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MORPHOLOGICAL COLLATION OF SPECIES AND ACCESSIONS OF *PLUMBAGO* L. USING NUMERICAL ANALYSIS

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This study examines relationship of species and accessions of *Plumbago* found in Maharashtra (India). This is achieved by phenetic clustering based on morphological characters. Three species and two accessions were collected and identified. For each sample 16 qualitative and 16 quantitative characters were scored and data were subjected to numerical taxonomy analysis. The result of the present study clearly indicates that there are 2 subsections basically grouped as *Plumbago auriculata, Plumbago zeylanica* and their accessions on one branch where as *Plumbago rosea* on the separate branch, clearly indicating the relationship among the three species and accessions of *Plumbago.* In support principal coordinate analysis PCO (Gower General Similarity Coefficient) and the biplot Principal Correspondence Analysis (PCA-biplot) was carried out and showed significant result.

Keywords: Morphodiversity, Numerical taxonomy, Phenogram, *Plumbago.*

The family Plumbaginaceae is sometimes referred to as the leadwort family or the *Plumbago* family. Family includes 27 genera and 730-836 species (Simpson 2010). *P. auriculata* Lam. synonyms *P. capensis* Thunb. (1794) is commonly known as blue colored Lead wort (English), Neela Chitrak (Hindi), Neela Kodiveli (Tamil) and Krishna chithraka (Sanskrit) (Ariyanathan *et al.* 2011). *Plumbago rosea* L. syn *P. indica* known as rose colored Lead wort (English), Rakt chitrak (Hindi), Koduvelli (Tamil) and Lal Chitrak (Marathi) (Datta and Mishra 2012). *P. zeylanica* occurs widely growing in wild. *P. zeylanica* known as Ceylon Lead wort (English), Chitra (Hindi), Chitramoolam (Tamil), Chitrak (Marathi) and Krishna Chithraka (Sanskrit). All the species of Plumbago are highly medicinal. *P. auriculata, P. rosea* and *P. zeylanica* contain Plumbagin, Apigenin, Luteolin and their Glycosides and Flavanoids as effective as an emetic. Roots are employed as a styptic in scrofula (Kurian *et al.* 2001). The Naphthoquinones: Plumbagin and Epi-isoshinanolone; Steroids; Sitosterol and 3-glucosylsitosterol, Plumbagic and Palmitic acids have been isolated from *P. auriculata* (Paiva *et al.* 2005). Plumbagin is used in the treatment of early leucoderma and baldness, used as abortifacients and in lowering blood pressure. It has got anti-rheumatic, anticancerous and antimicrobial properties. It is also used as a preservative for non alcoholic beverages.

Taxonomy is a part of systematics that includes four main components: description, identification, nomenclature and classification of taxa (Simpson 2010). All species of *Plumbago* are morphologically diverse. Diversity includes the phenetic and genetic variation among the genera. Morphological markers are the traditional markers. Morphological mutant traits in a population are mapped and linked to detect a desirable or undesirable trait and indirect selection is carried out using the physically identifiable mutant for the trait. Generally plants were examined for different characters in morphological studies. The plant morphology was defined as the study of the anatomical and cytological features of the life histories of plants expressed in a taxonomic framework (Donald *et al.* 2001).

Numerical taxonomy (phenetics), as a new approach, was created in 1960's by Robert R.

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Sokal & Peter H.A. Sneath as a reaction to that situation. Their main aim was to classify organisms based on their overall similarity and all available characters without any weighting, by clearly defined numerical procedures, in order to avoid high level of subjectivity that exists in previous systems of classification. Therefore, numerical taxonomy was defined as a: "Grouping by numerical methods of taxonomic units on the basis of their character states" (Sneath and Sokal, 1973). Literature study reveals that very few taxonomic comparative studies ever done on the species of *Plumbago* in India. For the first time the species and accessions of *Plumbago*, existing in Maharashtra, India, has been evaluated together, by the aid of morphological characters applying numerical taxonomy. Main aim is to see linear arrangement of species and accessions. The maximum possible number of the character and character states was used, in order to obtain the most objective result and to produce long lasting natural grouping

MATERIALS AND METHODS

Initial step in numerical taxonomic analysis is to select Operational Taxonomic Units (OTUs). OTUs could be individuals, populations, species, genera, etc. OTUs in this study are species of *Plumbago* present in Maharashtra, India. In total 5 OUTs (three species and two accessions) of *Plumbago viz. Plumbago auriculata* Lam., *Plumbago rosea* Linn., and *Plumbago zeylanica* Linn were selected. For convenience selected species and accessions were coded as species *P. auriculata* (*Pa1*), *P. rosea* (*Pr*) *P. zeylanica* (*Pz1*); accessions as *P. auriculata* (*Pa2*) and *P. zeylanica* (*Pz2*) respectively.

Plumbago auriculata and *Plumbago zeylanica* were obtained from garden of forest department at Parbhani, District Parbhani (Latitude 19° 16' 0" N, Longitude 76° 64' 59" E, Altitude 410 meter or 1345 ft) and Melghat Tiger Reserve Chikhaldara Dist. Maharashtra (Latitude 21° 24' 3.0143" N, Longitude 77° 19' 41.286" E; Altitude 1100 meter or 3608 ft) (Species and accession *Pa1* and *Pa2* respectively). *Plumbago rosea* was obtained from Gavhe, Dapoli, Dist. Ratnagiri (Latitude 17° 46' 0" N, Longitude 73° 11' 0" E, Altitude 243 meter or 800 ft). No accession for the *P. rosea* was collected as there were no or scanty of rainfall for two consecutive year of the study. The collected plantlets were planted in pots containing equal volume of compost and soil and watered daily. Herbariums of different species were prepared in 3 sets for each species and accessions. The specimens were identified by referring to the information in the *Flora of Marathwada* and the *Flora of Maharashtra*, Vol. 7(Almeida 1997), (Naik 1998), (Singh *et al.*2000). They were authenticated from Botanical survey of India (BSI), Western Regional Centre 7, Koregaon Road, Pune- 411 001. The voucher numbers were *P. auriculata* (PJG - 1), *P. rosea* (PJG - 2) and *P. zeylanica* (PJG - 3).

Second step is selection of characters and coding of the character states. It is advisable to select characters from all parts of the OTUs. Characters are usually combination of quantitative and qualitative features. It is important in numerical taxonomy that all characters should be unweighted or equally weighted, without giving much importance to some of the features. In support to the principles of numerical taxonomy 32 characters were selected for this study (16 qualitative and 16 quantitative). All morphological characters were chosen from all part of OTUs: general characters like life form, life length, stem characters, leaf characters, inflorescence character, flower characters, calyx characters, corolla characters, characters regarding stigma and style features, fruit characters and root characters (Table 1).

Data analysis: All the analyses were performed using MVSP Version 3.22 A Multi-Variate Statistical Package by Kovach Computing Services. Genetic distance matrix

Table 1. List of characters used in numerical taxonomic analysis.

A. Life length and forms	
1.	Perennial Shrub (0), Perennial herb (1)
B. Plant pattern	
2.	Height (ft)
C. Stems	
3.	Texture Herbaceous at apex woody at base(0), Herbaceous (1)
4.	Erect trailing (0), Erect Climbing (1)
5.	Internodal length (cm)
D. Leaves	
6.	Leaf length (cm)
7.	Leaf width (cm)
8.	Shape Obvate (0), Ovate (1)
9.	Apex obtuse (0), acute (1)
10.	Base Attenuate (0), Cuneate (1)
11.	Petiole amplexicaul (0), Exauriculate (1)
D. Inflorescence	
12.	Raceme (0), Spike (1)
13.	Inflorescence length (cm)
14.	Bract length (mm)
15.	Peduncle (cm)
16.	Pedicel (mm)
E. Flowers	
17.	Flower length (cm)
18.	Calyx length (cm)
19.	Sepal colour dark green (0), red (1), yellow green (2)
20.	Glands on calyx less in no. (0), More in No. (1)
21.	Corolla length (cm)
22.	Petal colour faint blue (0), Dark blue (1), red (2), white (3)
23.	Lobes obvate (0), obvate oblong (1)
24.	Apex mucronate (0), Acute (1)
25.	Stamen length (cm)
26.	Filament length (mm)
27.	Fruit absent (0) Present (1)
28.	Fruit length (cm)
29.	Seed length (cm)
F. Root	
30.	Seed per fruit Zero (0), one (1)
31.	Root tap (0), tuberous (1)
32.	Root length (ft)

were calculated by Euclidean distance and further pooled to cluster analysis using unweighted pair group method with arithmetic average (UPGMA). The principal coordinate analysis (PCO) was performed essentially as described by Meudt and Bayly based on the correlation matrix. Gower's General Similarity Coefficient was used as the data was mixed data, in which some variables are measurements whereas others are binary. In addition PCO case score was performed on the pooled data of PCO analysis (Podani 2000).

OBSERVATIONS AND RESULTS

When the phenogram was studied, it was observed that all the species and accessions were composed of two major groups. The species of the first group, namely *P. rosea* appear to be very restricted and found on separate cluster. On the other hand it is worth noting that species and accessions of *P. zeylanica* and *P. auriculata* shared the same cluster.

Both our previous molecular study (Gadge and Nathar 2015) and the current morphological analysis determined a close relationship between the species and accession of *P. auriculata* (Pa1 and Pa2) followed by *P. zeylanica* (Pz1 and Pz2), whereas *P. rosea* showed distinctly relationship with the other (Fig 1).

The studied PCA biplot of morphological characters showed that studied individuals had varied morphological characteristics which were helpful in distinction of the species and accessions. For example, in species and accession of *P. zeylanica* Ch-17 was prominent (Fig 2). PCA plot of morphological features showed that some characters, such as Ch-2 (Plant height), Ch-5 (Internodal length), Ch-6 (Leaf length), Ch-13 (Inflorescence length), Ch-15 (Peduncle length) Ch-27 (Fruit Present/absent) and Ch-28 (Fruit length) were separated from others and the remaining placed near or overlapped (Fig 3) (Table 2).

The principal coordinate analysis (PCO) showed clear separation of the species and the accessions on the axes (Fig 5). All the vectors with an eigenvalue explained 49.228%, 83.997% and 96.775% of the variation in the data (Table 3), respectively. Mostly consistent with phenogram generated in cluster analysis, the PCO plot shows that the 5 species and accessions could be clearly separated from each other. It also shows the resemblance among the species and accessions viz. *P. auriculata* and *P. zeylanica* (Fig 4).

The phenogram obtained from the UPGMA clustering of similarity matrix (Fig 1), types of

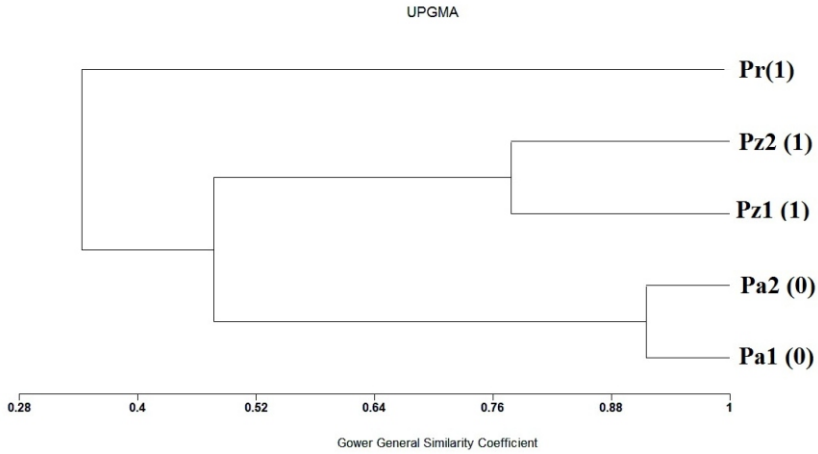


Figure 1: Cluster analysis of species and accessions of *Plumbago*

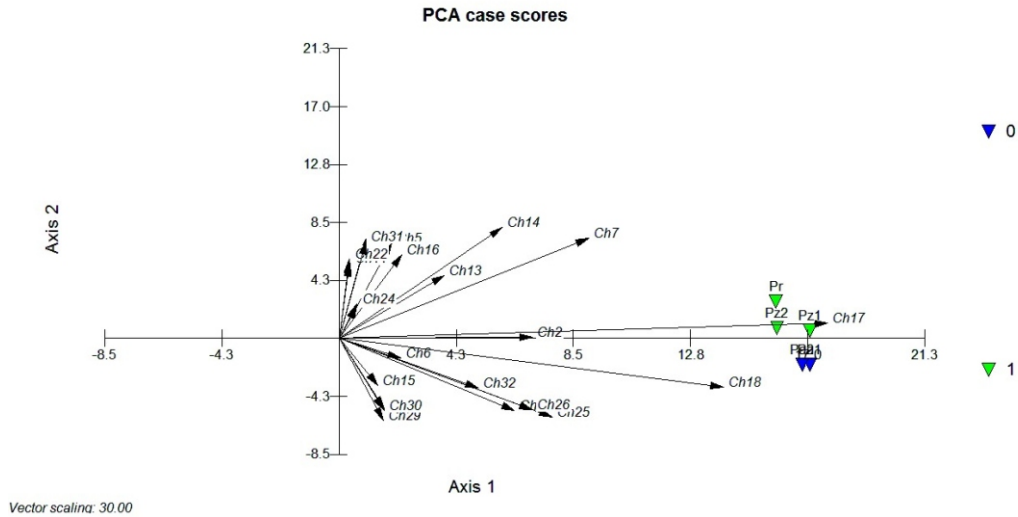


Figure 2:PCA Biplot showing characters

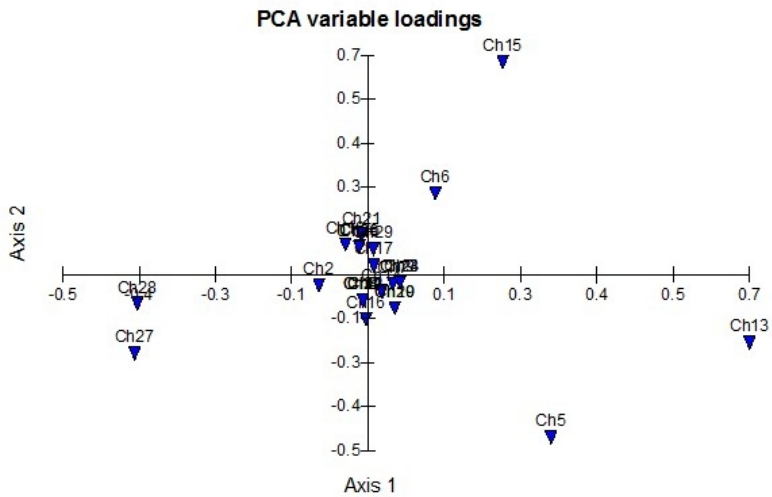


Figure 3:PCA analysis showing characters details

Table 2: cluster analysis (UPGMA) Gower General Similarity Coefficient

Node	Group 1	Group 2	Similarity	Objects in group
1	Pa1 (0)	Pa2 (0)	0.915	2
2	Pz1 (1)	Pz2 (1)	0.779	2
3	Node 1	Node 2	0.477	4
4	Node 3	Pr (1)	0.343	5

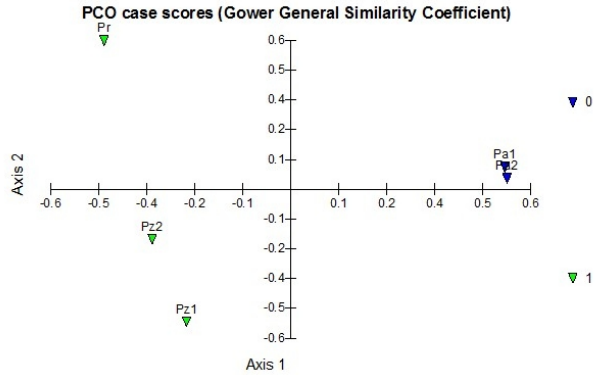
Table 3: Principal Components Analysis Imported data (Analysing 30 variables x 5 cases) Tolerance of eigenanalysis set at 1E-007 Eigenvalues

	Axis 1	Axis 2
Eigenvalues	56.600	16.095
Percentage	71.952	20.460
Cum. Percentage	71.952	92.413

	Axis 1	Axis 2
A-Ch2	**-0.087**	**-0.033**
B-Ch3	-0.009	-0.080
C-Ch4	-0.009	-0.080
D-Ch5	**0.324**	**-0.500**
E-Ch6	**0.119**	**0.251**
F-Ch7	0.044	-0.028
G-Ch8	-0.009	-0.080
H-Ch9	0.056	-0.023
I-Ch10	0.047	-0.102
J-Ch11	-0.009	-0.080
K-Ch12	-0.009	-0.080
L-Ch13	**0.676**	**-0.209**
M-Ch14	0.024	-0.051
N-Ch15	**0.238**	**0.655**
O-Ch16	-0.004	-0.137
P-Ch17	0.010	0.028
Q-Ch18	-0.040	0.093
R-Ch19	0.047	-0.102
S-Ch20	0.047	-0.102
T-Ch21	-0.012	0.124
U-Ch22	-0.009	-0.080
V-Ch23	0.056	-0.023
W-Ch24	0.056	-0.023
X-Ch25	-0.015	0.089
Y-Ch26	-0.016	0.086
Z-Ch27	**-0.414**	**-0.241**
a-Ch28	**-0.409**	**-0.089**
b-Ch29	0.009	0.080
c-Ch30	-0.009	-0.080

grouping with PCA analysis (Fig 2) and PCO analysis (Fig 3) showed significant similarity in the results.

Figure 4: PCO analysis showing similarity



CONCLUSION

Although this investigation added new findings to the literature, it is somewhat limited to the known species and accessions. Most of the studies in past were done in the regional level, as a part of the floristic studies. A comprehensive characterization covering all *Plumbago* species be found to be necessary to create a more satisfactory classification. It will more authentic if one can handle morphological data with application of molecular data in combination.

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