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Pollen morphometrics in the genus *Indigofera* L. from Karnataka

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Abstract
In the present palyno-morphometric studies in the genus *Indigofera* from Karnataka were analyzed with the support like principal component analysis, principal co-ordinate analysis, cluster analysis and light microscopy technique. Pollen dimensions were measured by considering quantitative characters viz. equatorial diameter, polar diameter, P/E ratio and numbers of apertures. It was observed that the mean polar dimension ranges from 2.13 µm to 3.35 µm while equatorial dimension ranges from 1.68 µm to 3.65 µm. Based on the obtained results correlation matrix reveals positive significant relationship between polar diameter and equatorial diameter in contrast with this distance matrix shows great similarities between *Indigofera cordifolia-Indigofera linifolia, Indigofera hirsuta- Indigofera linifolia, Indigofera hochstetteri-Indigofera karnatakana- Indigofera uniflora, Indigofera karnatakana-Indigofera uniflora, Indigofera tinctoria-Indigofera trita*. Among all species *Indigofera caerulea -Indigofera glandulosa* shows greater variation, whereas *Indigofera karnatakana-Indigofera uniflora* shows closely positive significant relationship. In conclusion the study was demonstrated the usage of such analysis in taxonomic research will solve the doubtful grouping of the species.

Keywords: dendrogram, *indigofera*, principal component analysis, principal co-ordinate analysis

Introduction
The Fabaceae, Leguminosae (Papilionaceae) commonly known as the legume pea or bean family are a large and economically important family of flowering plants. The family is widely distributed, and is the third-largest land plant family in terms of number of species, behind the Orchidaceae and Asteraceae, with about 751 genera and some 19,000 known species (Christenhusz and Byng, 2016) [6]. The five largest of the genera are *Astragalus* (over 3,000 species), *Acacia* (over 1000 species), *Indigofera* (around 700 species), *Crotalaria* (around 700 species) and *Mimosa* (around 500 species), which constitute about a quarter of all legume species. Recent molecular and morphological evidence supports the fact that the Fabaceae is a single monophyletic family (Lewis *et al*., 2005) [20]. This point of view has been supported not only by the degree of interrelation shown by different groups within the family compared with that found among the Leguminosae and their closest relations, but also by all the recent phylogenetic studies based on DNA sequences (Wojciechowski *et al*., 2004) [33]. These studies confirm that the Fabaceae are a monophyletic group that is closely related to the Polygalaceae, Surianaceae and Quillajaceae families and that they belong to the order Fabales under rosids (APG, 2016) [1].
Although a comprehensive palynological data embarrassing a large number of taxa from a single geographical region is important from the systematic and phylogenetic perspective, such information on the Papilionaceae is very much lacking. The available information is too fragmentary and scattered. The family is abundantly represented in the tropical South Indian region, this group is almost unexplored palynologically. The Papilionaceae are represented in the Peninsular India by 658 species in 95 genera under 10 tribes (Hooker, 1879) [17]. Gamble (1918) [15] has described 345 species in 59 genera under eight tribes from South India.
The main characters of taxonomic value in pollen grains are the number and position of furrows, pollen wall morphology, symmetry and shape and size of pollen. The Palyno-Morphometric studies in the genus *Indigofera* has not been investigated before. Morphometric studies have great importance and play significant role in distinct grouping or segregation of closely associated species. The processes like principal component analysis, principal co-ordinate analysis and cluster analysis produces hierarchical classification of entities based on similarity matrix, distance matrix and dendrogram. Such morphometric and cladistics analyses based on morphological characters have been carried out in a number of genera and families (Gomes-da-Silva *et al*. 2012) [16]. Morphometric studies received considerable attention for species relatedness in different genera (Bolourian and Pakravan, 2011) [4]. Although such studies were carried out in different legume genera, for example, *Cassia* (Boonkerd *et al*., 2005) [5], *Indigofera* (Soladoye *et al*., 2010a) [30], *Daniellia* (de La Estrella *et al*. 2009) [7].
Recently, Soladoye *et al*. (2010b) [29] made a morphometric study of eight species of *Senna* from south-western Nigeria

and using 13 morphological characters they showed that *S. sophera* is closely related to *S. hirsuta*. The results revealed that three out of the thirteen characters employed contributed significantly in differentiating each of the species from the other at 95% level of significance. The species have great similarities hence their grouping under the same genus (Soladoye, 2010a) [30]. Similarly, the same species were analyzed by Rahman *et al*. (2013) [25] in Bangladesh. The highest similarity was found between *S. obtusifolia* and *S. tora*, while the highest variation is observed between *S. alata* and *S. hirsuta*. A close relationship was found between *S. multiglandulosa* and *S. sophera*, and between *S. siamea* and *S. timoriensis*. Other than this, Rahman and Rahman (2012) [26] evaluated phenetic variation among 14 species of *Desmodium* Desv. in Bangladesh. A total 36 floral and vegetative characters were selected morphological similarity and variation within *Desmodium* and the genus *Cassia* L., *Exacum* L., *Clerodendrum* L., *Caesalpinia* L. (Deshmukh *et al.,* 2011, 2012, 2012, 2013) [9, 10, 11, 12] by taking an account of the morphological quantitative characters only. Again, Deshmukh *et al*. (2014) [13] performed the palyno-morphometric study in the eight species of *Cassia* L. for Maharashtra State. Very recent, Awaradi *et al*, (2017) [2] analyzed the relationship among thirteen species of *Indigofera* using eight quantitative characters based on both fresh and herbarium specimens. The results of Principal Component Analysis (PCA) and Cluster Analysis divulged that only three characters examined accounted for about 78% importance in the delimitation in the taxa. The present investigation here concerns about 12 species of *Indigofera* considering the objectives; i) to assemble pollen morphology of a fair sample of the group occurring in the Karnataka using Light Microscopy (LM) ii) to evaluate the relationship in between *Indigofera* species on the basis of Palyno-morphometric analysis.

Materials and Methods

The *Indigofera* species were collected from various localities of Karnataka and identified with the aid of available literatures (Kotresha *et al*., 2012; Kotresha and Kambhar, 2016) [19, 18] and also pollen slides were prepared by using fresh as well as herbarium specimens (Table 1). For the present study, the 12 species of *Indigofera* were studied. Pollen grains from each plant were collected by teasing out from fresh as well as herbarium specimens collected in fresh as well as on pollen morphology and their morphometric analysis of genus *Indigofera*.

Table 1: Voucher information and distribution of fresh and herbarium specimens of *Indigofera* species studied

Plant species	Location	Voucher no.
Indigofera caerulea Roxb.	Bagewadi, Gadag	5514
Indigofera coultea (Burm.f.) Merr.	Chikkanargund, Gadag	5517
Indigofera cordifolia Heyne ex Roth	Binkadkatti, Gadag	5520
	AWU, Vijayapur	474
Indigofera glandulosa J.C. Wendl.	AWU, Vijayapur	472
Indigofera hirsuta L.	Bhairapur, Gadag	5521
Indigofera hochstetteri Baker	Jyalwadgi, Gadag	6063
Indigofera karnatakana Sanjappa	Bhairapur, Gadag	5528
Indigofera linifolia (L.f.) Retz.	AWU, Vijayapur	260
Indigofera linnaei Ali	Kappat hill, Gadag	5522
Indigofera tinctoria L.	Kappat hill, Gadag	5529
Indigofera trita L.f.	Tumkur	5531
	Hesargundagi, Chincholi	473
Indigofera uniflora Buch.-Ham. ex Roxb.	Chikkanargund, Gadag	5535

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For palynological studies, only the mature anthers of flowers at anthesis were used. They were collected by means of forceps that were previously cleaned and wiped dry before making every collection. It may be noted, that the flowers selected were of the same plant, the twigs of which were used to make voucher herbarium sheets. The anthers were first fixed in acetic-alcohol (1: 3) and then preserved in 70% alcohol until future use. Each pollen collection was given an appropriate voucher number. In order to make preliminary observations pollen slides were prepared following the Wodehouse method. However, in order to measure the size and to study the structure and sculpture of exine, only acetolysed and non-chorinated pollen prepared following the Erdtman's acetolysis method (1960) were used.

LM studies, microphotographs were taken with COOL PAD (A8) camera attached to Monocular Magnus MLX (220-240V~0.2A 50/60Hz) microscope. Both morphological observations and measurements of pollen grains and the structural features of the exine were made with microscope using a 10X ocular micrometre calibrated with a 100 pm stage micrometre and 40 X oil immersion objective. It may be noted, that all the measurements with light microscopy (LM) are a mean of 10 measurements made on 10 randomly selected pollen grains.

Results

Pollen dimensions were measured and different quantitative characters viz. equatorial diameter, polar diameter, P/E ratio and numbers of apertures and represented in the form mean and SD. Correlation between the Pollen dimensions were studied by using Karl-Pearson's Correlation Coefficient. The cluster analysis was used to Identifying groups of individuals or objects that are similar to each other but different from individuals in other groups can be intellectually satisfying, profitable, or sometimes both.

Table 2: Pollen Dimensions ranges in *Indigofera*

	Polar Diameter (µm)	Equitorial Diameter (µm)	P/E Ratio (µm)	Aperture Number
	Mean±SD	Mean±SD	Mean±SD	Mean
Indigofera caerulea	1.35±0.21	1.68±0.24	0.81±0.06	3.00
Indigofera coultea	2.83±0.35	3.20±0.47	0.89±0.06	3.00
Indigofera cordifolia	3.05±0.16	3.48±0.18	0.88±0.05	3.00
Indigofera glandulosa	3.35±0.38	3.65±0.29	0.92±0.04	3.00
Indigofera hirsuta	2.83±0.24	3.45±0.20	0.82±0.08	3.00
Indigofera hochstetteri	2.35±0.34	2.73±0.25	0.86±0.06	3.00
Indigofera karnatakana	2.43±0.24	2.83±0.21	0.86±0.05	3.00
Indigofera linifolia	2.93±0.33	3.50±0.31	0.84±0.07	3.00
Indigofera linnaei	2.50±0.31	3.05±0.16	0.82±0.10	3.00
Indigofera tinctoria	2.23±0.22	2.50±0.20	0.89±0.03	3.00
Indigofera trita	2.13±0.13	2.43±0.17	0.88±0.08	3.00
Indigofera uniflora	2.45±0.31	2.78±0.43	0.89±0.05	3.00

The mean polar dimension ranges from 2.13 µm to 3.35 µm while the mean equatorial dimension ranges from 1.68 µm to 3.65 µm (Table 2). Palyno-Morphometric studies based on the correlation matrix reveals positive significant relationship between polar diameter and equatorial diameter (Table 3) while principal co-ordinate analysis (distance matrix) shows great similarities between *Indigofera cordifolia-Indigofera linifolia, Indigofera hirsute- Indigofera linifolia, Indigofera hochstetteri-Indigofera karnatakana- Indigofera uniflora, Indigofera karnatakana-Indigofera uniflora, Indigofera tinctoria-Indigofera trita* (Table 4). On the basis of cluster analysis (Dendrogram) the species dissimilarity greater in *Indigofera caerulea -Indigofera glandulosa* i.e 7.913 while in between *Indigofera karnatakana-Indigofera uniflora* i.e. 0.04 indicating both the species closely related (Figure 1).

Table 3: Principal Component Analysis

	Polar Diameter (µ)	Equatorial Diameter (µ)	P/E Ratio (µ)	Aperture Number
Polar Diameter (µ)	1.000	0.981**		
Equatorial Diameter (µ)	0.981**	1.000		
P/E Ratio (µ)	0.457	0.283	1.000	
Aperture Number	0.000	0.000	0.000	0.000

Table 4: Principal Co-ordinate Analysis

Case	Squared Euclidean Distance											
	A	B	C	D	E	F	G	H	I	J	K	L
A	0.000											
B	4.508	0.000										
C	6.135	0.126	0.000									
D	7.913	0.479	0.122	0.000								
E	5.326	0.067	0.055	0.325	0.000							
F	2.105	0.452	1.053	1.859	0.753	0.000						
G	2.481	0.301	0.814	1.540	0.552	0.016	0.000					
H	5.812	0.103	0.018	0.209	0.013	0.932	0.706	0.000				
I	3.213	0.133	0.487	1.092	0.266	0.130	0.058	0.383	0.000			
J	1.453	0.850	1.631	2.589	1.267	0.067	0.147	1.493	0.383	0.000		
K	1.169	1.091	1.958	3.003	1.544	0.141	0.250	1.797	0.535	0.016	0.000	
L	2.427	0.321	0.850	1.576	0.601	0.013	0.004	0.754	0.083	0.126	0.228	0.000

A. *Indigofera caerulea* B. *Indigofera coultea* C. *Indigofera cordifolia* D. *Indigofera glandulosa* E. *Indigofera hirsuta* F. *Indigofera hochstetteri* G. *Indigofera karnatakana* H. *Indigofera linifolia* I. *Indigofera linnaei* J. *Indigofera tinctoria* K. *Indigofera trita* L. *Indigofera uniflora*

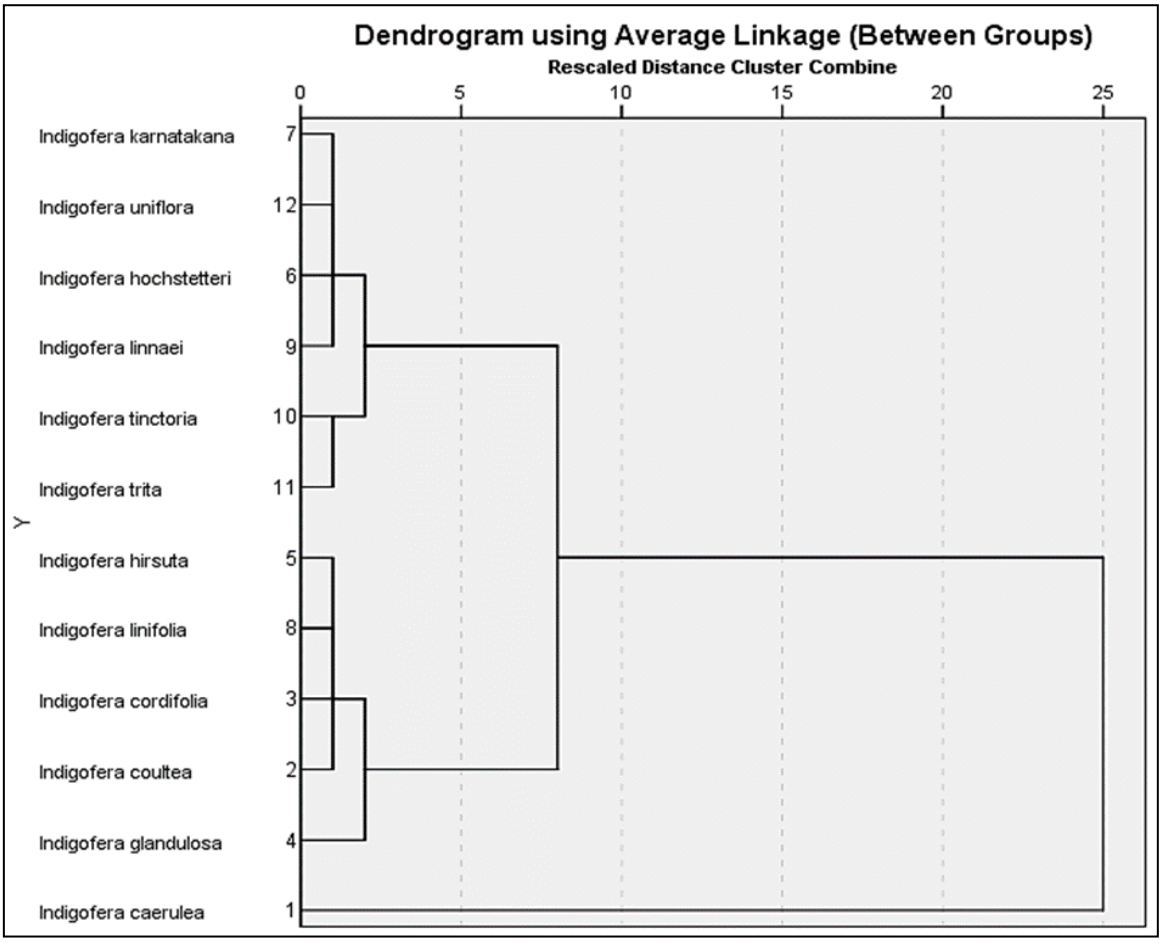

Fig 1: Dendrogram based on the palynological characters

Discussion and conclusion

In most taxonomic studies in the real world have both quantitative as well as qualitative variables; the challenge however is the integration of many variables in the development of cluster clarifications. It was therefore necessary to use the steps for cluster analysis to come up with a cluster solution but at the same time supported the results with the others taxonomic evidences such as anatomy, embryology, cytology, phytochemistry and even morphometric analysis (Soladoye *et al*., 2008) [28]. Morphometric analyses are commonly performed on organisms or species to analyze the fossil record. Morphometric value will help to add a quantitative element to existing descriptions and make it allow more rigorous comparisons.

The palynological investigations on plant relationship and phylogeny are depend on their form, number, distribution, and position of the apertures, exine structure details can be used in plant identification (Stuessy, 2009) [31]. Pollen grains also providing best criteria which is often constant and visible under the microscope and have been applied to place genera within families (Backlund and Nilson, 1997) [3]. The variations in shape, aperture character, Polar axis, equatorial axis, P/E ratio, Pollen size, shape class have been the source of delimitation of various taxa (Nowicke and Skavarla, 1979) [22]. The ability of palynology to provide non-molecular data to support the molecular studies in the resolution of some contested phylogenies, as well as its success in the past studies of Caesalpinoideae and Mimosideae (Ferguson, 1981) [14] Papilionoideae (Wael, 2009) [32] validates its application in this study of *Indigofera*, a member of the subfamily Papilionoideae. Numerical taxonomy enables classification based on either one or a few characters or one set data (Sneath and Sokal, 1973) [27].

In the existing results by Awaradi *et al*, (2017) [2] shows that the numerical analysis of thirteen *Indigofera* species significant correlation existing between number of Pl length and No of seeds, Lf length and lf width and Infl length and Fr length shows that these characters carry more weight in the overall analysis. Also the average linkage between the groups on agglomeration schedule and cluster analysis show that greater affinity exist between *Indigofera coultea* and *I. wightii, I. oblongifolia* and *I. trita, I. hirsuta* and *I. hochstetteri* and *I. cordifolia* and *I. glandulosa* than between *I. caerulea* and *I. linnaei*. In contrast with this, the palyno-morphometric clusters showing the species dissimilarity greater in *Indigofera caerulea -Indigofera glandulosa* i.e 7.913 while in between *Indigofera karnatakana-Indigofera uniflora* i.e. 0.04 indicating both the species closely related.

This approach of cluster solution can assist in the identification of species more precise in situations where there is no clear cut means of plant species placement along the hierarch of plant identification as in the genus *Indigofera*. Natural classification of species is attained with clarity of

affinity in the taxonomic level. Further research in the usage of cluster solution would solve plant identification questions especially where the species is placed in the doubtful grouping of the species. It is time consuming taxonomic exercise but worth the research work process and taxonomic purpose. Thus, the functional significance of pollen characteristics has been used to solve identification placement of species in different plant groupings like Papilionoideae. With few exceptions, ten pollen types recognized in the Papilionoideae (Perveen and Qaiser, 1998) [23] correspond to Polhill and Raven's tribal classification Polhil (1981) [24] and most of the tribes are easily distinguished using pollen types 1, III, and IV types. These types are common in the tribes Enddl. Indigofereae and Desmodieae (Wu and Huang, 1995; Perveen and Qaiser, 1998). [21, 23] Advancement in the microscopy should refine the studies of palynology and its application in science, especially taxonomy.

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