

# Digital Morphometrics: A Tool for Leaf Morpho-Taxonomical Studies

Kavi K. Oza, Rinku J. Desai, Vinay M. Raole



**Abstract:** Leaves are most important part of the plant and can be used for the identification of a taxon. An appropriate understanding of leaf development in terms of shape and responsible abiotic factors is necessary for improvement in plant. Leaf shape variation could be evaluated successfully, and the symmetrical and asymmetrical elements of the overall shape variation could be detected. The aim of the present study was to establish a quantitative analysis method of leaf shape by elliptic Fourier descriptors and principal component analysis (EF-PCA). EF-PCA describes an overall shape mathematically by transforming coordinate information concerning its contours into elliptic Fourier descriptors (EFDs) and summarizing the EFDs by principal component analysis. We can be able to extract six variables by using leaf specimen images from field and herbarium specimens. In the present study, total leaf area with respect to notch area is more variable within species. Within a species the major source of the symmetrical elements may be governed by genotypic features and the asymmetrical elements are strongly affected by the environment. We could discuss the value of morphometrics to detect subtle morphological variation which may be undetectable by human eye.

**Keywords:** Convolvulaceae, EFA, Morphometrics, PCA, SHAPE.

## I. INTRODUCTION

As a beginning, we offer a quotation from MacLeod (2002): "The fundamental observation of biology is morphology. Morphological data form the basis of virtually all systematic descriptions". That this is so reinforced by the fact that most systematic studies begin by grouping organisms based on morphological similarity. Angiosperm leaves are the prime photosynthetic organ of the plant and tremendously inconsistent in their form. The variation in leaf morphology suggests the multifaceted role of genetics, development, and environment. In addition to genetic and developmental effects, role of the abiotic and biotic factors also influences leaf shape. For example, at higher temperature fluctuation plants show higher incidence of leaf shape plasticity (Little et al. 2010), and colder climates can be associated with more highly dissected leaf margins (Royer et al. 2009) To study the development of different

leaf forms and shapes among closely related species, well-preserved morphological features can take into considerations, which are not clearly noticeable in the mature form. Morphometrics, the measurement (metron) of shape (morphé), is a subfield of statistics with history going back to very beginning of this discipline. One of the pioneers who worked on shape analysis is by Bookstein (1991),

who described it, as "the existence of a new specialty: morphometrics, the biometry of shape". The term geometric morphometrics suggested by Marcus & Corti (1996) used "geometric" as a necessary modifier to differentiate the new from the old, wherein basis is the identification of a set of landmarks and its origin. In geometric morphometrics, analysis is based on the anatomical landmarks expressed as a coordinate to quantify the shape and size (Slice et al., 1996).

Leaf morphology is central to plant taxonomy and systematics and it has mostly been studied using traditional morphometrics. In the last decade, however, there has been an increasing interest in the use of modern geometric morphometrics (GMM) to study the form of leaves. Leaves of plant taxa are particularly suitable to landmark based geometric morphometrics. Yet, most plant scientists do not have taken advantage of this set of methods in their morpho-taxonomic studies even at population level (Viscosi and Cardini, 2011). Genetics and morphometrics can fruitfully interact as complementary tools to understand the origin of phenotypic differences within the population and biogeographical variations which can further be used taxonomy and in plant developmental biology (Klingenberg, 2010).

In recent years, methods of morphometric analysis have undergone significant revolution due to various computer software and changed from multivariate to geometric morphometrics (Adams et al., 2004). In these methods, landmark data and shape functions are also fitted to curves or surfaces are now routinely used by researchers i.e. Elliptical Fourier Analysis (Viscosi&Fortini, 2011; Iwata, 2002; Jensen et al., 2002). Elliptical Fourier Analysis (EFA) represent an accurate method for outline characterization and able to capture outline information very proficiently (Kuhl and Giardina, 1982; Lestrel, 1997; Jensen, 2003).

Hickey (1973) has described various shapes of leaves in detail to employ it for taxonomy. Until now such shape descriptors are used largely for qualitative comparisons only. However, qualitative descriptors do not provide for an unambiguous definition of such outlines. Simple quantitative descriptors, such as ratios calculated from linear dimensions

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(length, width, thickness, etc.) discriminate poorly between outward appearances. Therefore, in this study, we are trying to assess accurate desired leaf characters in vegetative state. Variability in leaf morphology has led to the use of suites of leaf measures for studies of inter- and intra-generic variation.

The objective of this study was to test the extent to which the species of Convolvulaceae could be distinguished using quantitative measures of leaf outline shape taken from field samples.

Leaf development was tracked from initiation to maturity to identify how developmental

trajectories diverge and what is conserved across species. The development of leaf from young to maturity is of prime importance to understand the shape and size variability in different Convolvulaceae members.

### II. MATERIAL AND METHODS

Ten Commonly growing species belonging to four genera of family Convolvulaceae from Vadodara, Gujarat, India were selected for the above study. Identification of selected taxa was done through routine observation of reproductive structures and from available literature (Hooker, 1882; Cooke, 1958; Shah, 1978). Leaf development was tracked from initiation to maturity in order to identify how developmental trajectories diverge and what is conserved across species. Undamaged leaves of all the species from the initial stage of growth to the mature ones were collected and then dried in different stages of growth at different time. Leaves were kept into paper to blot dry and also to remain flat for further analysis. Then leaves were arranged from initial stage to mature one according to their size and shape. Freshly collected leaf specimens were placed on paper and color image of leaf outline of abaxial surface were captured with digital camera. The voucher specimens were deposited in BARO herbarium, department of Botany, The M S University of Baroda, Vadodara. (BARO/KO 21, 34, 41, 52, 61, 71, 79, 86, 91, 96)



**Figure 1: Arrangement of Leaves for Processing (Black square is scale of 30mm x 30mm)**

### III. DATA SAMPLING:

Young to mature leaves from each selected species were collected, pressed dried. The leaves were photographed with a scale marker (30mm × 30mm) using a digital camera (Nikon D5200) with an abaxial surface facing upwards (Fig. - 1). The digitized images of the leaves are converted into bitmap file (BMP format) image. The bitmap images are

then processed with SHAPE v.1.3d, image processing software (Iwata, 2002) to implement an Elliptic Fourier Analysis and Principal Component analysis (EF-PCA). Each colour image was converted into a binary image, from which the outline was traced and then transcribed in chain-code using the ChainCoder software program (Iwata and Ukai 2002). Outlines were then reduced to the coefficients of elliptic Fourier descriptors (EFDs, Kuhl and Giardina, 1982) of 50 harmonics and the size and orientation of each contour was standardized by the longest radius method using the Chc2Nef software program (Iwata and Ukai, 2002), whereupon the coefficients effectively became shape variables. These coefficients are mathematical descriptors of forms that can be statistically analysed by routine methods (Kuhl and Giardina, 1982).

In Elliptic Fourier Analysis (EFA), shapes of the sample were analysed in frequency domain (Kuhl & Giardina, 1982) to describe outline shape and provide shape measures (Andrade et al., 2008). The prepared images were processed to the Chain Coder program to obtain black and white images. This program extracts the contours of images and stores the relevant information as chain-code and the chain-codes are analysed by the program Chc2Nef where it calculates the EFD information. The EFD information analysis uses Max Harmonic No. of 20, the number of harmonics form the Fourier descriptor that represent shapes independently of the orientation (Andrade et al., 2008). The calculated normalized EFD is analysed by PrinComp program where EFD coefficient becomes larger. The PrinComp program visualizes the shape variation (Fig. - 2) acquired by each component where the EFD was calculated.

In this study, we approximated the shape by the first 20 harmonics. Because the coefficients of an elliptic Fourier descriptor are not invariant in size, rotation, shift and starting point of chain-coding about a contour, we standardized the Fourier coefficients on the basis of the work of Kuhl and Giardina (1982). The entire data collection procedure (i.e., image acquisition and landmark digitization) on the sample of leaves was repeated twice to estimate measurement error. The repetition was performed two weeks after the first round of data collection.

### IV. RESULTS

The standardized elliptic Fourier coefficients of 1440 leaves from the 10 species of family Convolvulaceae were calculated. The mean leaf shape of each species was then drawn using the mean values of the standardize Fourier coefficients within each species. The first three principal components (Table - 1) of the groups provide a good summary of the data. For this visualization, the coefficient of the elliptic Fourier descriptors was evaluated inversely using an eigen-vector matrix, letting the score on a particular principal component be equal to the mean  $\pm 2$  s.d. (standard deviation), while the score on the remaining components remained at the mean.



The study reveals that among the first five significant principal components scores of the coefficient of leaf mean shape, the major proportion of total variation was found in the first three principal components only. In terms of shape variables, the first three principal components accounted for over 80% of the total variance and described distinct and easily recognized trends of leaf outline shape change. The next 3 principal components expressed ambiguous shape variations, and which were not easily figure out visually. The variations refer for the surrounding of the notch separating the posterior lobes in the base of the leaves and the correlation of leaf length to width respectively. Shape variations of the first three PCs of each species are described in Table 1 and also can be seen in Figure 2.

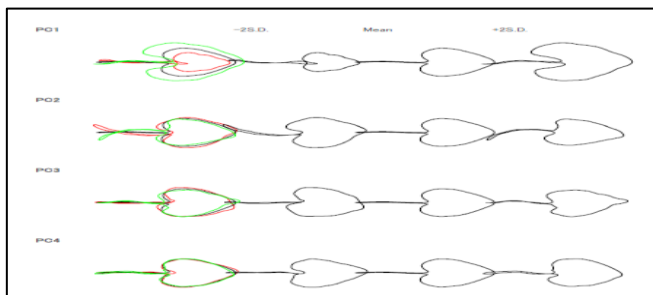


Figure 2: Reconstructed Leaf outline (Green line: +2SD, Red line: -2SD, Black line: Mean Leaf Shape)

First and second principal components are good measures of the aspect ratio and the depth of the head notch. The third component is associated with the bluntness of the distal part of the leaf and the fourth component expresses the bluntness of the side and proximal parts of the leaves. The fifth and sixth component is related to variation that cannot be ascribed to the first four components.

The first principal component is the most conspicuous characteristic as increase in length to width ratio is reasonably depicted. In this PCA transition from immature to mature leaves is taking place and it is capable enough to suggest an asymmetry at the leaf base which becomes better defined in the transition. The next PCA is illustrating mostly by the increasing difference in the width and area of the two sides of the leaf lamina. The third principal component shape variable is characterized primarily due to migration of the widest width axis giving a trend from elliptic to ovate or vice versa.

Table 1: Principal Component Analysis and Eigen value matrix

Sr. No.	Name of the species	PC	Eigenvalue	Proportion (%)	Cumulative (%)
1	Argyreia sericea St. Lag.	1	9.27E-02	80.38	80.38
		2	2.49E-02	18.66	95.11
		3	4.56E-03	5.06	99.63
2	Evolvulusnummularius(L.) L.	1	1.98E-02	68.38	68.38
		2	4.50E-03	19.11	87.49
		3	1.88E-03	7.32	94.81
3	Ipomoea marginata(Desr.) Verdc.	1	7.93E-02	58.5	58.5
		2	3.00E-02	21.44	83.84
		3	1.54E-02	10.33	94.17
4	Ipomoea pes-tigridis L.	1	1.70E-01	57.49	57.49
		2	9.13E-02	31.76	83.94
		3	4.83E-02	16.02	99.96
5	Ipomoea quamoclit L.	1	6.71E-02	48.23	48.23
		2	4.60E-02	32.31	80.54
		3	2.52E-02	18.16	98.69
6	Ipomoea aquatica Forssk.	1	2.81E-02	62.0118	62.011
		2	1.36E-02	29.986	91.997
		3	3.62E-03	8.0021	100
7	Ipomoea batatas (L.) Lam.	1	8.67E-02	72.22	72.22
		2	3.04E-02	22.45	91.2
		3	1.05E-02	9.57	97.84
8	Ipomoea cairica(L.) Sweet	1	1.60E-01	47.39	47.39
		2	1.01E-01	29.92	77.31
		3	6.52E-02	19.42	96.73
9	Merremia emarginata (Burm. f.) Hallier f.	1	6.08E-02	69.25	69.25
		2	2.29E-02	21.81	91.06



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		3	9.42E-03	8.94	100
10	Operculinaturpethum (L.) Silva Manso	1	0.02179	71.0018	71.0018
		2	0.00572	18.6471	89.6489
		3	0.00187	6.0986	95.7476

The first PC, in *A. sericea*, variation accounts for 80%, *I. batatas* for 72%, *I. aquatica* for 62%, *I. marginata* for 58%,

*I. pes-tigridis* for 57%, *I. quamoclit* for 48%, and *I. cairica* for 47%. The observed variance in *O. turpethum* for 87%, *M. emarginata* for 69%, and, *E. nummularius* for 68% of total variance, was related to the variation of the notch separating the posterior lobes on the base of the leaves. The second PC, for all the species characterized mainly by the change the correlation of leaf length to width. In contrast to overall components described by leaves of all the species still described by the first 2 PCs accounted for 80% of was related to the variation of the apical region of the leaves and a total variance of 100% was characterized mainly by the change in correlation of leaf length to width.

Six species of *Ipomoea* viz., *I. aquatica*, *I. batatas*, *I. marginata*, *I. pes-tigridis*, *I. quamoclit*, and *I. cairica* show a range in leaf forms from simple to highly dissected. Resulted cladogram output of shape data clearly segregates the genera based on the leaf morphology. *I. carica* and *I. pes-tigridis* are having 5-7 lobes as well as less width of lamina and *I. quamoclit* nearby because of dissected compound leaves. *I. aquatica* and *I. batatas* having ovate to lanceolate shape which is bringing them in close association

with *Merremia* has digitate leaves having acuminate apex and *Argyria* having the deltoid, ovate to lanceolate, apex acute-acuminate, base truncate. *Operculina*, *Evolvulus* and *I. marginata* are in one clade due to ovate, oblong, or oblong elliptic shape and truncate or cordate at the base.

The Kruskal-Wallis (1952) test result (Table 2) reveals that among the three principal components of the first two principal of identified in *Ipomoea* and capable enough to segregate 4 species of *ipomoea* from other 2 species *I. marginata* and *I. aquatica*. The *Ipomoea*, *Argyria* and *Merremia* fall within the same range except the *I. pes-tigridis*. Moreover, *Evolvulus* (4.50E-03) and *Operculina* (6.41E-03) depicted different range values. This implies that out of 83% of related variation of the notch separating the posterior lobes on the base of the leaves. The Kruskal-Wallis values (Table - 2) are suggesting the relationship of developmental stages of the leaf. Leaf development depends on intrinsic and extrinsic factors. PCA 1, 2, 3 in large number of individuals if calculated then it can give us a clue about the various sub-parts of the leaf i.e., epipodium, mesopodium and hypopodium in developmental studies at macro

**Table 2: Kruskal-Wallis test results**

Name of the species	PC	Kruskal-Wallis Value	P-Value	Remarks
<i>Argyria sericea</i> St. Lag.	1	23.35	8.25E-06	significant difference
	2	25.81	2.49E-06	significant difference
<i>Ipomoea quamoclit</i> L.	1	21.35	2.23E-05	significant difference
	2	28.47	6.51E-07	significant difference
<i>Ipomoea aquatica</i> Forssk.	1	2	3.68E-01	no significant difference
	2	2	3.68E-01	no significant difference
<i>Ipomoea batatas</i> (L.) Lam.	1	17.36	1.66E-04	significant difference
	2	20.48	3.56E-05	significant difference
<i>Ipomoea marginata</i> (Desr.) Verdc.	1	29.35	4.18E-07	significant difference
	2	39.13	3.18E-09	significant difference
<i>Ipomoea cairica</i> (L.) Sweet	1	17.4	1.66E-04	significant difference
	2	23.14	9.43E-06	significant difference
<i>Ipomoea pes-tigridis</i> L.	1	17.36	1.66E-04	significant difference
	2	20.48	3.57E-05	significant difference
<i>Merremia emarginata</i> (Burm. f.) Hallier f.	1	3.714	1.56E-01	no significant difference
	2	4.57	1.02E-01	no significant difference
<i>Evolvulusnummularius</i> (L.) L.	1	27.35	1.13E-06	significant difference
	2	36.47	1.21E-08	significant difference
<i>Operculinaturpethum</i> (L.) Silva Manso	1	5.6	6.08E-02	no significant difference
	2	7.2	2.41E-02	significant difference

morphological level. The shape of mature leaves results from three overlapping, yet distinct processes: first, initiation on the flank of the shoot apical meristem; second, a brief period of primary morphogenesis when the major regions and elements of Shape are defined; and third, a longer period of expansion when initial leaf shape may be altered by allometric expansion (Dengler and Tsukaya, 2001). Cluster dendrogram was constructed after getting the PCA data. The

dendrogram (Fig. - 3) depicted that this method can successfully be used in species identification. The cluster dendrogram was constructed by calculating Euclidian distance.

The first group which get separated in the dendrogram is of three species *I. cairica*, *I. pes-tigridis* and, *I. quamoclit* respectively. These three plant species are having lobed or very dissected leaves while rest of all the species are having simple leaves.

The second big cluster is consisting of plant species which are having acute or acuminate leaf tip and their leaves are somewhat lanceolate or arrow shaped. The third cluster consist of two species i.e., *E. nummularius* and *O. turpethum*. These two are having oval shaped or cordate shaped leaves and blunt leaf tip. In all totality 10 species of 4 different genera have taken into consideration. The traditional data available in flora and literature has been

taken into consideration for comparison with software generated data.

Moreover, we have been able to establish the relationship with traditional way of classification with digital morphometrics. Our results are in accordance with the earlier reports of Demandante et al. (2014) for *Cairica* papaya as well as Andrade et al. (2008) for *Monstera* populations as well as for *Anthurium* leaf outline shape (Andrade et al., 2008). The variations refer for the surrounding of the notch separating the posterior lobes in the base of the leaves and the correlation of leaf length to width respectively. The comparison suggests doing more observations in different populations is necessary to come down to any explicit conclusion.

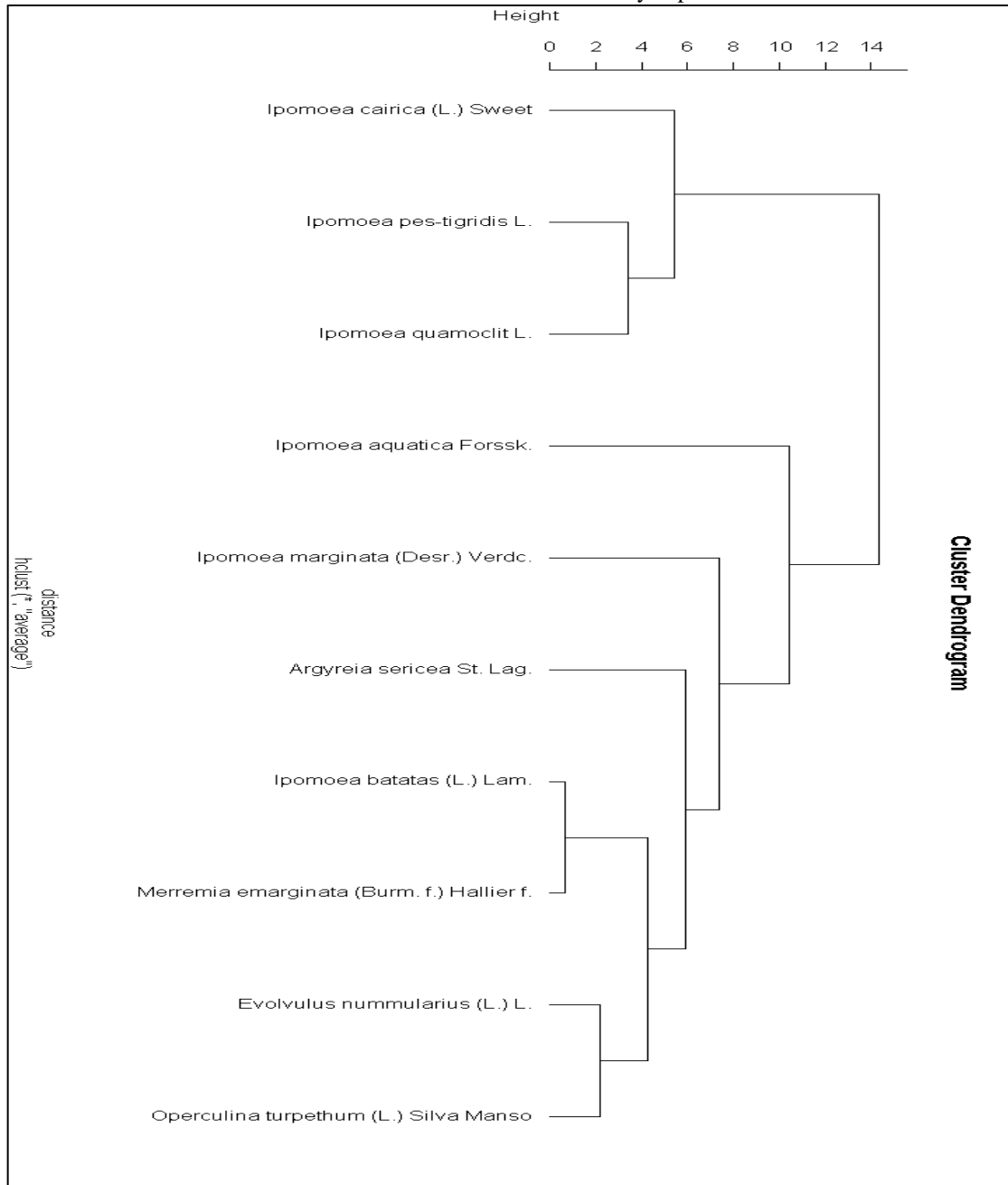


Figure 3: Cluster Dendrogram of PC analysis depicting segregation of species based on leaf shape

## V. DISCUSSION

Plants in nature are extremely diverse in shape, size and color which are mostly used for delimiting the nearby populations as well as species. A method that works very well on one group may rely on features that are absent in another taxon. Morpho-variants observed and collected from fields gives an idea of environmental proxies affecting the ecological and evolutionary forces acting within the species, genera or the family (MacLeod 2005). However, a morphological character predominantly decides the taxonomic categories as it offers by far reachable data having great systematic value (MacLeod, Benfield & Culverhouse, 2010).

In recent years computer-based software's are providing technological and methodological advancements

for multidimensional studies. Such semi-automated computerized tools possibly will soon provide more efficient ways of detecting biodiversity and discriminating taxonomic groups using shape data (Adams et al., 2011, Caridini and Elton, 2009; McCleod et al., 2010). Good morphometrics and phylogenetic studies can go hand-in-hand to understand the specific interrelationship and reexamination of phenotypic characters. Image analysis is a new tool for morphometrics and systematics as it facilitates the outline from leaves. It has also been used for Elliptical Fourier Descriptors as they are capable enough to depict the subtle differences among very nearly and similar shapes. Rohlf's (1998) seminal paper on the biological interpretation of shape variables has been used for taxonomic studies on group differences in botany and other disciplines. It is a classical application to shape data of methods commonly used in traditional multivariate morphometrics (Marcus, 1990; Neff and Marcus, 1980). Its main aim is to provide taxonomists with little or no experience in GMM with a clear, simple and easy to follow step-by-step protocol that may help them to familiarize with the method avoiding some of the most common pitfalls.

Our results produced two major insights. First, EFA is a reliable tool to analyze and compare the subtle variations in leaf shape. Secondly, EFA revealed that in three out of the six species studied differed in terms of the shape and form of leaves. However, one would be well advised to exercise caution in speculating on the nature of the processes underlying the morphological differences observed, or on whether they might have arisen from natural selection or from random drift, i.e., whether they should be considered adaptive or neutral (Holderegger et al., 2005). For example, landmarks may be readily definable and identifiable for some taxa, such as those with distinctive lobes, but not for others (Viscosi and Cardini, 2011), An analysis of relevance for each feature acquired in these two scenarios, the notch separating and length to width ratio is presented. The results obtained demonstrate the promising potential of the soft-biometric approach. Finally, an optimal system configuration according to each scenario is obtained.

The investigation of new approaches to identification is potentially valuable for identification and conservation of natural populations of different taxa is extracted from forest and non-forest area that forms the basis for an important

taxonomic study. It is also suggested that the value of geometric morphometrics is suitable for detecting subtle morphological variations which may be undetectable to the human eye. Such variations if studied in detail at large number it may be useful in understanding evolutionary implications. Cope et al. (2012) in his paper discussed a number of species identification systems that rely on both domain knowledge and on a wide range of morphometric methods. It should be clear that no single method provides a panacea for all problems, but rather that appropriate methods must be chosen for each task at hand. But this soft-biometric is useful for identification of plant species even at vegetative morphology in field. As this method is nondestructive in nature, it can be used for studying restricted and threatened plant taxa. Moreover, in terms of economic plants and its habitat relationship can be established without performing any physiological study (Michael Farris, 1984).

## VI. CONCLUSION

The differences in shape and form among organisms are the most visible manifestations of evolution. The morphological change according to environmental variability gives phenotypic plasticity. According to Gielis (2003) the morpho-spaces of an organism are due to different genetic and developmental channels which can explore the shape space and still be evolutionarily viable. The present study shows that closely related species can be distinguished at least partially by a quantitative comparison of their leaf shape. It is quite pertinent to the present observation for different genera of family Convolvulaceae. The method can be used successfully in a real-world application to find, for an unknown leaf, similar classes from a database of classified leaf images representing different plant taxa at family and/or generic level. Given the large-scale nature of botanical morphometrics and image processing automation is essential in addition to efficacy of this analytical software's, leaf morphology and developmental biology for plant identification at vegetative state can be done with the help of shape software. Elliptic Fourier analysis and Principal Component's analysis were very helpful tools in quantitative measurements differentiating leaf morphology of different species studied in this work. We could discuss the value of morphometrics to detect subtle morphological variation which may be undetectable by human eye.

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