have been protected by the Convention on International Trade of Endangered Species (CITES) of Wild Flora and Fauna. *Paphiopedilum* is known as Lady's Slipper orchids, due to its lip shape's resembles to pouch like. The basic chromosome number of *Paphiopedilum* is 2n = 26 (9, 14).

Paphiopedilum Maudiae was belongs to the Maudiae type, resulted from the crossing of *Paph. callosum* x *lawrenceanum*. Maudiae types usually use specieses such as *Paph. callosum* var giganteum, *Paph. lawrenceanum* "Florafest", *Paph*. Maudiae "Magnificum" (*Paph. callosum* x *lawrenceanum*), *Paph*. Claire de Lune "Edgar van Belle" (16). *Paph*. Maudiae was first made in the 1900's, but nothing much improved in this line of breeding until the 1960's when the two darkly coloured clones of *Paph. callosum* were collected and introduced into the breeding lines. This primary *Paph* are very attractive,

having pleasing, and mottled leaves. The colour of Maudiae type range from light bronzy pink deep rose to very dark vinicolor. Breeding of vinivolor Paph began with the discovery of *Paph. callosum* 'Jac' and *callosum* 'Sparkling Burgundy'. *Paph. callosum* is terrestrial in nature, growing under the shaded and moist conditions of the forest floor (5). *Paph lawrenceanum* was found on limestone rocks, terrestrial or lithophytic in Borneo, having 1 to occasionally 2 flowers, and maroon inflorescence. Easy growing of Maudiae type hybrids were evolved and paved gateway for path-breaking Barbata and Vini type breeding line (4). *Paph tonsum* was known as bald *Paphiopedilum*, refers to the flowers having no hairs. They were foundin shady pockets of leaf litter over limestone at elevation of 1000 to 1800 m asl with deep green mottled green leaves with purple undersides that blooms on an erect as a single flowered inflorescence. Including of *Paph tonsum* to the Maudiae type will give desirable feature like spotted petal, different shapes and flat flower. Taxonomic placement of *Paph tonsum* and *Paph lawrenceanum* were in the same section Barbata. Based on morphological, molecular phylogenetic and cytological data, *Paphiopedilum* has been divided into five sections *Pardalopetalum*, *Cochlopetalum*, *Coryopedilum*, *Barbata* and *Paphiopedilum* (3).

Phylogenetic relationship can be studied based on morphological data using phenetic and cladistic methods. Phenetic methods or numerical taxonomy was used to classify organisms based on their overall similarity to study lineages and evolution by creating group categorization (12). Simple way to study the relationship of plants were morphological data and morphometric data (3, 6). Genetic relationship between plants or population can be estimate based on the similarity some of characters or traits, assumed that different characters figure the difference of genetic constitution (10, 15). Morphological data were collected on several distinct characteristics. Clustering usually was estimated through character observed based on the similarity of morphology or phenotypic appearance, physiology, ecology and Molecular (7, 8). Data generated through this research were used as input data for statistical analysis, producing a phylogenetic dendrogram and genetic distance or similarity. The aim of the research was to estimate the relationship between parents (*Paph*. Maudiae "Black" & *Paph. tonsum*) and their 19 progenies. The result of the hybrid will be a new hybrid with combination between two section of *Paphiopedilum*.

2. Materials and Methods

2.1. Plant materials. Twenty-one Paphiopedilum accessions obtained from crossing of *Paph*. Maudiae x tonsum in 2010 in Indonesian Ornamental Crops Research Institute (IOCRI) were used in this study including their parents (figure 1 & figure 2). All plant materials were 8 years old and performed in IOCRI's screen house 1100 m asl.

2.2. Characterization. Method of the research was observation carried out on each genotype based on morphological and morphometrical traits. Leaf shape was determined based on the ration of the length and width of the leaf. Observations on morphometrical traits were determined in the form of category scores according to modified (13). Size of the flowers was observed in fully bloom. Flower color was standardized using the Royal Horticultural Society Color Chart.

2.3. Morphological and morphometric data analysis. 33 morphological qualitative traits and 13 morphometrical quantitative traits were used in multivariate analysis to perform dendrogram. Grouping of taxa was carried out utilizing the clustering analysis method (UPGMA) based on qualitative data and quantitative data characters of the leaf and flower (table 1). The morphological qualitative data was transformed and scored numerically as present (1) and absent (0), and then analyzed using NTSYS v.2.1 (11).

Figure 1. Seed pod parent (*Paphiopedilum* Maudiae). pollen parent (*Paphiopedilum tonsum*).

Morphometric traits data were used to analyse dendrogram and principal component, using NTSYS v.2.1 (11).

Table 1. Qualitative data (morphological characters) and quantitative data (Morphometrical characters) of the leaf and flower.

No.	Morphological characters	Description	No.	Morphometrical quantitative
1	Leaf shape	eliptic	1	Plant height (cm)
2	Leaf shape	oblong	2	Leaf number
3	Apical leaf shape	acute	3	Leaf length (cm)
4	Apical leaf shape	obtuse	4	Leaf width (cm)
5	Leaf base color	YG 147D	5	Flowering time (year)
6	Leaf base color	YG 147A	6	Dorsal sepal length (cm)
7	Leaf color pattern	YG 147A	7	Dorsal sepal width (cm)
8	Leaf color pattern	YG 144C	8	Flower length (cm)
9	Bractea shape	oblong	9	Flower width (cm)
10	Dorsal sepal shape	ovate	10	Petal length (cm)
11	Dorsal sepal shape	oblong	11	Petal width (cm)
12	Dorsal sepal color pattern	PG N 77a	12	Pouch length (cm)
13	Dorsal sepal color	N155C	13	Pouch width (cm)
14	Dorsal sepal color	YG 144A		
15	Dorsal sepal pattern	bergaris		
16	Petal color pattern	PG 79A		
17	Dorsal sepal curvature	Straight		
18	Synsepal shape	ovate		
19	Synsepal color	N78A		
20	Synsepal pattern	Line		
21	synsepal color pattern	PG 79A		
22	petal curvature	reflected		
23	Petal orientation	Arching		
24	Petal shape	lanceolate		
25	Petal base color	YG 144A		
26	Petal base color	N144D		
27	Petal base color	GRG 179C		
28	Petal pattern	Blotch		

29	Petal color pattern	N79B
30	Petal color pattern	YG 144d
31	Petal color pattern	PGN77a
32	Column staminiode shape	lobed
33	Lip color	PG N79A

3. Results and Discussion

3.1. Morphological character

Total progenies from the crossing between *Paphiopedilum* Maudiae x *Paphiopedilum tonsum* was 200 genotypes. Late flowering progenies were not including in the analysis. In general all progenies and the parents have the same type of leaves (green with line pattern), and single flower. except PH1.44. Genotype PH1.44 had *multi-floral* arrangement with two flowers (figure 9). The characteristic data showed that fifteen characters observed were monomorphic while 18 characters were polymorphic.

Cluster analysis was carried out to investigate the relationships among 19 progenies and their parents based on overall similarity. Similarity level can be figured as *dendrogram* and matrix (figure 2 and table 1). The result of cluster analysis showed that there are two main groups based on Dice similarity coefficient. The similarity level of that two groups are 0,941 (94,1%) based on 33 morphological traits. Group 1 was similar 100% to *Paph* Maudiae (female parent). They are 5 genotypes, PH1.01 (figure 3), PH1.16 (Figure 4); PH1.51 (figure 5), PH1.73, and PH1.78. Group 2 was consist of 10 genotype (PH1.42 (figure 6), PH.02 (figure 7), PH1.03, PH1.04, PH1.05, PH1.29, PH1.36 (figure 8), PH1.55, PH1.61 and PH1.77 were similar 94,1% with Group 1. *Paph. tonsum*, PH1.01A, PH1.06, PH1.07(figure 10) and PH1.44 (figure 9) are stand alone, but PH1.1A, PH1.06 and PH1.07 are closely related at 0,81 similarity coefficient. The similarity of each genotype based on morphological traits showed that PH1.44 was the most distinct among the progenies with the parents (0,74) (table 2, figure 9). PH1.44 has light colour of flower and different colour of leaf blotch.

Table 2. Similarity matrix of 21 genotipe of *Paphiopedilum* based on morphological traits data.

	PH1 01	PH1 73	PH1 78	PH1 51	PH1 16	PH1 42	PH1 02	PH1 61	PH1 36	PH1 29	PH1 77	PH1 55	PH1 03	PH1 04	PH1 05	PH1 07	PH1 06	PH1 01	PH1 44	Paph Maudiae	Paph tonsum
PH1_01	1,00																				
PH1_73	1,00	1,00																			
PH1_78	1,00	1,00	1,00																		
PH1_51	1,00	1,00	1,00	1,00																	
PH1_16	1,00	1,00	1,00	1,00	1,00																
PH1_42	0,96	0,96	0,96	0,96	0,96	1,00															
PH1_02	0,96	0,96	0,96	0,96	0,96	1,00	1,00														
PH1_61	0,96	0,96	0,96	0,96	0,96	1,00	1,00	1,00													
PH1_36	0,96	0,96	0,96	0,96	0,96	1,00	1,00	1,00	1,00												
PH1_29	0,96	0,96	0,96	0,96	0,96	1,00	1,00	1,00	1,00	1,00											
PH1_77	0,96	0,96	0,96	0,96	0,96	1,00	1,00	1,00	1,00	1,00	1,00										
PH1_55	0,96	0,96	0,96	0,96	0,96	1,00	1,00	1,00	1,00	1,00	1,00	1,00									
PH1_03	0,96	0,96	0,96	0,96	0,96	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00								
PH1_04	0,96	0,96	0,96	0,96	0,96	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00							
PH1_05	0,96	0,96	0,96	0,96	0,96	1,00	1,00	1,00	0,00	1,00	0,00	1,00	0,00	1,00	0,00						
PH1_07	0,87	0,87	0,87	0,87	0,87	0,91	0,91	0,91	0,91	0,91	0,91	0,91	0,91	0,91	0,91	1,00					
PH1_06	0,83	0,83	0,83	0,83	0,83	0,87	0,87	0,87	0,87	0,87	0,87	0,87	0,87	0,87	0,87	0,96	1,00				
PH1_01	0,78	0,78	0,78	0,78	0,78	0,83	0,83	0,83	0,83	0,83	0,83	0,83	0,83	0,83	0,83	0,91	0,91	1,00			
PH1_44	0,74	0,74	0,74	0,74	0,74	0,78	0,78	0,78	0,78	0,78	0,78	0,78	0,78	0,78	0,78	0,87	0,83	0,83	1,00		
Paph Maudiae	1,00	1,00	1,00	1,00	1,00	0,96	0,96	0,96	0,96	0,96	0,96	0,96	0,96	0,96	0,96	0,87	0,83	0,78	0,74	1,00	
Paph tonsum	0,91	0,91	0,91	0,91	0,91	0,96	0,96	0,96	0,96	0,96	0,96	0,96	0,96	0,96	0,96	0,87	0,83	0,78	0,74	0,91	1,00

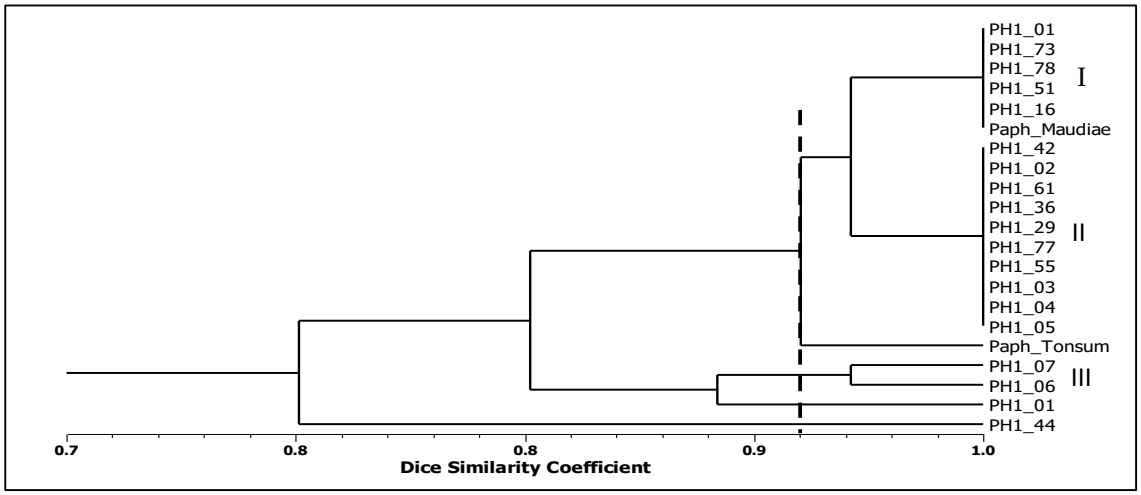

Figure 2. Dendrogram 21 genotipe *Paphiopedilum* Maudiae type based on morphological traits.

Figure 3. Progeny PH1.1.

Figure 4. Progeny PH1.16.

Figure 5. Progeny PH1.51.

Figure 6. Progeny PH1.42.

Figure 7. Progeny PH1.02.

Figure 8. Progeny PH1.36.

Figure 9. Progeny PH1.44

Figure 10. Progeny PH1.07.

Figure 11. Progeny PH1.29.

3.2. Morphometric character

Morphometric character observed was chosen only on size of plants, leaves and flowers. The consumer preference usually have different viewpoint. The size of plant was important because the segments should be in sufficient proportion to each other to create balance about both the vertical and horizontal axes of the plant. The size of the individual floral segments should be in proportion and not distort the overall shape. A synsepal had sufficient size to form a visually pleasing background for the pouch and preferably show a neat margin around and below the pouch. The pouch should be proportioned pouches in size.

According to data transformation was required to reduce the influence of different measurement scale and categories from different characters (1). The calculation on the formula of the average taxonomic distance between genotypes has various unit values scale and was presented in the form of average taxonomic distance matrix (Table 3).

The relationship between genotypes of *Paphiopedilum* based on morphometric trait was depicted in dendrogram. The distance between genotype was showed in matrix distance (table 4). Dendrogram based on morphometric trait constructed by cluster analysis showed that there were three groups with 1,49 of the average taxonomic distance. Group I formed into two clusters, i.e. first cluster (PH1.01A, PH1.77, PH1.78, PH1.16, PH1.03, PH1.44, PH1.06) was closely related to *Paphiopedilum tonsum,* the second cluster was PH1.02, PH1.05, and PH1.36. Group II was closely related to *Paphiopedilum Maudiae.* The third group was only two genotypes, i.e. PH1.01B and PH1.07 (figure 12). The higher the distance the farther the relationship is. The grouping as a dendrogram was a result in the genotypes with similar morphometric trait, so it was being classified into the same cluster.

Table 3. Taxonomic distance of 21 genotipe of *Paphiopedilum* based on morphometrical traits data.

	PH1 01	PH1 73	PH1 78	PH1 51	PH1 16	PH1 42	PH1 02	PH1 61	PH1 36	PH1 29	PH1 77	PH1 55	PH1 03	PH1 04	PH1 05	PH1 07	PH1 06	PH1 01	PH1 44	Paph Maudia e	Paph tonsum
PH1_01	0																				
PH1_73	1,36	0																			
PH1_78	0,99	1,18	0																		
PH1_51	1,47	1,13	1,37	0																	
PH1_16	0,95	1,19	0,5	1,5	0																
PH1_42	1,47	1,66	1,73	1,5	1,78	0															
PH1_02	1,29	1,61	0,79	1,8	0,94	2	0														
PH1_61	1,19	0,93	1,17	0,8	1,13	1,5	1,59	0													
PH1_36	1,25	1,63	0,89	1,7	1,02	2	0,66	1,55	0												
PH1_29	1,22	1,11	1,09	1,2	1,13	1,2	1,59	0,81	1,6	0											
PH1_77	0,52	1,55	1,22	1,7	1,08	1,6	1,57	1,39	1,4	1,41	0										
PH1_55	1,64	0,96	1,34	1,4	1,31	1,9	1,69	1,05	1,64	1,18	1,83	0									
PH1_03	0,8	1,29	0,84	1,5	0,71	1,7	1,16	1,15	1,01	1,12	0,78	1,5	0								
PH1_04	1,44	0,87	1,17	1,4	1,21	1,4	0,17	1,13	1,61	0,96	1,61	1	1,38	0							
PH1_05	1,41	1,72	1,09	1,9	1,3	2	0,81	1,67	1,29	1,57	1,81	2	1,44	1,84	0						
PH1_07	1,32	1,45	1,41	1,2	1,53	1,9	1,87	1,3	1,81	1,7	1,46	1,7	1,63	1,74	2,09	0					
PH1_06	1,07	1,28	0,92	1,2	1	1,6	1,25	1,29	1,38	1,25	1,2	1,7	0,84	1,57	1,32	1,51	0				
PH1_01	1,62	1,78	1,26	1,4	1,39	2,1	1,77	1,51	1,65	1,72	1,73	1,9	1,64	1,63	2,1	1,22	1,69	0			
PH1_44	0,88	1,38	0,8	1,4	0,68	1,7	0,98	1,19	1,1	1,19	1	1,7	0,55	1,55	1,19	1,64	0,68	1,63	0		
Paph Maudiae	1,75	1,34	1,53	1,1	1,59	2	1,85	1,17	1,82	1,45	1,9	1,1	1,58	1,67	2,11	1,49	1,53	1,83	1,63	0	
Paph tonsum	0,98	1,22	0,73	1,3	1	1,8	0,94	1,19	0,86	1,16	1,23	1,6	0,85	1,4	1,03	1,52	0,98	1,63	0,92	1,65	0

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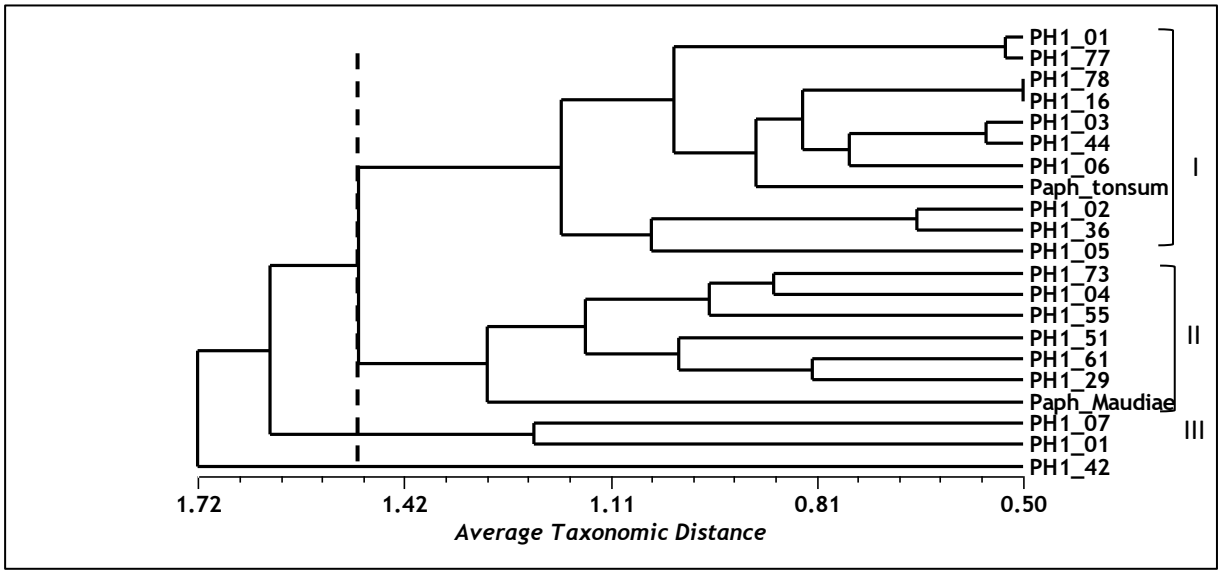

Figure 12. Dendrogram of 21 genotipe *Paphiopedilum* based on morphometric traits.

Principle Component Analysis (PCA) involving 33 morphological characters allows a better illustration of the effectiveness of each trait concerning the variation of 21 genotypes. Therefore the data was continue analysed with PCA with the same data and program to understand which characters contributed to the grouping, and to know the relative position of PH1_42, which in dendrogram did not joint in the groups. The result showed in Figure 13 that in two dimention of PCA, the grouping was similar to the dendrogram, but PH1_42 was joint in group II.

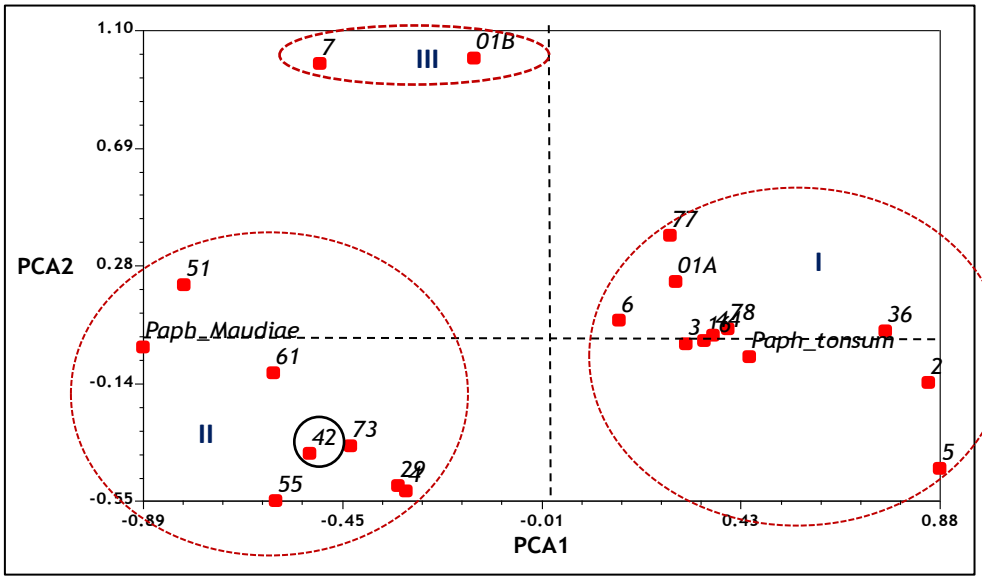

Figure 13. Relative position of 21 genotypes of *Paphiopedilum* split into three group in two dimension of the principle component based on morphometric characters.

The analysis of the principle components, conducted to reduce all 13 characters, revealed four PCA that had eigenvalue (λ) higher than 1, it was explained by 74.192% of the data variability (table 4). The analysis of 4 principal components proves that PCA1 was the main and most effective component, consisted four characters, i.e. plant height, leaf length, flowering time and pouch width which explained

30,171% of total variation with eigenvalue 6.04. PCA 2 consisted 2 characters, i.e. leaf number and leaf width which explained 17.631% of total variation with eigenvalue 3.530. PCA 3 consisted two character, i.e. dorsal sepal length and flower width which explained 16.686% of variation with eigenvalue 3.340. the last PCA 4 consisted one character, i.e. petal length which explained 9.704% of variation with eigenvalue 1.940.

Table 4. Principle Component value of 13 characters contributed in differentiating 21 genotypes of *Paphiopedilum.*

Characters	PCA1	PCA2	PCA3	PCA4
Plant height	**8.989**	-3.128	6.327	1.537
Leaf number	4.058	**-6.219**	2.714	2.427
Leaf length	**8.716**	1.115	6.412	1.165
Leaf width	-1.474	**-5.416**	-1.826	1.721
Flowering time	**9.884**	-3.462	-6.724	-7.977
Dorsal sepal length	3.608	7.405	**-8.255**	-3.676
Dorsal sepal width	-4.340	2.113	-5.146	3.422
Flower length	6.328	5.299	2.052	-2.053
Flower width	-1.201	-2.529	**6.055**	-7.605
Petal length	-3.508	3.100	4.675	**5.159**
Petal width	-3.871	-1.692	5.665	-4.996
Pouch length	5.276	-1.956	-3.053	-4.762
Pouch width	**-7.728**	3.221	-1.896	-2.650
Eigenvalue	6.04	3.530	3.340	1.940
variation (%)	30.171	17.631	16.686	9.704
cummulative (%)	30.171	47.802	64.488	74.192

*PCA (Principle Component Analysis)

3.3. Discussion

Paphiopedilum Maudiae was a primary Paph, very attractive, having pleasing mottled leaves. All Maudiae type hybrid, except Paph Maudiae (*Paph. callosum* x *lawrenceanum*) itself are Novelty hybrid. The definition of Maudiae in *Paphiopedilum* was used as an honor of botanist Stephen Troyte Dunn's wife Maud Dunn. *Paph.* Maudiae came in two types, green or alba type and vinicolor (red of purple) (16). According to International Union for Conservation of Nature (IUCN) red list *Paph. tonsum* was uncommon, rare and very local with a distribution restricted to Sumatera. The population trend was decreasing and the abundance of the species has been significantly reduced during recent years. There for *Paph. tonsum* was assessed as Endangered. The crossing between *Paphiopedilum* Maudiae x *Paphiopedilum tonsum* has already registered in Royal Horticulture Society (RHS) as *Paphiopedilum* Chief Joseph Baker & Chantry 1996. *Paph* Chief Joseph having 100% subgenus Sigmatopetalum and 50% section Punctatum, 50% section Barbata.

The different result of clustering derived from morphologic and morphometric data indicating that those characters each reveal different genomic regions. But morphological data was better differentiated of genotype. Therefore, it was better to combine data revealing from morphology and agronomy data.

Higher standard deviation of morphometric traits indicated a high level of morphological plasticity present among that genotype. Therefore, Principle Component Analysis (PCA) was performed to identify the real structure of the parent and their progenies. Since first four principal components estimated about 74.192% of the morphological variation, the representing morphometric traits (plant height, leaf length, flowering time, pouch width, leaf number, leaf width, dorsal sepal length, flower width and petal length) are recommended to consider the useful of this morphometric characteristics for further studies.

4. Conclusion

Clustering analysis of interspesific hybrid of *Paphiopedilum* using morphological data was succeded to estimate progenies variation. Five genotype i.e. PH1.01, PH1.16, PH1.51 PH1.73, and PH1.78 had morphological resemblance with *Paph*. Maudiae and ten genotype (PH1.42, PH.02, PH1.03, PH1.04, PH1.05, PH1.29, PH1.36, PH1.55, PH1.61 and PH1.77) had morphological resemblance with *Paph*. Maudiae and *Paph. tonsum*. Based on morphometric data, 11 genotypes had quantitative trait resemblance with *Paph. Tonsum*, And 7 accessions had quantitative trait resemblance with *Paph*. Maudiae. Morphological trait of *Paph*. Maudiae was dominant inherited to their progenies.

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