Analysis of Petal and Sepal Shape Variation in Orchids of the Genus *Vanda* (Orchidaceae) by Elliptic Fourier Descriptors and Principal Component Analysis[1]

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Abstract

The shapes of the sepals and petals are important for orchids because of the commercial value of its flowers, and their form is a target characteristic for breeding. This study introduces a method that allows for the quantitative evaluation of the structures using elliptic Fourier descriptors and principal component analysis (PCA). This method describes petal and sepal shapes mathematically by transforming chain-coded contours into elliptic Fourier descriptors and summarizing it by PCA. To examine differences among the orchids, one-way analysis of variance was performed on the principal component scores. Results of the analysis showed that the mean shapes can be evaluated successfully and for use in systematics and classification of orchids. A number of significant principal components were generated to summarize shape differences-left sepals (7); right sepals (6); center sepals (5); left petals (8); right petals (7). Ordination of the first two principal components showed overlaps between the convex hulls delineating the different groups of orchids. However, ANOVA of the principal component scores was successful in detecting independent significant characteristics that vary significantly among

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the orchids. Among the most variable traits include the aspect ration (length-width ratio) and the shape of the sinuses at the base of each petal and sepal. The overall shapes of the structures were also decomposed into symmetric and asymmetric components. The results were discussed in relation to the relative contribution of genetics and environmental factors in determining the shapes of petals and sepals; and that within an orchid flower the major source of the symmetrical elements is genotypic and the asymmetrical elements are strongly affected by the environment.

Keywords: Vanda, geometric morphometrics, elliptic Fourier analysis, chain codes

Introduction

The family orchids (*orchidaceae*) comprising a total number of about 25,000 known species worldwide is one of the most widespread of all plant families. Their adaptation to extremely diverse environments has produced great variation among them. Orchids come in all shapes and colors. An orchid flower consists of 3 sepals which are usually different from sepals in shape but not in color. There are also 3 petals but one has been greatly modified into a lipan extremely complex, which is quite different from the other two petals, (Soon, T.E., 1989).

Vanda is one genus of the orchid family (*orchidaceae*). Members of this genus are mostly epiphytic but sometimes lithophytic or terrestrial orchids and are distributed in India, Himalaya, Southeast Asia, Indonesia, Philippines, New Guinea, Southern China and Northern Australia. This genus is one of the five most horticulturally important orchid genera because it has some of the most magnificient flowers to be found in the entire orchid family. The shapes of their sepals and petals are important because of the commercial value of their flowers and their forms are the target characteristic for breeding.

The colors and shapes of the sepals and petals are often used to determine the variations among orchid varieties. However, these qualitative evaluations are all rough estimates by human visual judgment which were sometimes deceived and misled by size factors. Furthermore, the variations are generally continuous and quantitative measurements are essential. Therefore, quantitative evaluation of sepals and petals shape is important to understand variations among orchid varieties.

This study introduces a method that allows for the quantitative evaluation of the structures using elliptic Fourier descriptors (EFDs) and principal component analysis (PCA). Elliptic Fourier descriptors (EFDs) Kulh and Giardana (1982) is one of the method which is commonly used to describe the shape quantitatively and have been applied effectively to the evaluation of various biological shapes in animals and plants. This method can delineate any type of shape with a closed two-dimensional contour. It describes an overall shape mathematically by transforming coordinate information concerning its contours into Fourier coefficient. Rohlf and Archie (1984) suggested principal component analysis for summarizing the elliptic Fourier descriptors. Furthermore, they reported that it is possible to analyze the shape quantitatively by using the principal component scores as ordinary quantitative characters. Quantitative evaluation of shape based on EFD has been successfully conducted in several plant species such as Citrus leaf (Iwata, et al, 2002); Radish root (Iwata, et al., 2004); Primula seiboldii petal (Yoshioka, et al., 2004); Soybean leaflet (Furuta , et al.,1995); Buckwheat kernel (Ohsawa, 1998) and Yam tubers (Toyohara, et al., 2000). The development of computer program package "SHAPE" by Iwata and Ukai (2002), based on elliptic Fourier descriptors made quantitative evaluation of biological shape possible.

The objectives of this study are to examine the petals and sepals shape variations in orchids particularly among genus vanda using elliptic fourier descriptors and principal component analysis, establish a quantitative method for the evaluation of variations among vanda orchids and to test the affectivity of EFD and PCA in analyzing the variations among vanda varieties using petals and sepals.

Materials and Methods

Six (6) varieties of vanda orchids taken from orchids grower of Iligan City and from JC Garden, Orchids and Green at Carpenter Hills, Koronadal City were considered in this study. These are the V. JVB Thongchai Gold, Velt. Mammo, Velt 406 x Dr. Anek, Velt. Penang Manila , Velt. Charles Good Fellow and Velt. Dr. Anek. A total of 236 vanda flowers belonging to six (6) vanda varieties were collected. The 3 sepals and 2 petals were separated using a cutter. These were arranged accordingly between two pieces of 5" x 5" clear glass of 1/16 inch thick. A scanner (HP Scanjet 2400) was used to capture the image of the sepals and petals using a resolution of 600dpi. The images of the sepals were then grouped accordingly based on their position, left, right and center. Similarly, the petals were also grouped into left and right. Using a chain coder, the full color images of the sepals and petals were converted to a binary (black and white color) images.

The contours of the sepals and petals were then traced and were recorded as chain code (Freeman, 1974). The CHC2NEF software was then used to calculate the normalized EFDs from the chain code information. The 80 coefficient were also classified into two groups related to symmetrical and asymmetrical variations for the central axis of each petal and sepal (Iwata, et al., 1998). The coefficients a and d represents symmetrical while b and c represents asymmetrical variations.

A program named princomp was used to visualize the shape variations accounted for by each principal component. The coefficients of the elliptic Fourier descriptors were calculated, then the sepal and petal shapes were reconstructed from the coefficients by inverse Fourier transformation (Furuta et al., 1995). This visualization method is helpful in understanding the morphological mean of the variations evaluated by each principal component. Using the normalized elliptic Fourier coefficients, the principal component analysis were performed and the scatter plot and box plot graphs were generated. The outline of the method used is shown in figure A.

To further compare the shapes of sepals and petals, one way ANOVA (Kruskal Wallis) and Dunn's Multiple Comparisons Test were performed using the scores of the components as characteristics of petals and sepals shape.

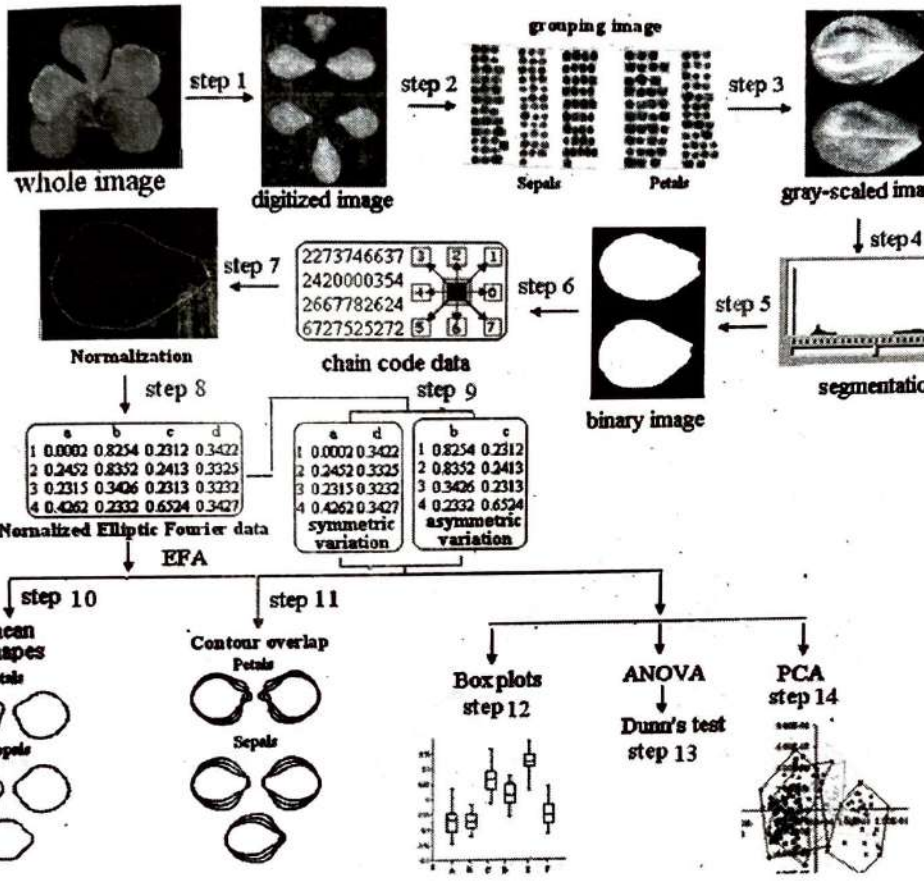


Figure A. Outline of the method used in the study

Results

The standardized elliptic Fourier coefficients of the sepals and petals of the 236 vanda flower belonging to 6 varieties were calculated. The mean shapes of the 3 sepals (left, right and center) and 2 petals (left and right) of each variety were drawn using the mean values of the standardized Fourier coefficient. Figure 1 shows the comparison of mean shape of the 3 sepals and 2 petals of the six (6) vanda varieties. The figure shows that variations occurred among sepals and petals of the vanda varieties.

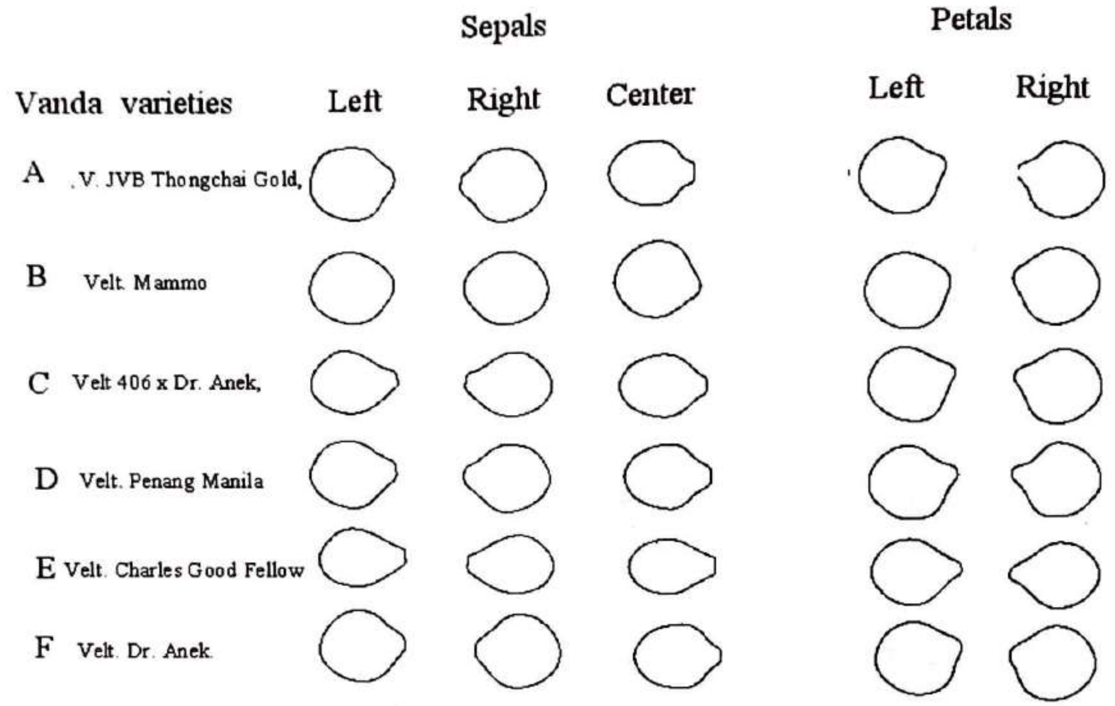


Figure 1. Mean shapes of sepals and petals of the six (6) vanda varieties.

Tables 1 and 2 show the results of the principal component analysis and the contributions of each component to the variations of the sepals and petals shape. A number of significant components were generated to summarize shape differences – left sepals 7; right sepals 6; center sepals 5; left petals 8 and right petals 7. Among sepals (left, right and center), these significant components accounted for 93.10%, 92.42% and 92.13% variations, similarly for petals (left and right) they accounted for 92.36% and 91.57% variations respectively. The results also show that great variations among sepals and petals were summarized in component 1 while the other principal components detect the minor or subtle variations which cannot be detected by merely comparing the sepal and petal structures qualitatively.

Table 1. Eigenevalues and the contributions of the effective principal components to the shapes of sepals of the six (6) vanda varieties.

COMPONENTS	LEFT SEPALS			RIGHT SEPALS			CENTER SEPALS		
	Eigen values (10^{-4})	Proportion	Cumulative	Eigen values (10^{-4})	Proportion	Cumulative	Eigen values (10^{-4})	Proportion	Cumulative
1	66.62	74.81	74.81	64.87	76.87	76.87	64.24	82.05	82.05
2	4.72	5.30	80.12	4.08	4.84	81.71	2.87	3.67	85.73
3	4.15	4.66	84.79	3.29	3.90	85.62	1.86	2.38	88.11
4	2.70	3.03	87.82	3.06	3.62	89.25	1.75	2.24	90.36
5	2.01	2.26	90.08	1.53	1.82	91.07	1.39	1.77	92.13
6	1.54	1.73	91.81	1.13	1.34	92.42			
7	1.14	1.29	93.10						
Total variance	84.04			84.39			78.29		

Table 2 . Eigenvalues and the contributions of the effective principal components to the shapes of petals of the six (6) vanda varieties.

COMPONENTS	LEFT PETALS			RIGHT PETALS		
	Eigen values (10^{-4})	Proportion	Cumulative	Eigen values (10^{-4})	Proportion	Cumulative
1	39.43	49.48	49.48	41.93	51.87	57.87
2	13.36	16.77	66.26	10.55	13.06	64.93
3	9.91	12.44	78.70	9.56	11.83	76.76
4	4.48	5.63	84.34	6.83	8.45	85.22
5	2.24	2.81	87.15	2.23	2.76	87.98
6	1.54	1.93	89.09	1.80	2.23	90.22
7	1.40	1.75	90.85	1.09	1.35	91.57
8	1.20	1.50	92.36			
Total variance	79.67			80.83		

Reconstructed shapes show contour overlaps in the sepals and petals indicating variations among the different varieties of vanda orchids (figure 2). The figure shows greater contour overlaps occurred in component implying that this component contributed greatly to the variations of both sepals and petals. Among the most variable traits include the aspect ratio (length-width ratio) and the shape of the sinuses at the base of each petal and sepal. The variation in length-width ratio as summarized by component 1 accounted for 74.81%, 76.87% and 82.05% for left, right and center sepals, respectively (tables 1 and 2). The other principal components had minor contour overlaps indicating minor variations or subtle variations in the shape of sepals and petals. These said variations cannot be detected based on estimates using human visual judgment.

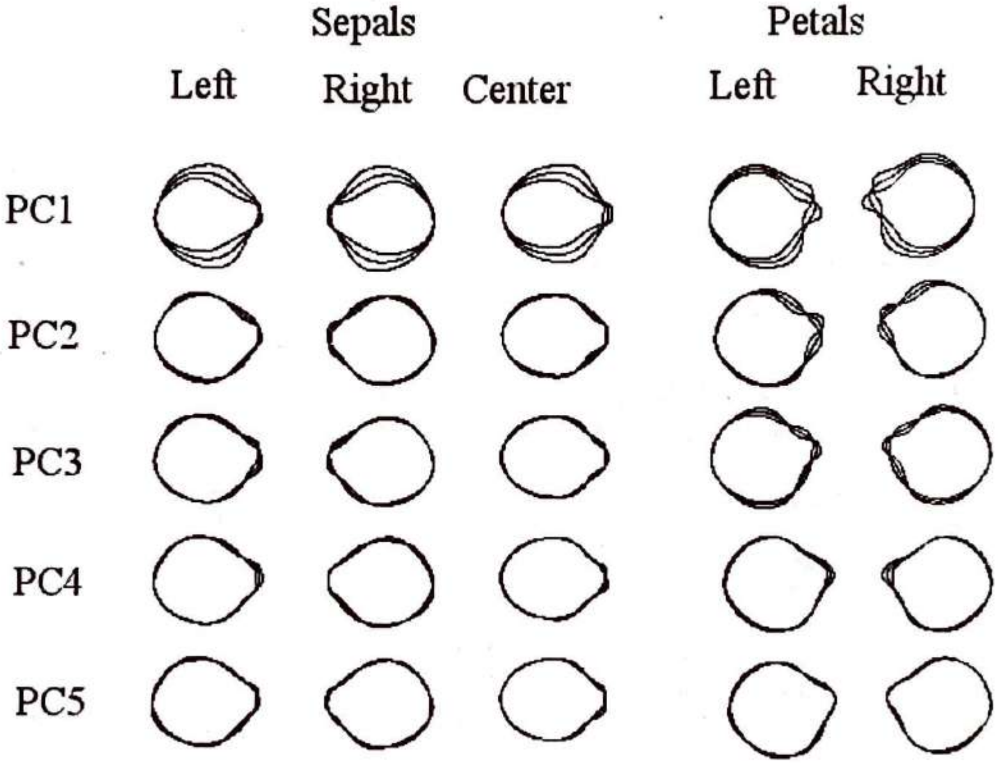


Figure 2. Effects of the effective principal components on each petal and sepal shapes of the six (6) vanda varieties

The overall shapes of the structures were also decomposed into symmetrical and asymmetrical components. Among sepals, the contributions of the symmetrical components were greater than that of the asymmetrical components. The variations accounted for by symmetrical components of the left, right and center sepals were 93.03%, 94.47% and 94.48%, while the variations accounted for by asymmetrical components were 85.02%, 89.21% and 88.49% for left, right and center sepals respectively. Similarly, the variations accounted for by symmetrical components of the left and right petals were 93.10% and 92.09% which were higher than that of the variations accounted for by the asymmetrical components of the left and right petals which were 88.87% and 87.77% respectively (Tables 3a&b and 4a&b).

Table 3a. Eigenvalues and contributions of the symmetrical group to the shapes of sepals of the six vanda varieties.

COMPONENTS	LEFT SEPALS			RIGHT SEPALS			CENTER SEPALS		
	Eigen values (10^{-4})	Proportion	Cumulative	Eigen values (10^{-4})	Proportion	Cumulative	Eigen values (10^{-4})	Proportion	Cumulative
1	66.23	84.59	84.59	64.75	87.23	87.23	34.52	79.94	79.94
2	4.39	5.61	90.21	3.46	4.67	91.90	6.28	14.54	94.48
3	2.20	2.81	93.03	1.90	2.57	94.47			
Total variance	78.29			74.23			43.18		

Table 3b. Eigenvalues and contributions of the asymmetrical group to the shapes of sepals of the six vanda varieties.

COMPONENTS	LEFT SEPALS			RIGHT SEPALS			CENTER SEPALS		
	Eigen values (10^{-4})	Proportion	Cumulative	Eigen values (10^{-4})	Proportion	Cumulative	Eigen values (10^{-4})	Proportion	Cumulative
1	3.86	35.91	35.91	3.29	32.42	32.42	19.54	44.09	44.09
2	2.53	23.53	59.44	2.94	28.97	61.39	0.79	17.87	61.96
3	1.26	11.78	71.23	0.96	9.53	70.93	0.55	12.42	74.39
4	0.66	6.20	77.44	0.64	6.32	77.25	0.29	6.65	81.04
5	0.46	4.45	81.79	0.49	4.88	82.14	0.21	4.81	85.86
6	0.34	3.22	85.02	0.36	3.59	85.73	0.11	2.63	88.49
7				0.35	3.47	89.21			
Total variance	10.75			10.16			4.43		

Table 4a. Eigenvalues and contributions of the symmetrical group to the shapes of petals of the six vanda varieties.

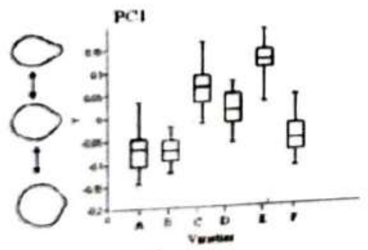
COMPONENTS	LEFT PETALS			RIGHT PETALS		
	Eigen values (10^{-4})	Proportion	Cumulative	Eigen values (10^{-4})	Proportion	Cumulative
1	38.61	66.47	66.47	40.77	66.36	66.36
2	9.53	16.40	82.88	9.16	14.92	81.28
3	4.33	7.46	90.35	6.64	10.81	92.09
4	1.59	2.75	93.10			
Total variance	58.08			61.43		

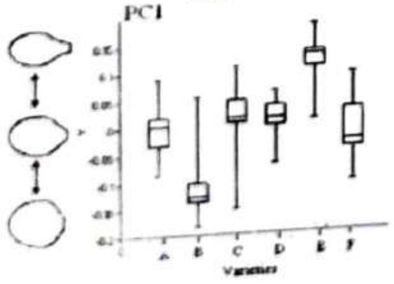
Table 4b. Eigenvalues and contributions of the asymmetrical group to the shapes of petals of six (6) vanda varieties.

COMPONENTS	LEFT PETALS			RIGHT PETALS		
	Eigen values (10^{-4})	Proportion	Cumulative	Eigen values (10^{-4})	Proportion	Cumulative
1	12.98	60.12	60.12	10.57	54.50	54.50
2	2.35	10.92	71.05	2.38	12.30	66.81
3	2.08	9.65	80.71	1.88	9.72	76.53
4	1.12	5.19	85.91	1.17	6.03	82.56
5	0.63	2.96	88.87	1.00	5.20	87.77
Total variance	21.59			19.39		

The box plot graphs of the first five components of sepals and petals are shown in figures 3 and 4. The PC 1 showed wide variations in sepal and petals mean shape as exhibited by scatter box plots, while minor variations among mean sepal and petal shapes were shown by principal components (PC) 2-5.

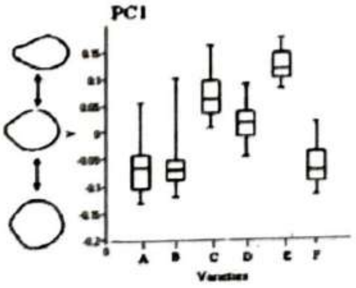
A. Left sepals

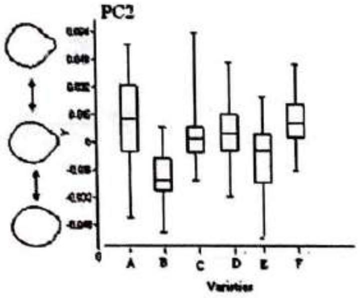




B. Right sepals

C. Center sepals




PC2
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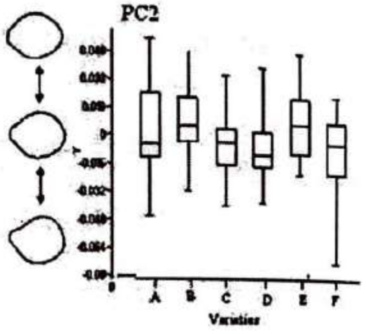
Varieties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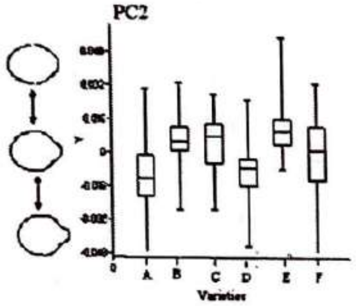
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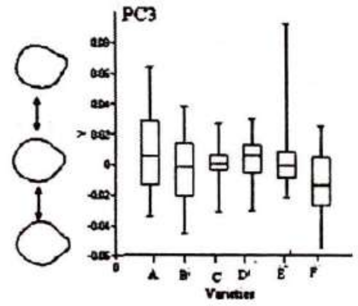
PC3
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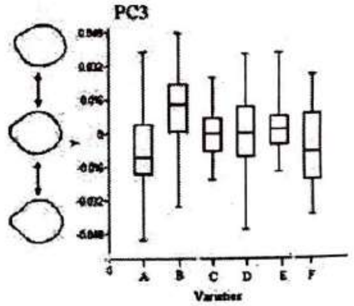
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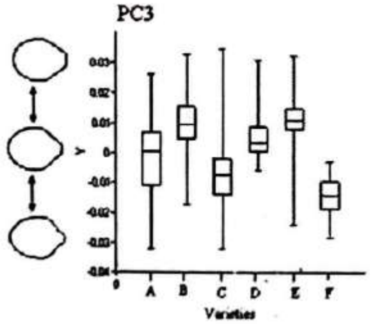
PC3
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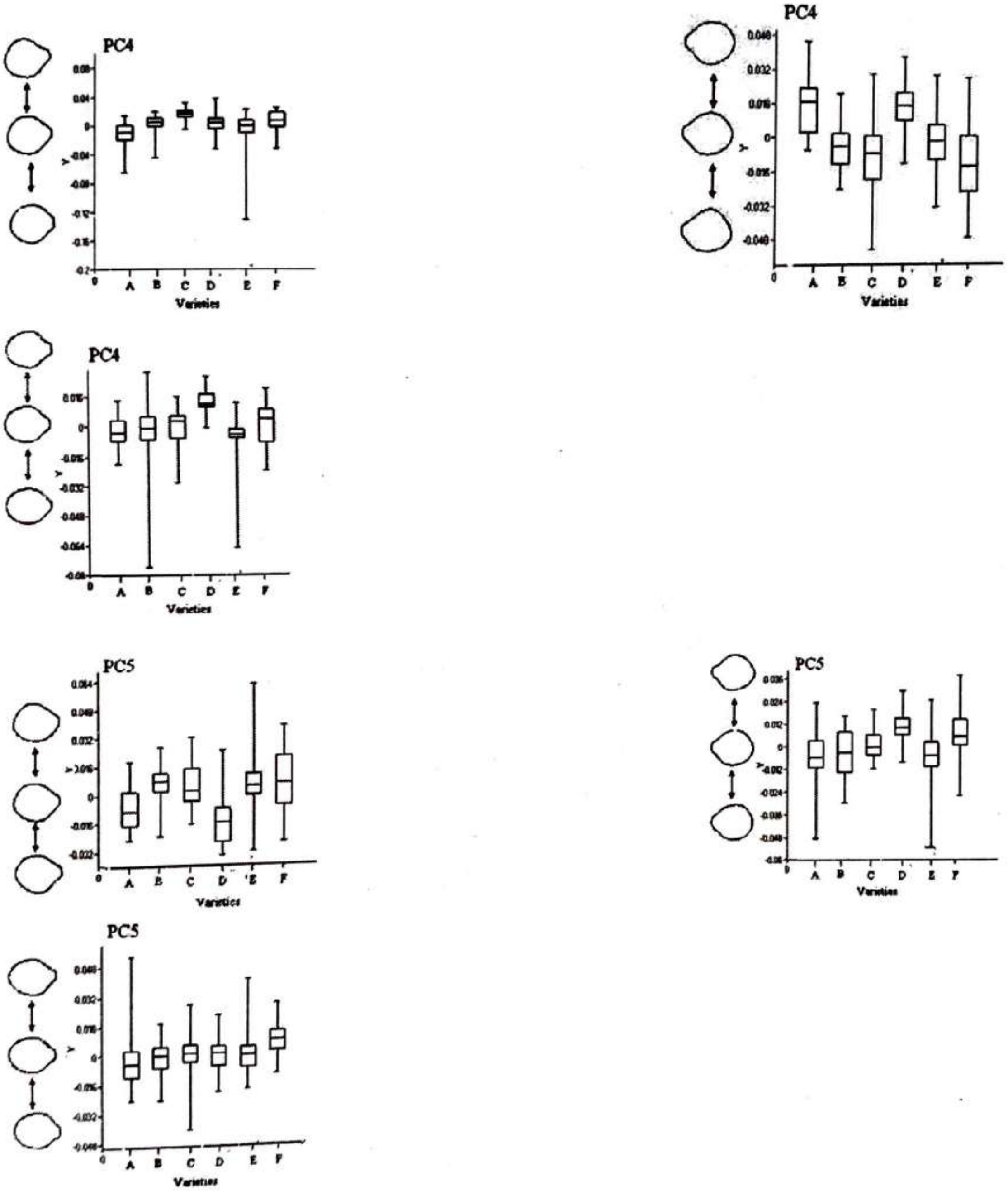
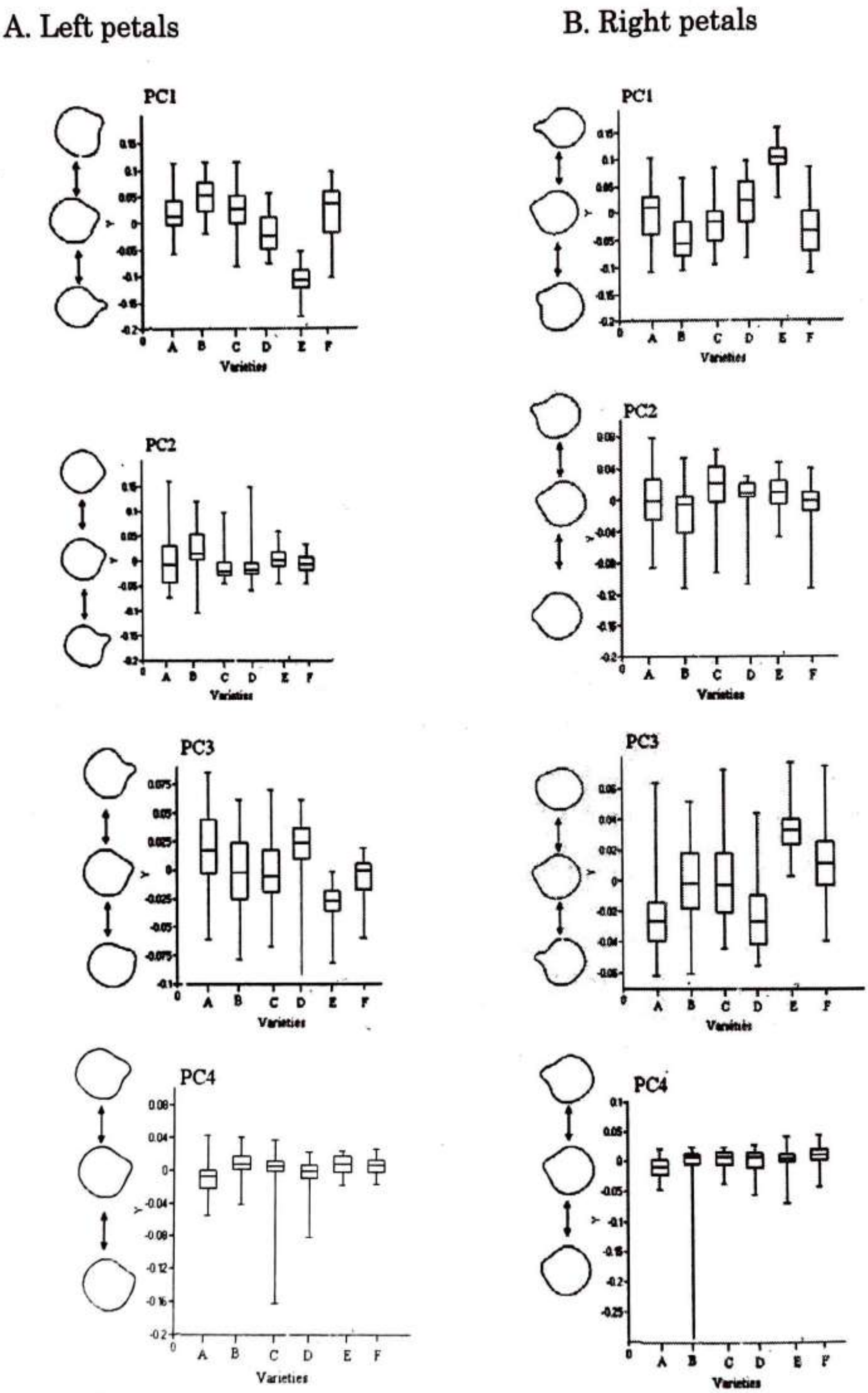


Figure 3. Box plot graphs of the effective principal components of sepals of the six vanda varieties. (A) V. JVB Thongchai Gold; (B). Velt. Mammo; (C). Velt 406 x Dr. Anek; (D). Velt. Penang Manila ; (E). Velt. Charles Good Fellow ; (F). Velt. Dr. Anek.



A. Left petals

B. Right petals

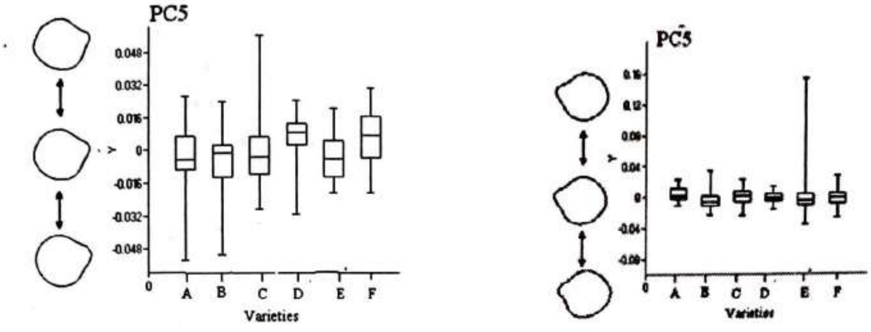
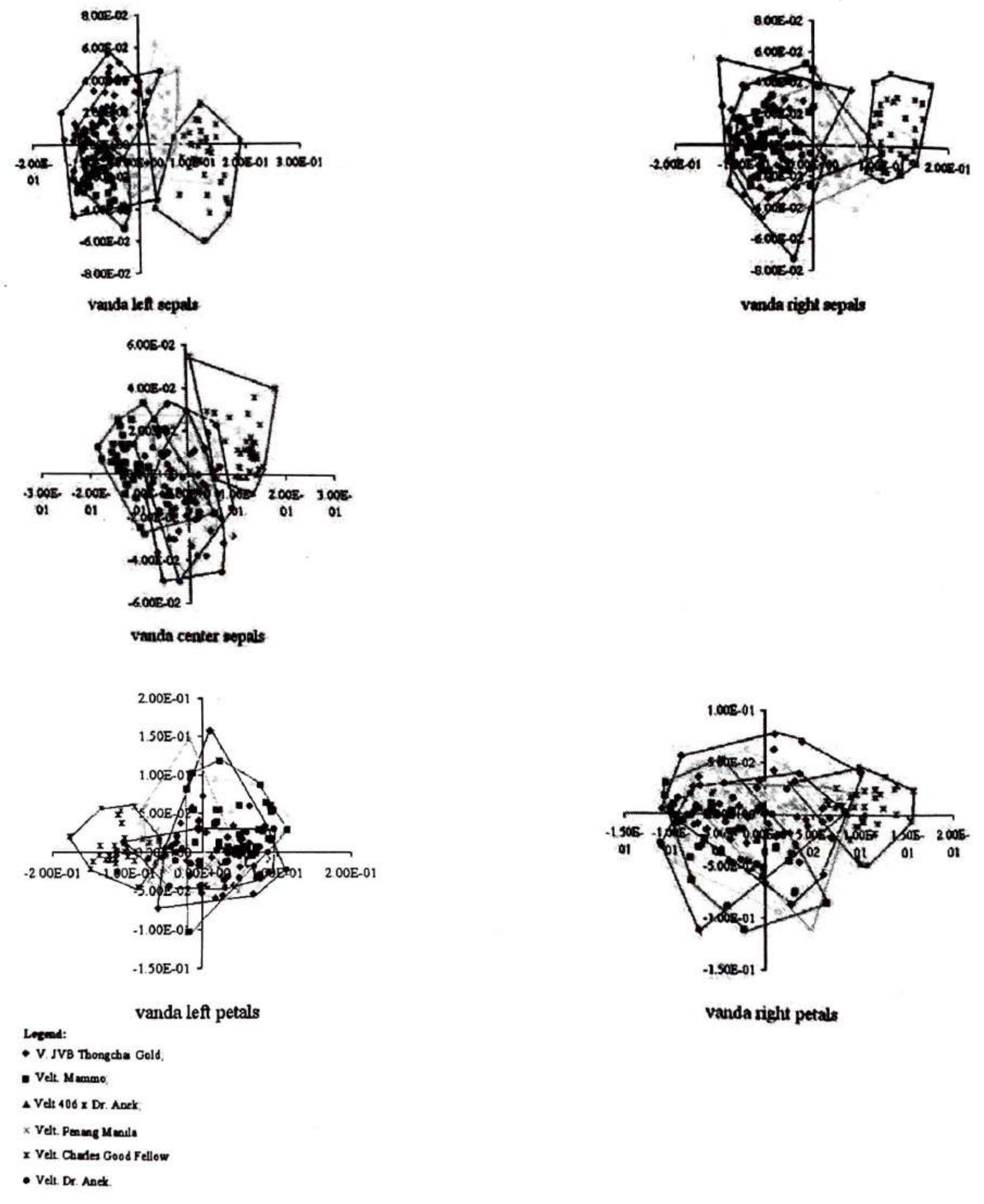


Figure 4. Box plot graphs of the effective principal components of petals of the six vanda varieties. . (A). V. JVB Thongchai Gold; (B). Velt. Mammo; (C). Velt 406 x Dr. Anek; (D). Velt. Penang Manila ; (E). Velt. Charles Good Fellow ; (F). Velt. Dr. Anek.

Ordination of the first two principal components showed convex hulls delineating the different vanda varieties (figure 5). Varieties overlapping in their convex hulls indicated that the said varieties share common characteristics in the shape of their sepals and petals. Moreover, varieties with convex hulls separated indicated that they possessed a distinct petal and sepal shape characteristics.



vanda left sepals

vanda right sepals

vanda center sepals

vanda left petals

vanda right petals

Figure 5. Results of the principal component analysis showing convex hulls delineating the six vanda varieties based on the shapes of their sepals and petals.

Table 5 shows the results of the one way ANOVA (Kruskal-Wallis) of the effective components of the 3 sepals (left, right and center) and 2 petals (left and right). The results showed that the effective principal components of both sepals and petals were all extremely significant except for component 3 of the left sepals and 5 of the right petals which were very significant and significant respectively.

Table 5. One way ANOVA (Kruskal-Wallis) of the effective components of sepals and petals of the six vanda varieties.

COMPONENTS	LEFT SEPALS	RIGHT SEPALS	CENTER SEPALS	LEFT PETALS	RIGHT PETALS
1	173.76***	173.48***	155.65***	119.45***	111.20***
2	71.01***	25.32***	58.06***	43.93***	32.73***
3	17.47**	33.22***	116.48***	64.08***	92.02***
4	67.85***	83.45***	69.37***	41.42***	32.05***
5	71.42***	62.65***	21.50***	30.54***	12.01*

***- P value <0.0001- extremely significant

**- P value =0.0037 - very significant

* - P value = 0.0346 - significant

Discussion

Quantitative evaluation of the shapes of sepals and petals of the vanda varieties has been successfully established by using principal component scores obtained from standardized elliptic fourier descriptors. The principal components were independent and can be observed visually how each affects the shape by drawing the contours under particular score value conditions, demonstrating that PC scores can be used as new shape characteristics of sepals and petals of vanda orchids. The study in P. seiboldii by Yoshioka (2004) also demonstrated that PC scores were also used as new shape characteristics of *P. sieboldii* sepals.

The generation of mean shape (figure 1) and the reconstruction of the contour shapes based on recalculated coefficient (figure 2) made us visualized the variations among Vanda varieties. The results indicated that the variations among sepals and petals as revealed by principal component (PC) 1 were greatly due to the length and width ratio as well as differences in the convex hulls at the base of both sepals and petals. This variation accounted for 74.81%, 76.87% and

82.05% for the left, right and center sepals, while for the left and right petals these accounted for 49.48% and 51.87% respectively, (tables 1 and 2). PC 2-5 described other causes of variations which were not described in PC 1. The detection of these minor causes of variations made the methods EFD and PCA very advantageous for these minor variations could not be detected by mere qualitative evaluation. Furthermore, EFD and PCA are also very effective methods for the evaluation of shapes for they can quantitatively analyze the shape of the image/ object independent from their size. The capability of this method to do such is a great advantage in performing such analysis, for accurate evaluation of shape variations could not be attained by using qualitative method because human visual judgment on shape of the object / image were often influenced or deceived by the effect of size factor.

The results in tables 1 and 2 revealed that left and right sepals showed greater variations than that of the center sepals which were 89.04 x 10^{-4}, 84.39 x 10^{-4} and 78.29 x 10^{-4} respectively. The results of total variations of the sepals from that of the petals, the total variations of the sepals were generally higher than that of the petals with total variations of 79.67x10^{-4} and 80.83x10^{-4} for both left and right sepals respectively. This implied that the use of the left and right sepals to discriminate variations among Vanda varieties is better than that of the center sepals. Furthermore, the use of left and right sepals is also more effective in discriminating shape variations among Vanda varieties than using the left and right petals.

The results of the symmetrical and asymmetrical analysis of both sepals and petals showed that the total variations of the sepals (left, right and center) which are 78.29x10^{-4}, 74.23x10^{-4} and 43.18x10^{-4} of the symmetrical group were higher than that of the total variations of the asymmetrical group which are 10.75x10^{-4}, 10.16x10^{-4} and 4.43x10^{-4} for left, right and center sepals respectively. Similar results were also observed among petals wherein the left and right petals of the symmetrical group have higher total variations which are 58.08x10^{-4} and 61.43x10^{-4} than that of the total variations of the left and right petals of the asymmetrical group with 21.59x10^{-4} and 19.39x10^{-4} respectively. These results implied that variations among the orchid flowers can be attributed by both genetic as well as environmental factors, wherein genetic factors play a greater role than that of the environmental factors. Within an orchid flower, the major source of the symmetrical elements is genotypic and the asymmetrical elements are strongly affected by the environment. The wide variations in shape of petals and sepals among vanda varieties are greatly contributed by the genetic factors which direct the activities of the cells during cell division and cell elongation. The differences in the extent of cell activities during cell divisions are major factors which determine the shape of sepals and petals. According to Meyerowitz (1997) the quantitative characters such as the size and shape of the floral organ, tend to vary continuously among individuals within populations and can arise from both environmental and genetic factors. Since plant cells do not migrate during development, variations in size and shape of the floral organs is probably the result of variations in patterns of cell division and cell elongation among genotypes. In addition, development of the floral organs was largely controlled by homeotic genes which also controlled organ identity by regulating the appropriate differentiation of organs and specific tissues, (Nakayama et al., 2005).

Environmental effects also contributed variations among sepals and petals of the Vanda varieties as shown by the result of the asymmetrical analysis. The contribution of environmental factors to variations can be divided into levels ; general environmental variation which arises from permanent or non-localized circumstances such as nutritional and climatic factors and special environmental variation which arises from temporary or localized circumstances operating during development (Falconer and Mackay, 1996; Ukai 2002).

The one way ANOVA of the principal component scores was successful in detecting independent significant characteristics that vary significantly among the orchids.

In conclusion, EFD and PCA can be effectively used in analyzing the shape variations of sepals and petals of Vanda orchids quantitatively. It is also an effective method used in the study of variations among Vanda varieties by using the sepals and petals of the orchid flower. In the study of variation among Vanda, the use of sepals are better than that of petals and among sepals it is best to use the left and right sepals for they can discriminate better the variations among different Vanda varieties.

References Cited

Falconer, DS and Mackay TFC. 1996. Introduction to quantitative genetics 4th edn. Essex, UK: Longman.

Freeman, H. 1974. Computer processing of line drawing images. Computing Surveys. 6:57-97.

Furuta, N, S. Nnomiya, S. Takahashi, H. Ohmori and Y. Ukai. 1995. Quantitative evaluation of soybean (*Lysine max L.., Merr*) leaflet shape by principal component scores based on elliptic fourier descriptor. Breeding Science 45 : 315-320.

Iwata, Hiroyoshi, Satoshi Niikura, Seiji Matsuura, Yasushi Takano and Yasuo Ukai. 2004. Genetic control of root shape at different growth stages in radish (*Raphanus sativus L.*). Breeding Science 54:117-124.

Iwata, Hiroyoski, Hirohisa Nesumi, Seishi Ninomiya, Yasuishi Takano and Yasuo Ukai. 2002. Diallel analysis of leaf shape variations of citrus varieties based on elliptic fourier descriptors. Breeding Science 52: 89-94.

Iwata, Hiroyoski, Hirohisa Nesumi, Seishi Ninomiya, Yasushi Takano and Yasuo Ukai. 2002. The evaluation of genotype x environment interactions of citrus leaf morphology using image analysis and elliptic fourier descriptors. Breeding Science 52:243-251.

Iwata, H and Y. Ukai 2002. SHAPE: A computer program package for quantitative evaluation of biological shapes based on elliptic fourier descriptors. Journal of Heredity 93:384-385.

Iwata, H., S. Niikura, S. Matsau, Y. Takano and Y. Ukai. 1998. Evaluation of variation of root shape of Japanese radish (Ramphanus sativus L.) based on image analysis using elliptic fourier descriptors. Euphytica 102:143-149.

Kuhl, FP. and C.R. Giardina. 1982. Elliptic fourier features of a closed contour . computer graphics and image processing 18:236-258.

Meyerowitz, E. M., 1997 Genetic control of cell division patterns in developing plants. Cell 88:299-308.

Nakayama, Naomi, Juana M. Arroyo, Joseph Simorowski, Bruce May, Robert Martienssen and Vivian F. Irish. 2005. Gene trap lines defining domains of gene regulation in *Arabidopsis* petals and stamens. Plant Cell 17 (9): 2486- 2506.

Ohsawa, Ryo, Tadahiro Tsutsumi, Hideyuki Uehara, Hyoji Namai and Seishi Ninomiya. 1998. Quantitative evaluation of common buckwheat (*Fagopyrum esculentum Moench*) kernel shape by elliptic fourier descriptor. Euphytica 101 (2): 175-183.

Rohlf FJ. and Archie JW. 1984. A comparison of fourier methods for the description of wing shape in mosquitos (*Ritera culicidae.)* Systematic Zoology 33:322-317

Soon, Teoh Eng. 1989. Orchids of Asia. Times Book International. Times Center, Singapore.

Toyohara, H, K.Irie, W Ding, H. Iwata, H. Fujimaki, F. Kikuchi and Y. Ukai. 2000. Evaluation of the tuber shape of Yam *(Dioscoreaalla L..)* cultivars by image analysis and elliptic fourier descriptors. SABRAO Journal of Breeding and Genetics, 32:31-37.

Ukai, Y. 2002. Genetic analysis of quantitative traits. Tokyo: Igaku-shuppan Inc. 15-47.

Yoshioka, Yosuki, Hiroyoshi Iwata, Ryo Oshawa and Seiehi Ninomiya.2004. Analysis of petal shape variation of *Primula seiboldii* by elliptic fourier descriptors and principal component analysis. Annals of Botany 94: 657-664.