Comparative Use of Meristic and Geomorphometric Analyses of Vegetative and Floral Characters in Studying Intraspecific Variation in *Portulaca grandiflora* (Hook)

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ABSTRACT

Meristic and geometric morphometric analyses were applied to 150 *Portulaca grandiflora* (Hook) specimens in order to examine shape variations in vegetative and floral characters between and among the morphotypes of the species. Canonical Variate Analysis of both meristic and geomorphometric data show significant ($p<0.05$ and $p<0.01$, respectively) variation of leaf shape, specifically that of the petiole shape, between "single" flowered and "double" flowered varieties. Plants with single flowers possess leaves that are generally wider, especially toward the apex, while those of plants with double flowers are narrower. Differences were also observed in the size and shape of the petiole such that "double" flowered varieties had a more distinct petiole compared to the "single" flowered varieties. Petal shape was also significantly variable between the two varieties. Principal Component Analysis of both meristic and geomorphometric data also show significant variation within the individuals which may explain the occurrence of the different morphotypes of the plant. Cluster Analysis using meristic data again showed separation between "single" flowered and "double" flowered varieties and further separation between petal colors. In general, meristic and geomorphometric data showed comparable results. Description of shape variation in the vegetative and floral characters studied was consistent in the different analyses used.

INTRODUCTION

Morphometrics is a tool in which morphological characteristics are transformed into generalized shapes and quantitative data so that variations in and among biological structures can be compared without the bias of an experimenter. The goal of morphometrics is to illustrate patterns of variation in morphological shape so that they may be available for analysis (Macleod, 1991). Traditional methods of morphometrics make use of meristis data, i.e., linear measurements, angles and ratios. Several problems however are encountered in traditional morphometrics. First, it is very time consuming and usually requires killing a specimen. Second, the question of actual shape variation is being asked since variables obtained in traditional morphometrics are non-indicative of the shape (Zelditch *et al*., 2004). A relatively new type of morphometric analysis is geometric morphometrics (GM). It tries to solve problems of size and proportion that are encountered in traditional morphometrics by using landmark-based analysis. It makes use of landmarks so that topological analysis of the specimen may be studied and geometric relationships can be determined. True landmarks are usually chosen based on homological structures.

Portulaca grandiflora (Hook), commonly known as rose-moss, moss-rose, purslane or portulaca, is a widely cultivated species all over the world. The plant usually self-pollinates instead of relying on insect-mediation or cross pollination. Although this genus has been studied for seed variations (Matthews et al., 1994), no extensive studies have been done about its other morphological features. Moreover, no studies have been found to correlate the different species and variants of this genus using molecular data. Since molecular studies are relatively expensive to conduct, geomorphometrics offer a relatively cheaper and faster kind of analysis that may aid in the proper taxonomic treatment of this highly polymorphic species (Mereda et al., 2008).

This study determined patterns of variation within the different morphotypes of *P. grandiflora* by measuring significantly chosen vegetative and floral characteristics and compared the usefulness of meristic and geometric morphometric analyses in order to differentiate the morphotypes from each other.

MATERIALS AND METHODS

Collection of Samples

Five morphotypes of *P. grandiflora*, namely, Double Orange, Single Pink, Double Pink, Double Pink-White and Single Yellow, were obtained from a single site in Bicol, Philippines to eliminate variations due to ecological conditions. Thirty individuals were sampled for each morphotype.

Data Processing and Digitization

A total of sixteen (16) vegetative and floral characters were collected for each individual in triplicate measurements using a Vernier calliper or count method. The six (6) vegetative and ten (10) floral characters considered were: stem width of base, stem width of first leaf, internode between first and second leaf, maximum leaf length, maximum leaf width, petiole length, number of inflorescence, number of petals, maximum petal length, maximum petal width, number of sepals, maximum sepal length, maximum sepal width, floral diameter, pedicel length, and number of stamens, These characters were chosen based on the study of Mereda and co-workers (2008) which also involved intraspecific variation in a single species.

The first leaf, a sepal and the first petal behind the sepal were also collected for each individual. These were photographed using either a Nikon D60 or scanned using a Brother DCP-165C Scanner and the digitized specimens were assigned with landmarks using tpsDig2 (by F.J. Rohlf, available at http://life.bio.sunysb.edu/morph/).

For the leaves, ten (10) landmarks were assigned as seen in Figure 1. The landmarks used were based on Type I or homologous landmarks (2, 5, 8 and 10) of Jensen and co-workers (2002) and were added with

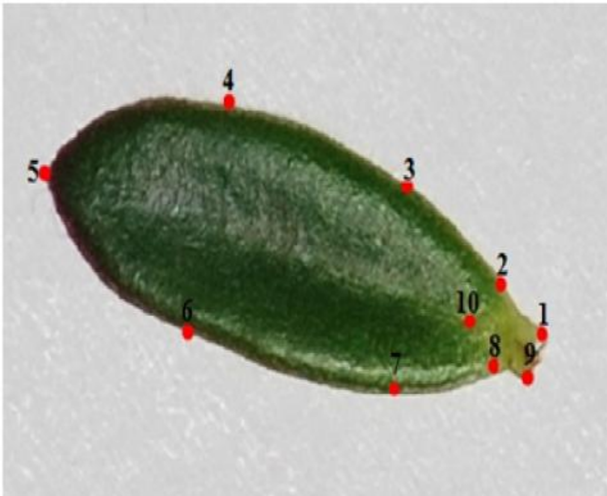

Figure 1. A sample leaf from a plant of single pink morphotype with ten (10) landmarks.

Type II or pseudo landmarks (1, 3, 4, 6, 7, 9) which show maximum curvatures of the leaf (Volkova, *et al*., 2005).

For the sepals, four (4) landmarks were assigned as seen in Figure 2. The following landmarks used were Type II. Landmarks 1, 2 and 3 are the bases of the primary parallel veins while landmark 4 shows the apex of the sepal.

Figure 2. A sample sepal from a plant of single pink morphotype with four (4) landmarks.

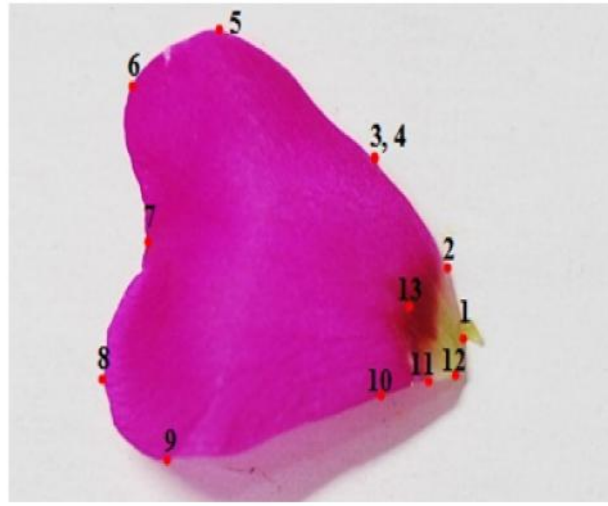

Figure 3. A sample petal from a plant of single pink morphotype with thirteen (13) landmarks.

For the petals, thirteen (13) landmarks were assigned as seen in Figure 3. The following landmarks used show the maximum curvatures of the petal.

The tps files produced for each specimen were processed in Coordgen6f to apply Generalized Procrustes Analysis (GPA) which was needed to eliminate differences in shape, size and orientation, maintaining only shape differences (IMP, by H.D. Sheets, available at http://www2.canisius.edu/~sheets/ morphsoft.html).

Data Analyses

Ordination analysis was done to examine shape variations. Landmark-based analysis made use of CVA-MANOVA (Canonical Variate Analysis-Multivariate Analysis of Variance) and PCA (Principal Component Analysis) using CVAGEN61 and PCAGen6n, respectively (IMP, by H.D. Sheets, available at http://www2.canisius.edu/~sheets/ morphosoft.html). Ordination analysis of traditional continuous data made use of CVA-MANOVA followed up by Discriminant Analysis and PCA using the SPSS 19 Software. Clustering was also done for traditional continuous data using Hierarchical Clustering in SPSS 19 Software.

RESULTS AND DISCUSSION

Five (5) different morphotypes of *P. grandiflora* considered in the study were: Double Orange, Single Pink, Double Pink, Double Pink-White and Single Yellow, as seen in Figure 4. Thirty (30) individuals for each morphotype and an added Double Pink individual (for GM Analysis) were obtained amounting to one hundred fifty (150) individuals all in all. Each individual was measured for sixteen (16) vegetative and floral meristic and nominal characters.

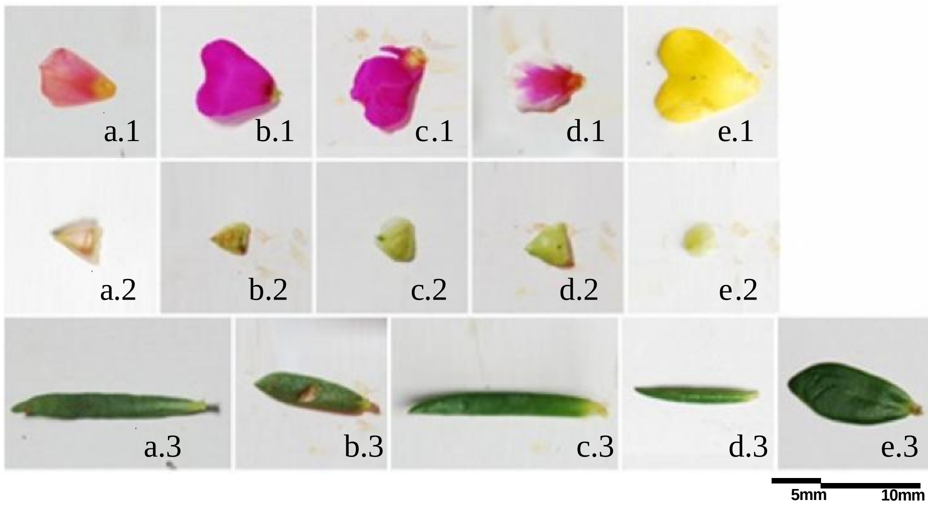

Figure 4. Samples of each morphotype of *P. grandiflora* (Hook).
(a.1.) Double Orange Petal, (b.1.) Single Pink Petal (c.1.), Double Pink Petal (d.1.), Double Pink-White Petal (e.1.), Single Yellow Petal (a.2.), Double Orange Sepal (b.2.), Single Pink Sepal (c.2.), Double Pink Sepal (d.2.), Double Pink-White Sepal (e.2.), Single Yellow Sepal (a.3.), Double Orange Leaf (b.3.), Single Pink Leaf (c.3.), Double Pink Leaf (d.3.), Double Pink-White Leaf (e.3.), Single Yellow Leaf.

Shape Variation Between Different Morphotypes of *Portulaca grandiflora*

Multivariate analysis was applied to the meristic data obtained through measurement of vegetative and floral characters. Variables were first transformed using natural logarithm or through ratios since initial data as enumerated were not normalized. Also, variables which were not normalized through such transformations were discarded. Multivariate analysis using Wilk's lambda ($p<0.05$) showed that differences within the five groups were significant.

Follow-up analysis was done through discriminant analysis. Four (4) eigenvalues were generated from this analysis with only the first eigenvalue considered significant accounting for 67.6% of the observed variance while the next eigenvalue only accounted for 25.9% of total variation. Looking at the first distinct CV, difference seen in the multivariate analysis was accounted for by petiole length. Leaf ratio and petiole length gave the most contribution in the observed variations between groups. Eigenvectors of petiole length and floral diameter are 1.149 and 0.205, respectively, while absolute pooled correlations are 0.817 and 0.146, respectively, for each variable. A plot of the first versus the second canonical variant in Figure 5 also showed that "double" varieties gravitate toward the negative side of canonical variate 1 while "single" varieties clump together toward the positive side of canonical variate 1. No distinct separation can be made between groups based on canonical variate 2, as expected.

Canonical Variate Analysis-Multivariate Analysis of Variance was applied to the landmarks generated for all 151 individual samples to observe shape variation in the leaves, petals and sepals of the five

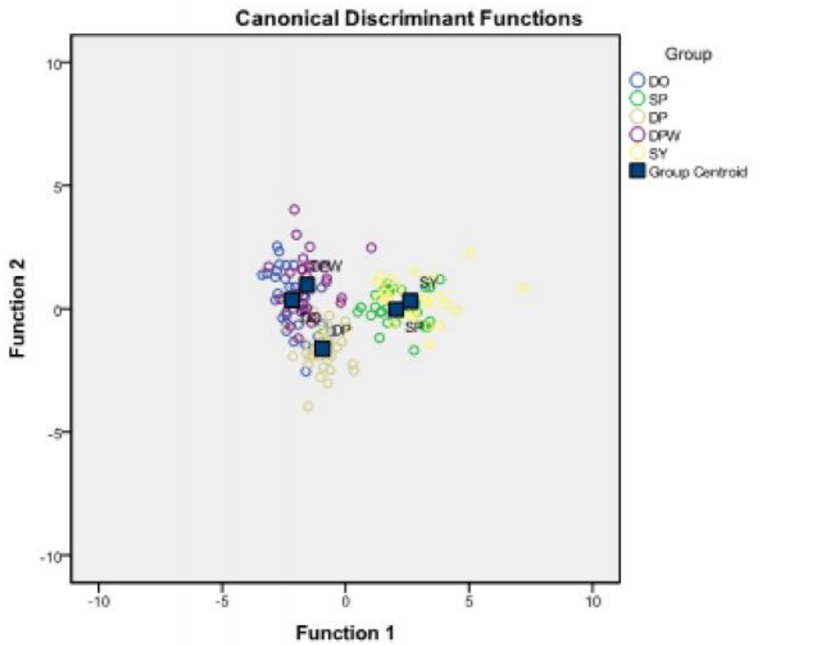

Figure 5. Plot of First Canonical Discriminant Function vs Second Canonical Discriminant Function of each individual from five (5) *P. grandiflora* morphotypes.

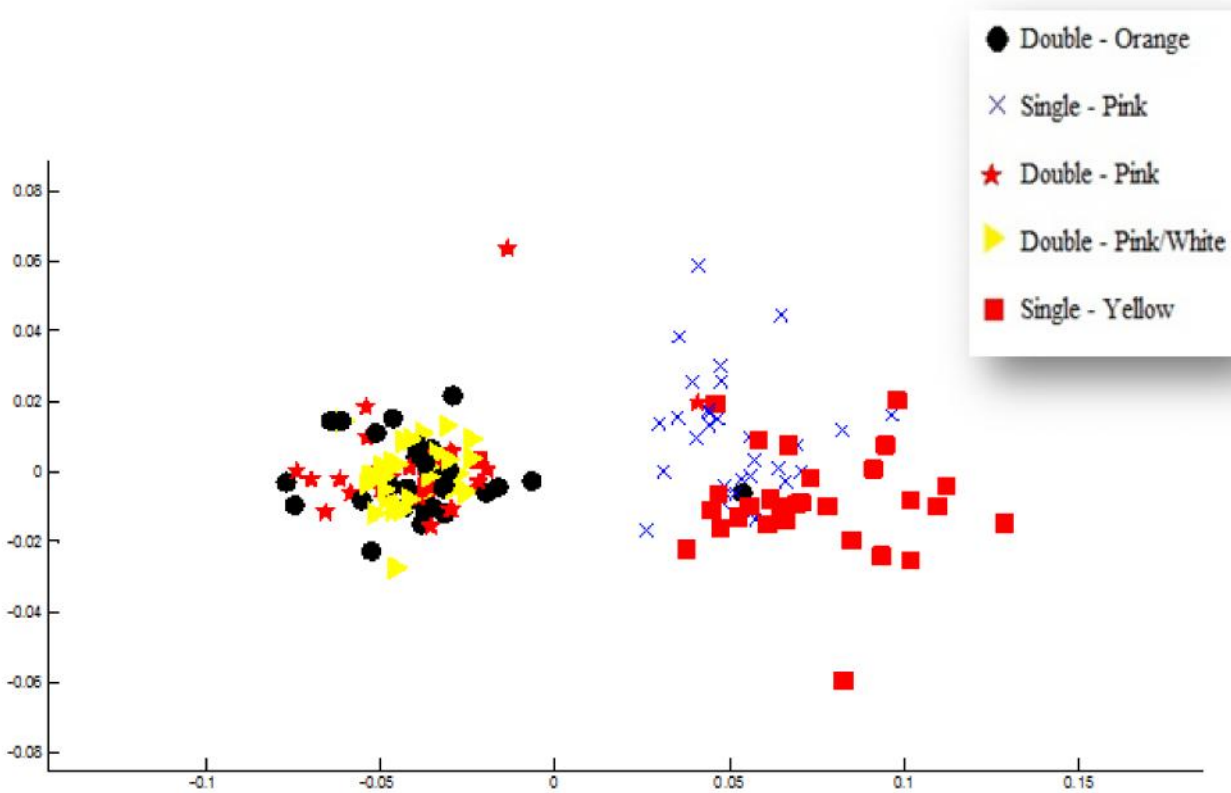

Figure 6. Canonical Variance Analysis on shape of the leaves of five (5) morphotypes (30 Double Orange, 30 Single Pink, 31 Double Pink, 30 Double Pink-White and 30 Single Yellow) of one hundred fifty-one (151) individuals of *P. grandiflora.*

morphotypes. In comparing leaf shape of all five morphotypes, one (1) distinct canonical variate (CV) was generated by the CVA program. A p-value of 2.22 x 10^{-16} (p<0.001) shows that there is a significant difference in shape between all five morphotypes. Figure 6 shows two (2) distinct groups formed, that of the "single" flower varieties, when plotting the first two canonical variates of all individuals. Generated procrustes deformation grids seen in Figure 9a showed that variation is due to differences in shape toward the petiole area.

Variation in petal shape can also be observed when comparing all five morphotypes, giving two (2) distinct canonical variates with the first CV giving a p-value of 2.62 x 10^{-7}. Figure 7 shows the CV1 vs CV2 plot for petal shape variation but does not exhibit distinct groupings as with the leaves despite significance of p-value. Overlapping between the groups can be seen. The procrustes deformation grids (Figure 10a) that were generated also showed that variation can be accounted for by differences in shape toward the basal and laminar area.

Shape analysis for sepals generated one (1) distinct CV but did not show significant shape variation between the five morphotypes with a p-value of 1.26 x 10^{-3}. This can also be seen in Figure 8 wherein the CV1 vs CV2 plot showed severe overlapping between members of the groups. Furthermore, no procrustes deformation grid could be generated for sepal shape.

When comparing shape variance between leaves of "single" flowered and "double" flowered varieties, results showed that there is a significant difference in the leaf shape between the two groups (p-value = 2.22 x 10^{-16}). Shape variation can also be accounted for by differences in petiole shape as seen in Figure 9b.

Variance in petal shape was also found to be significant (p-value = 2.29 x 10^{-5}) when comparing between

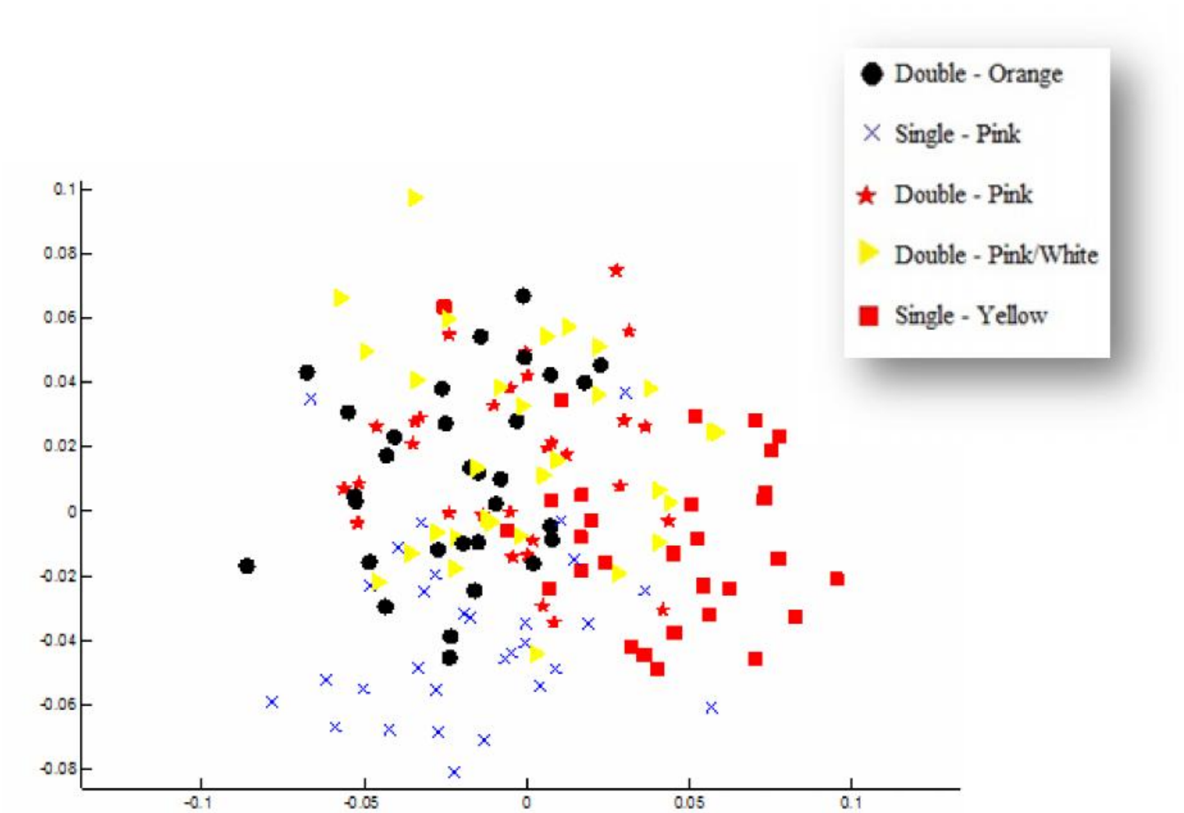

Figure 7. Canonical Variance Analysis on shape of the petals of five (5) morphotypes (30 Double Orange, 30 Single Pink, 31 Double Pink, 30 Double Pink-White and 30 Single Yellow) of 1one hundred fifty-one (151) individuals of *P. grandiflora.*

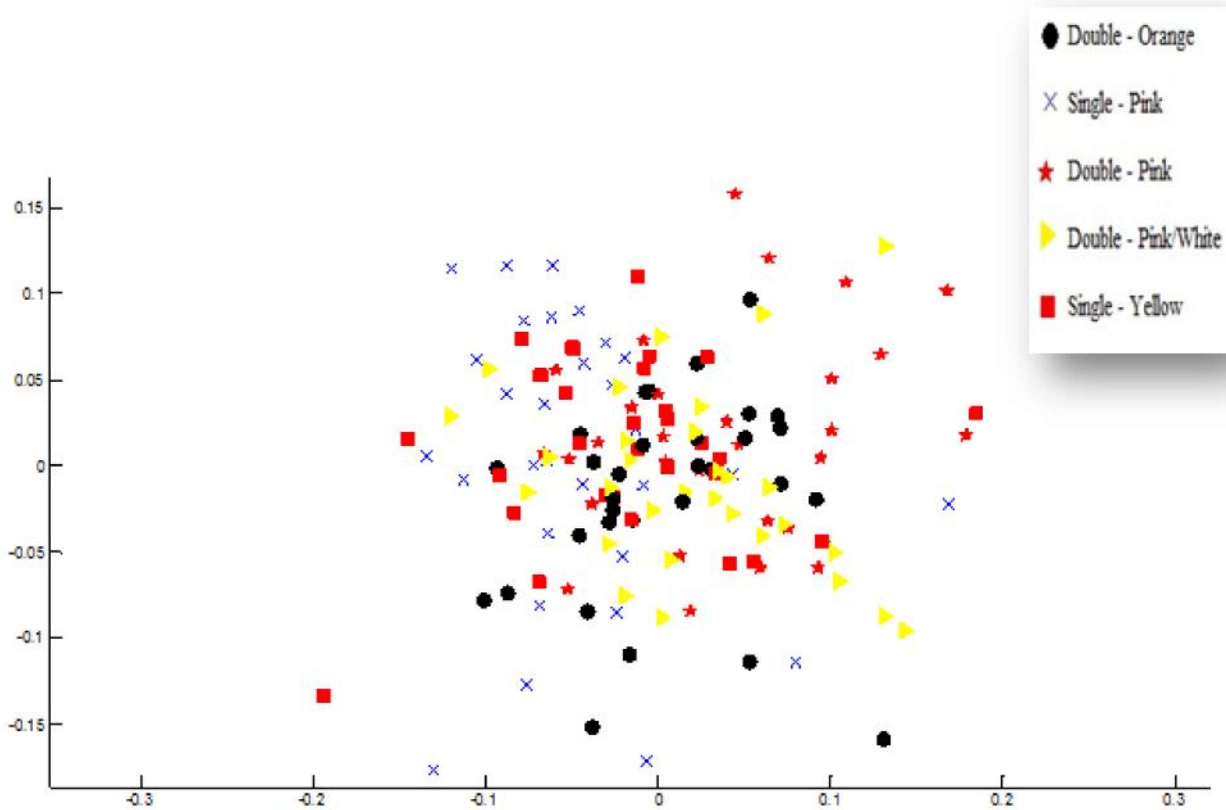

Figure 8. Canonical Variance Analysis on shape of the sepals of five (5) morphotypes (30 Double Orange, 30 Single Pink, 31 Double Pink, 30 Double Pink-White and 30 Single Yellow) of one hundred fifty-one (151) individuals of *P. grandiflora*.

"single" flowered and "double" flowered varieties with most of the variation coming from the basal and laminar area.

In comparing sepals of "single" flowered and "double" flowered varieties, a significant shape variation can be seen. CVA produced one distinct CV with p-value of 1.03 x 10^{-4} but no deformation procrustes grid can be produced.

Canonical variate analysis using both meristic continuous data and land-mark based data agree that there is a significant difference between the morphotypes most especially between "single" flowered varieties and "double" flowered varieties. Using traditional data, a follow-up discriminant analysis showed that there is one distinct eigenvalue accounting for most of the observed variation to petiole length followed by floral diameter. Land-mark based data also accounted for most of the observed variation to petiole length as shown by procrustes deformation grids of leaf land-marks. Significant deformation toward the basal end could also be observed in grids produced by petal land-marks while no deformation grid could be made for sepals. In land-mark based CVA, comparison of "single" and "double" flowered varieties was also made which only reaffirmed clusters made by comparison of the five different morphotypes. "Single" flowered variants formed into one cluster towards the positive end of the first CV axis while "double" flowered variants formed another cluster toward the negative end in terms of leaf shape. Shape variation in petals was accounted for by differences in the basal end and sepals did not show any significant distinction between the two groups.

The results of the different analyses clearly showed that the "single" flowered and "double" flowered variants are distinct from each other. Leaf size decreases as the number of petals increase in the floral whorl such that "single" flowered varieties showed leaves that are wider and "double" flowered varieties possessed leaves that are narrower. This agrees with the suggestion of Ashman and Majetic (2006) that there is a possible correlation in the genetic determination of floral and vegetative morphotypes, especially in actinomorphic (radially symmetric) flowers.

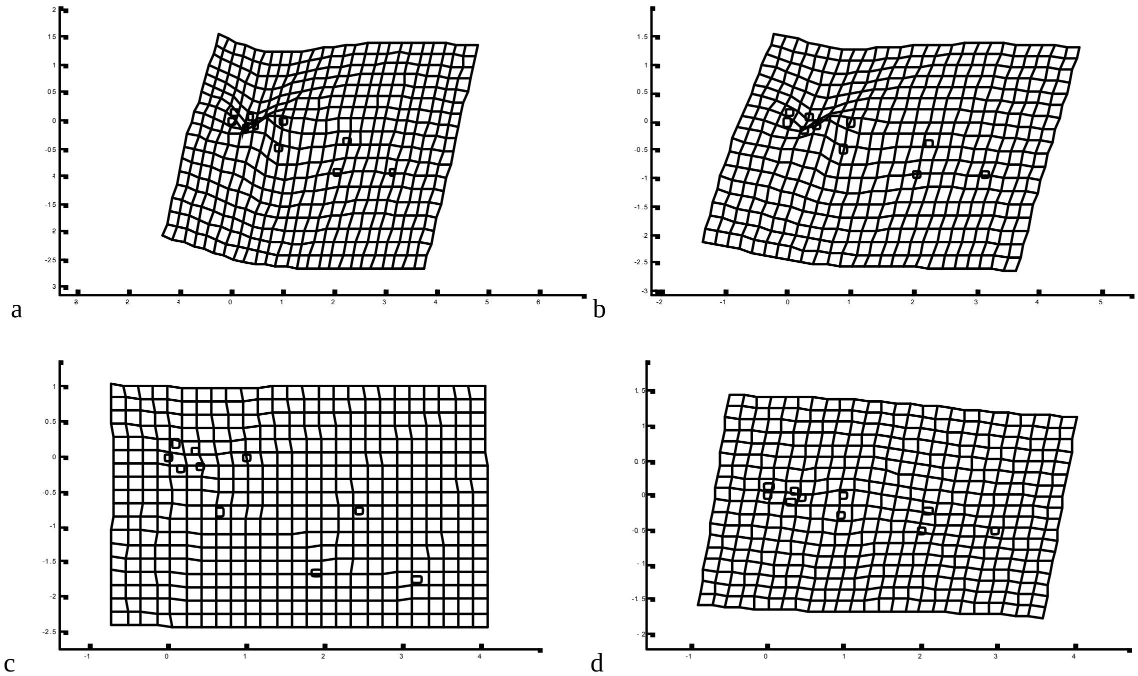

Figure 9. Procrustes deformation grids from Canonical Variate Analysis of leaf shape. (a) five morphotypes, (b) single and double varieties ,(c) single varieties, (d) double varieties

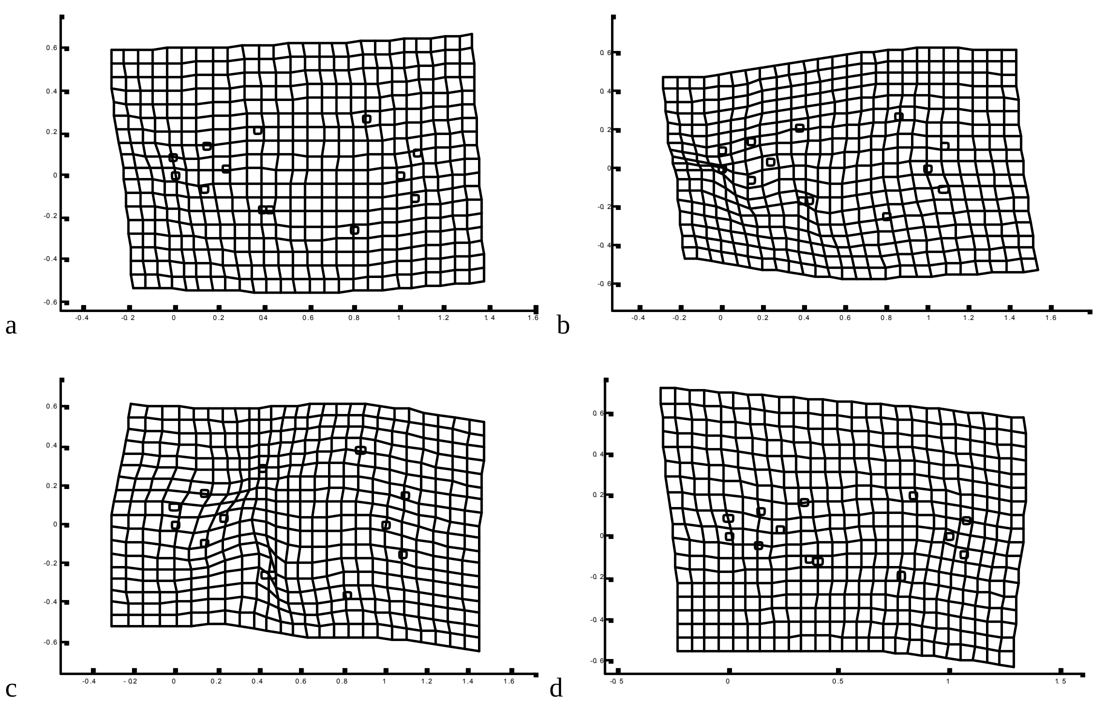

Figure 10. Procrustes deformation grids from Canonical Variate Analysis of petal shape. (a) five morphotypes, (b) single and double varieties, (c) single varieties, (d) double varieties

Shape Variation Within the Species of *Portulaca grandiflora*

Principal Component Analysis of merisitc data showed distinct variation within groups with Bartlett's test for spherecity having a significant value of 0.00. Factor extraction produced three distinct principal components cumulatively explain 60.63% of total variance. All variables included showed contribution to the first component, with leaf width to width length followed by petiole length having the most absolute contribution.

Principal Component Analysis of landmark-based specimen using the leaf as a factor in Figure 11 showed distinction between the "single" flowered and "double" flowered varieties with the "single" varieties concentrated towards the positive end while the "double" varieties concentrated toward the negative end. No further distinction can be made within the two groups formed. One distinct eigenvalue was generated which had a value of 2.32 x 10^{-5} showing distinct variation within the groups. A procrustes deformation grid in Figure 12 show variance is accounted for by shape difference in petiole area. Using petal as a factor in Figure 13, most of the individuals grouped together toward the positive end of principal component 1. However, several members from each of the five morphotypes deviated from the group and clumped together toward the negative end of principal component 1. Two distinct eigenvalues were generated having a value of 0.00133 and 0.00153, respectively, both of which suggest distinct variation within the groups. The generated procrustes deformation grid in Figure 12 accounts for variation from all sides of the petal.

Principal Component Analysis on sepals produced one distinct eigenvalue of 0.002526 showing distinct variation within groups as well. The principal component plot in Figure 15 shows completely scattered individuals. No deformation grid was generated for this analysis.

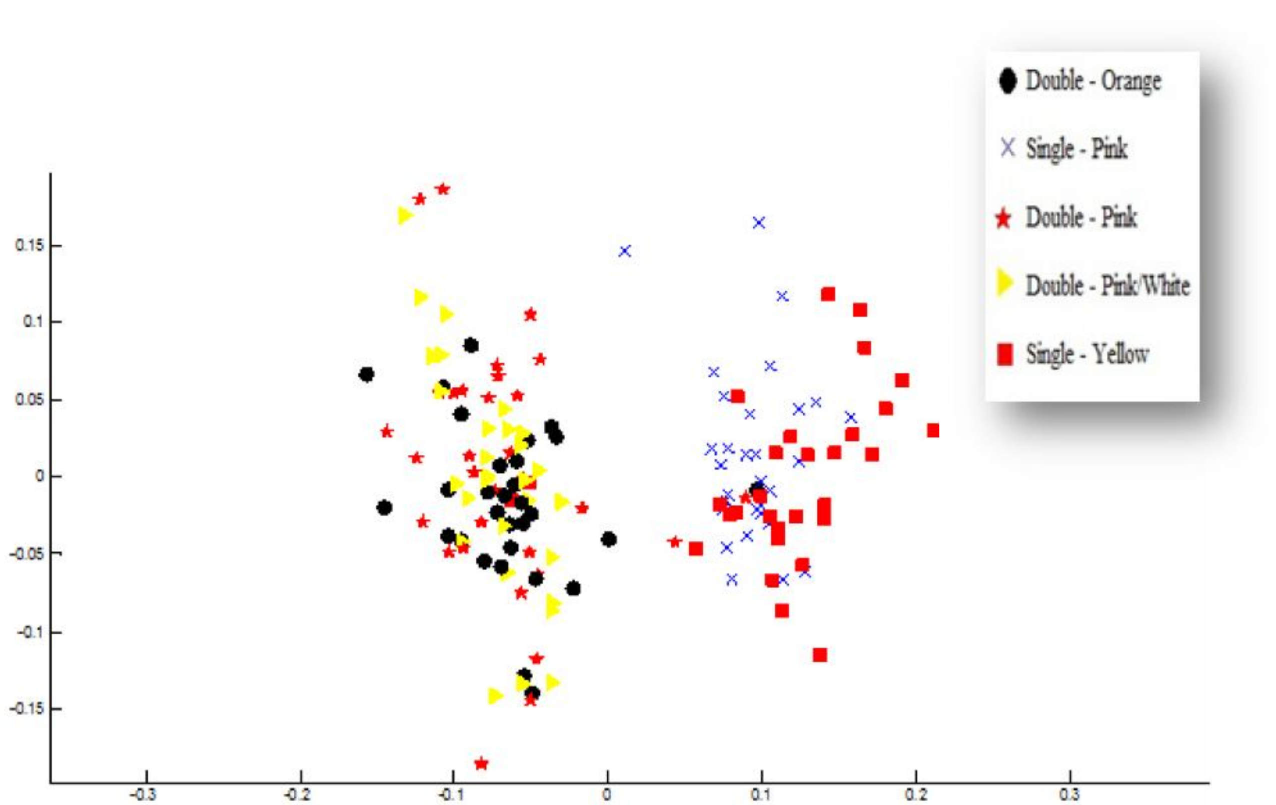

Figure 11. Principal Component Analysis on leaf shape of 5 morphotypes. (30 Double Orange, 30 Single Pink, 31 Double Pink, 30 Double Pink-White and 30 Single Yellow) of one hundred fifty-one (151) individuals of *P. grandiflora.*

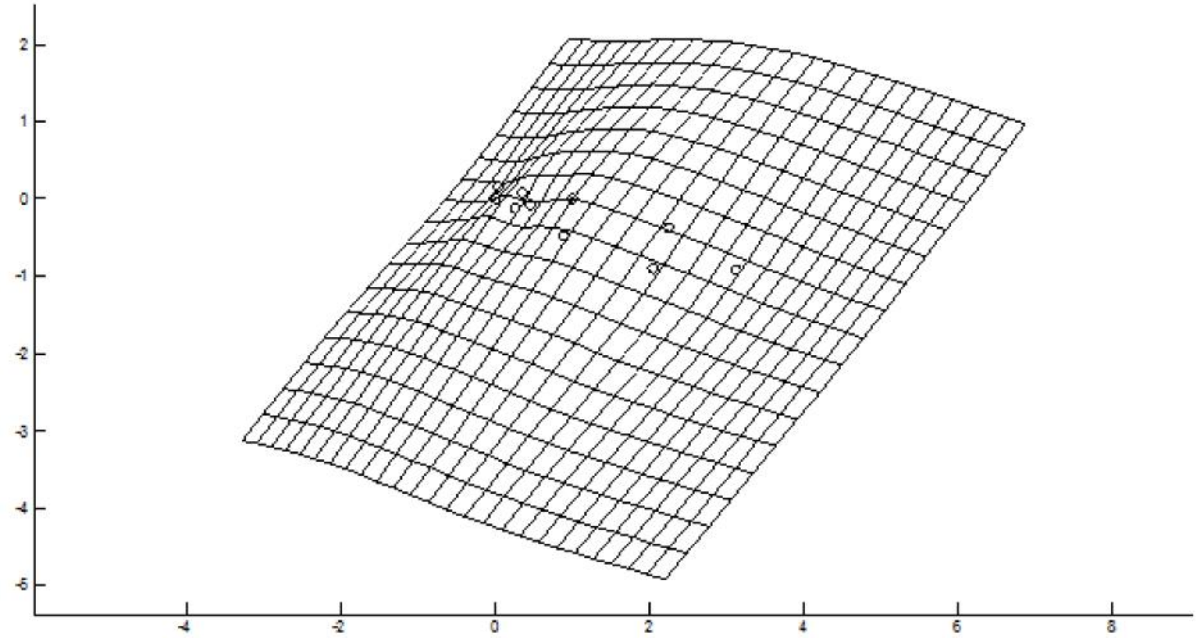

Figure 12. Procrustes deformation grids from Principal Component Analysis on leaf shape of 5 morphotypes (30 Double Orange, 30 Single Pink, 31 Double Pink, 30 Double Pink-White and 30 Single Yellow) of one hundred fifty-one (151) individuals of *P. grandiflora.*

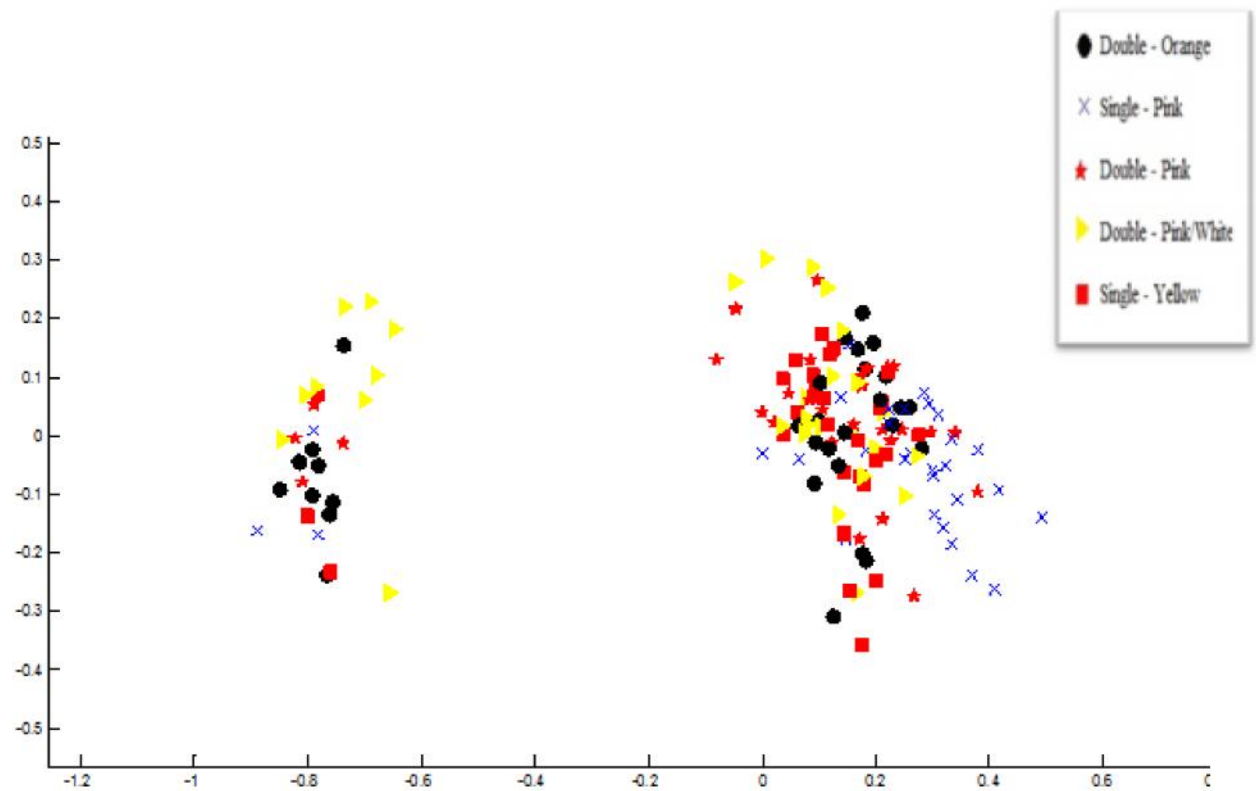

Figure 13. Principal Component Analysis on petal shape of 5 morphotypes. (30 Double Orange, 30 Single Pink, 31 Double Pink, 30 Double Pink-White and 30 Single Yellow) of one hundred fifty-one (151) individuals of *P. grandiflora.*

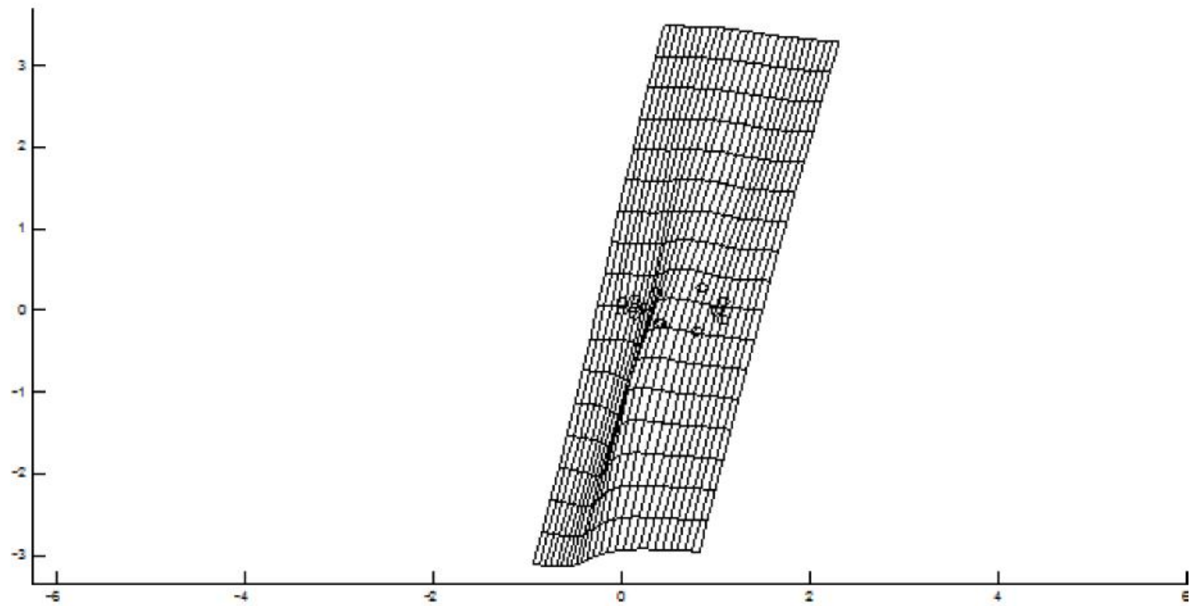

Figure 14. Procrustes deformation grids from Principal Component Analysis on petal shape of 5 morphotypes. (30 Double Orange, 30 Single Pink, 31 Double Pink, 30 Double Pink-White and 30 Single Yellow) of one hundred fifty-one (151) individuals of *P. grandiflora.*

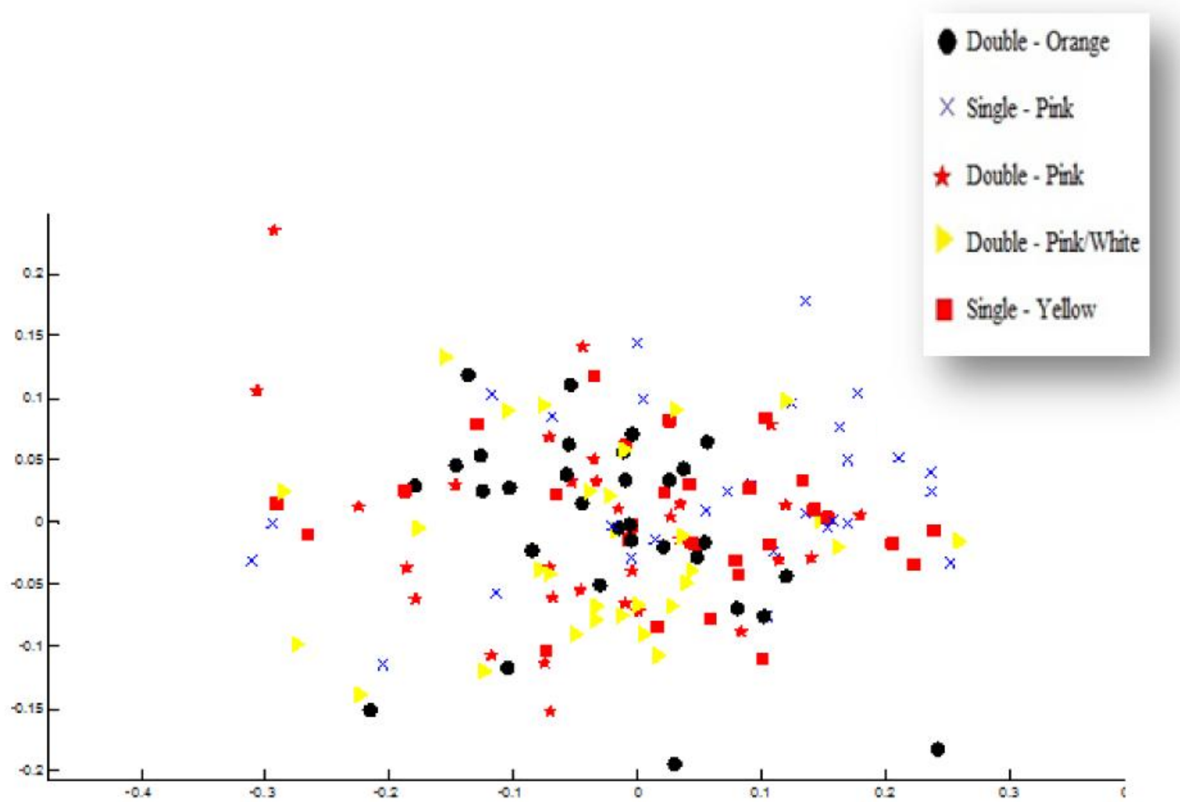

Figure 15. Principal Component Analysis on sepal shape of 5 morphotypes. (30 Double Orange, 30 Single Pink, 31 Double Pink, 30 Double Pink-White and 30 Single Yellow) of one hundred fifty-one (151) individuals of *P. grandiflora.*

Principal Component Analysis of both traditional meristic data and geometric landmark-based data both showed significant variation within the groups. Variation at the individual level is an important factor which can explain the high level of morphological variability between morphotypes as variation should first be present within the population before other selective forces such as pollinator preferences and other environmental forces can act on shaping the species (Galen, 1999). However, results of PCA do not fully agree with CVA as expected. Continuous data showed that the first principal component explains only 26.71% of variance and this variance could be accounted primarily to the ratio of leaf width to leaf length rather than petiole length.

In landmark-based data, leaf landmarks still show that variation is due to differences in petiole shape of "single" and "double" flowered varieties. Petal landmarks, on the other hand, show variation not only between morphotypes but among its members and the procrustes deformation grid accounts this to differences in the basal end of the petal as well as the margins of the petal. Still, shape variation in sepal landmarks among the individuals cannot be clearly seen.

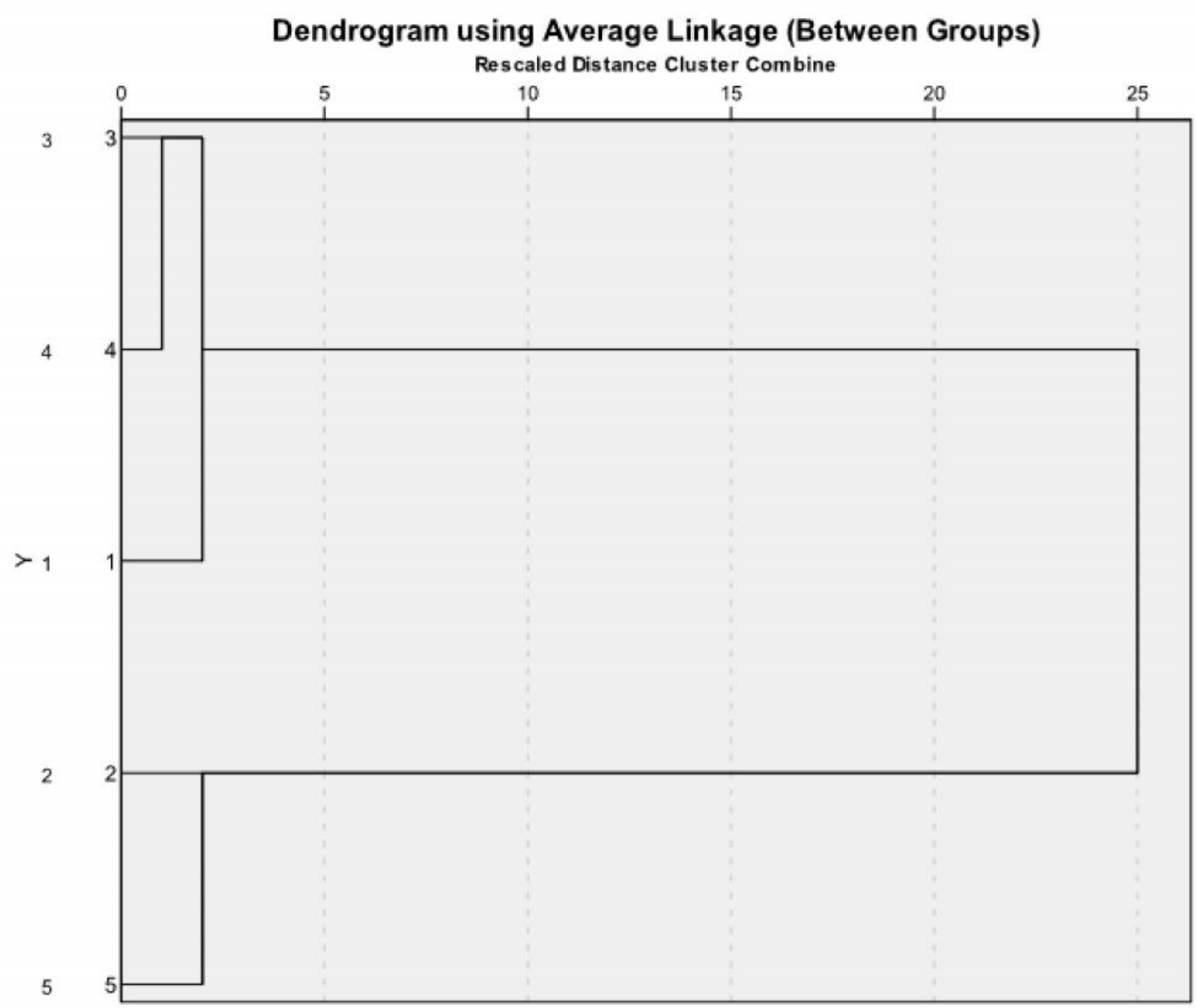

Figure 16. Dendrogram using Squared Euclidean Distances of Between Groups using meristic data of one hundred fifty-one (150) individuals of *P. grandiflora* (1: Double Orange 2: Single Pink 3: Double Pink 4: Double Pink/White 5: Single Yellow).

Cluster Analysis

Hierarchical cluster analysis using the means of each variable clearly showed that there is a separation between the "single" and "double" flowered plants with the two groups having a distance of 1.019. The two "single" flowered varieties were further separated having a distance of 0.136 between the two groups. Between the three "double" flowered varieties, Double-Orange was first separated from the group having a distance of 0.135 between the other two "double" varieties. Double-Pink and Double-Pink/ White had the least distance of 0.072 among all groups. A dendrogram of the analysis can be seen in Figure 16.

Although cluster analysis results should always be interpreted with caution due to the fact that there is a hierarchical assignment given to each group, it should be noted that results of the cluster analysis reiterate the fact that there is distinction between the "single" and "double" flowered varieties. Separation between the different floral colors could be due to the fact that individuals were assigned in such grouping and hierarchy was given to each group since earlier analyses were not able to discriminate between such factors. It is of interest however that between "double" flowered varieties, pink and pink/white varieties were closer to each other than that of the orange variety.

CONCLUSION

Although continuous data from traditional methods did not necessarily coincide with landmark data from geometric morphometrics, it is noteworthy to mention that results were fairly comparable across analyses. The "single" flowered varieties could be clearly distinguished from the "double" flowered varieties using Canonical Variate Analysis, Principal Component Analysis and Cluster Analysis. Given more time and resources, DNA studies can be done in order to correlate the data obtained with molecular genetic variation between and within morphotypes of this plant of interest.

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