Quest Journals
Journal of Research in Agriculture and Animal Science
Volume 3 ~ Issue 7 (2015) pp:11-17
ISSN(Online) : 2321-9459
www.questjournals.org

Research Paper

Numerical Assessment of Variable Importance in Palynological Characterization of Some *Indigofera* (Linn) Species at Maseno University Herbarium.

Sikolia[1], F. S. and Onyango[1], P
[1]*Botany Department, Maseno University, Kenya*

Received 20 November, 2015; **A**ccepted 30 December, 2015 © The author(s) 2015. Published with open access at **www.questjournals.org**

ABSTRACT:- Palynological characterization of 17 *Indigofera* species of the Maseno University Herbarium has been carried out in *I. tanganyikensis, l. dendroides, l. spicata, l. sabulata, I. volkensii, l. fulvopilosa, l. hirsute, l. drepanocarpa, l paracapitata, l. asparagoides, l ambelacensis, l vohemarensis, l subargentea, l. circinella, l. zenkeri, l. arrecta, and l. vicioides*, respectively. The results show that the best cluster solutions are either three or four at which the distance changes (Akaike Information Criterion) are 1.261 and 1.105, respectively. The amb and the polar axis are the most important pollen characteristics in both the three and four cluster solution on using both the qualitative and quantitative variables. The polar axis predictor importance value is higher in a three cluster solution than in a four – solution, while the amb shape is critically significant in cluster 3 of the four –cluster solution. aperture character was critically important in cluster 2 of the four cluster a variable wise assessment of various pollen characteristics showed the amb shape as most important character in cluster three of four cluster solution, and equatorial axis as the only quantitative character critically important in the characterization of the *Indigofera* in four –cluster solution. Equatorial axis is the only critically important parameter in cluster 2 of four cluster solution members. The importance of variables could be attributed to variance or chi square distribution of quantitative and qualitative variables respectively.

Keywords:-: - Palynology, variable importance, cluster solution, *Indigofera*

I. INTRODUCTION

The genus *Indigofera* has approximately 700 species, and it belongs to the family Leguminosae (Kokwaro, 1994) [1]. The *Indigofera* have great economic importance through; production of dyes, medicines, poisons and green mature (Takawira-Nyenya and Cordon, 2005) [2]. Previous taxonomic studies on the *Indigofera* (Ferguson and Strachan, 1982)[3]; Wu and Huang, 1995[4]; Perveen and Qaiser, 1998[5]; Soladoye and Lewis, 2003[6]), have either used only the morphometric, ecological or chemical characteristics, leaving behind the palynological aspects of have not included all the 17 representations of the genus *Indigofera* Linn hence the study of the *Indigofera* species at Maseno University herbarium. Stable classification system requires the inclusion of as many as possible pollen characteristics from a broad sampling of the members of the genus *Indigofera*.

Most palynological investigations on plant relationship and phylogeny are based on the form, number, distribution, and position of the apertures, Pollen exine details can be used in plant identification the way finger prints are used in the identification of human beings (Stuessy, 2009)[7]. Pollen grains also provide one of the best taxonomic criteria being often constant and visible under the microscope and have been used to place genera within families (Backlund and Nilson, 1997) [8]. At higher taxa levels pollen data has been used in suggesting relationships (Nowicke and Skavarla, 1979) [9]. Pollen has been used in the classification of the Caesalpinoideae (Graham and Barker, 1981) [10]. Pollen grains have also been useful in defining relationships at species level in the Dioscoreaceae [7]. The variation in shape, aperture character, Polar axis, equatorial axis, P/E ratio, Pollen size, shape class and amb have been the source of delimitation of various taxa[9]. 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)[11],[5], Papilionoideae (Wael, 2009) [12] justifies its application in this study of *Indigofera*, a member of the subfamily Papilionoideae. Numerical taxonomy enables classification based on either one or a few

*Corresponding Author: Sikolia[1]
[1]*Botany Department, Maseno University, Kenya*

characters or one set data (Sneath and Sokal, 1973[13]; Stace, 1989[14]). Other several advantages include integration of data from variety of sources, and automated processing of data which promotes efficiency. Keys can be developed from the data, and this has posed some fresh questions concerning initial classification and thus need for reexamination of certain classification systems (Guarcharan, 2004) [15]. Naturally, the selection of the characters will significantly affect the outcome in a cluster solution as should therefore be taken to avoid the inadvertent choice of one or more characters that may result in incorrect interpretations [13]. Determination of cluster numbers in a classification is one of the biggest problems in taxonomy. One of the earliest approaches was the use of phenon line [13]. The two-step clustering procedure automatically finds the optimal number of the clusters through the calculation of certain criteria statistics Akaike information criterion (AIC) and Swarchz-Bayesian information criterion (BIC), (Mooi and Sarstedt, 2010[16]; IBM SPSS, 2011[17]). No palynological study has previously been done on the 17 *Indigofera* species in the Maseno University Herbarium, The palynological studies in this research focuses on the pollen size, pollen shape, shape class, polar axis, equatorial axis and the P/E of the pollen of 17 species of the genus Indigofera and, the information obtained through cluster analysis will be of great use to plant taxonomists, as it will facilitate the reexamination of the past classifications, and thus help resolve the affinities of the Indigofera species affinities in conflict. The palynological data will also be available for integration with other data in order to come up with more natural classification of *Indigofera* species. This study is based on the hypothesis that although pollen characteristics are useful are more important than the others hence the need for a numerical assessment if the variable importance in various cluster solutions.

II. MATERIALS AND METHODS

The 17 curated and accessioned *Indigofera* species were obtained from Maseno University herbarium are shown in table l.

Fine tipped forceps, were used for the removal the pollen bearing flowers on the herbarium material [14].

Table 1. Inventory of *Indigofera* species used for palynological characterization

Taxon	Country	Altitude (m)	Date	ACC/No.	Collector
I. tanganyikensis	Kenya, Busia road, River Yala	2990	26/05/1967	DEKKER 142	William Dekker
I. dendroides	Uganda, Q. Elizabeth Park	2990	12/05/61	E.A.H. 703	William Dekker
I. spicata	Kenya Yala	2990	12/05/61	DEKKER 898	William Dekker
I. subulata	Kenya, coast Province, Ukunda	50	Aug. 1957	SYMES 170	Symes
I. volkensii	Uganda, Karasuk	4500	17/06/1959	SYMES 597	Symes
I. fulvopilosa	Uganda, Sese Islands	3900	11.10.1958	SYMES 488	Symes
I. hirsuta	Uganda, Bugalla	3900	11.10.58	SYMES 489	Symes
I. drepanocarpa	Uganda, Sese Islands	4000	12.10.58	SYMES 493	Symes
I. paracapitata	Uganda, Sese Islands	4000	12.10.58	SYMES 464	Symes
I. asparagoides	Uganda, Sese Islands	4000	12.10.58	SYMES 504	Symes
I. ambalacensis	Uganda, Queen Elizabeth Park	2990	12.5.61	SYMES 698	Symes
I. vohemarensis	Kenya, Coast Province	50	Aug. 1957	SYMES 168	Symes
I. subargentea	Kenya,Trannzoia	6400	8.9.61	SYMES 771	Symes
I. zenkeri	Kenya, Busia Road, Yala	2990	8.6.67	DEKKER 157	William Dekker
I. arrecta	Kenya, Yala	2990	28.2.1974	DEKKER 701	William Dekker
I. vicioides	Uganda, Karasuk	5900	17.6.59	SYMES 590	Symes

The instruments were cleaned with fresh ethanol after every use to avoid contamination and wiped with a fresh paper towel. Thick peduncle flowers were cut using a razor blade. Five flower of each species were put in the centrifuge tubes, boiled for five minutes in a boiling water bath (Typ: W600 DINI 2877-KI, GERMANY Model) with 5ml of 10% Potassium hydroxide stirring at intervals to soften and remove the pollen from the anthers. The polleniferous material was filtered through wire gauze of 200µm through gravity, centrifuged and then decanted using a pipette (Reitsima, 1969[18]; Reitsma, 1970[19]). The pollen filtrates obtained were acetolysed for 5minutes according to the method of Reitsima [18] using acetolysis mixture; 9 parts acetic anhydride; 1 part concentrated sulphuric acid prepared by adding sulphuric acid slowly to the acetic anhydride, 1ml of solution (5ml glycerin and 5ml distilled water) was added to first 2.5ml of polleniferous material and allowed to stand for 10 minutes, centrifuged and decanted. Light microscopy was done at Maseno University, Botany laboratory using Nikon Type-102 Microscope under 10X eyepiece, (E40, 0.65) and oil immersion (E100, 1.25). The eyepiece was calibrated using the ocular micrometer and the stage micrometer. Readings were taken in UM for quantitative characteristics (Polar axis, equatorial axis, and the P/E ratio). The mean values of polar axis, equatorial axis and pollen size were recorded, while the pollen size, aperture character, aperture position, shape class and the amb were coded as 1, 2, 3, or 4 according to table 2.

Table 2: Coding of qualitative characteristics used for cluster analysis and correlation analysis.

Character	State 1	State 2	State 3	State 4
Pollen size	Small	Medium	Large	Very large
Aperture character	Colpate	Porate	colporate	
Aperture position	Zonal	Global		
Shape class	Oblate	Oblate spheroidal	Spheroidal prolate	prolate
Amb	Angular straight	Angular convex	Non angular	

III. RESULTS

Table 3 shows the coded values for the qualitative characteristics (size, aperture position, shape class, and amb) and the mean quantitative characteristics (polar axis, equatorial axis and the P/E ratio). *I. tanganyikensis* has the highest mean polar axis and P/E ratio. *I. drepanocarpa* had the highest mean equatorial axis. Tables 4, the clusters formed were three and four at points at which the distance changes were 1.261 and 1.105, respectively.

Table 3. Summary table of the qualitative and the quantitative characteristics of the 17 *Indigofera* species studied.

Taxon	P(µm)	E(µm)	P/E	Sz	Ap.ctr	Ap.P	Sh.cl	Amb.
I. tanganyikensis	33.25	27.5	1.222	2	3	1	4	2
I. dendroides	28.34	24.94	1.15	2	3	1	3	1
I. spicata	29.25	25	1.17	2	3	1	3	3
I. sabulata	28	24.25	1.17	2	1	1	3	1
I. volkensii	25.88	24	1.09	2	1	1	3	3
I. fulvopilosa	30.58	26	1.18	2	3	1	3	1
I. hirsuta	25.45	21.75	1.17	2	3	1	3	1
I. drepanocarpa	37.56	34.34	1.11	2	3	1	3	2
I. paracapitata	28.75	24.31	1.15	2	1	1	3	2
I. asparagoides	36.31	31.06	1.17	2	3	1	3	2
I. ambelacensis	29.56	25.13	1.18	2	3	1	3	3
I. vohemarnsis	26.81	23.69	1.09	2	3	1	3	1
I. subargentea	25.56	24	1.08	2	3	1	3	2
I. circinella	27.44	22.25	1.28	2	1	1	4	2
I. zenkeri	26.75	24.69	1.1	2	2	1	3	1
I. arrecta	25.19	23.38	1.08	1	3	1	3	3
I. viciodes	23.63	17.5	1.14	1	3	1	3	3

Table 4. Akaike Information criterion for cluster solutions using mixed variables

Number of clusters	Akaike Information criterion (AIC)	AIC Change	Ratio of AIC changes	Ratio of distance measures
1	307.678			
2	286.242	-21.435	1.000	1.678
3	278.321	-7.921	.370	1.261
4	274.521	-3.800	.177	1.105
5	272.223	-2.298	.107	1.655
6	275.585	3.362	-157	1.538
7	281.969	6.384	-298	1.091
8	288.821	6.851	-320	1.018
9	295.762	6.942	-324	1.767
10	304.900	9.138	-426	1.759
11	315.273	10.373	-484	1.437
12	326.141	10.868	-507	1.085
13	337.097	10.956	-511	1.297
14	348.292	11.195	-522	2.206
15	359.927	11.635	-543	16.682
16	371.905	11.978	-559	D

a) The changes are from previous number of clusters in the table
b) The ratios of the changes are relative to the change for the two cluster solution
c) The ratios of distance measurers are based on the number of clusters against the previous number of cluster.
d) The distance at the current number of clusters is zero.

Figs. 1. and 2. Overall variable importance for three and four–cluster solution Based on all the pollen variables

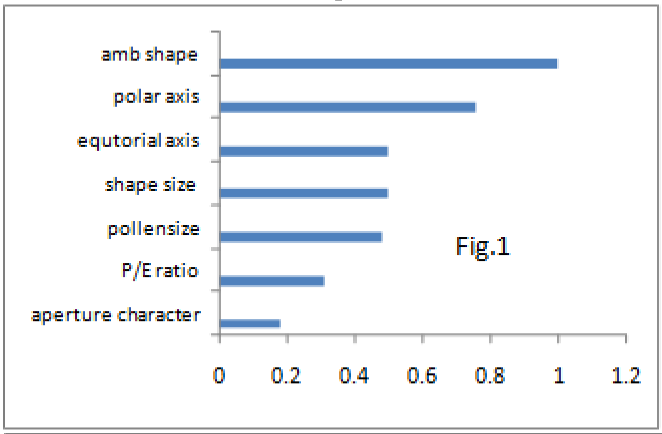


Fig.1

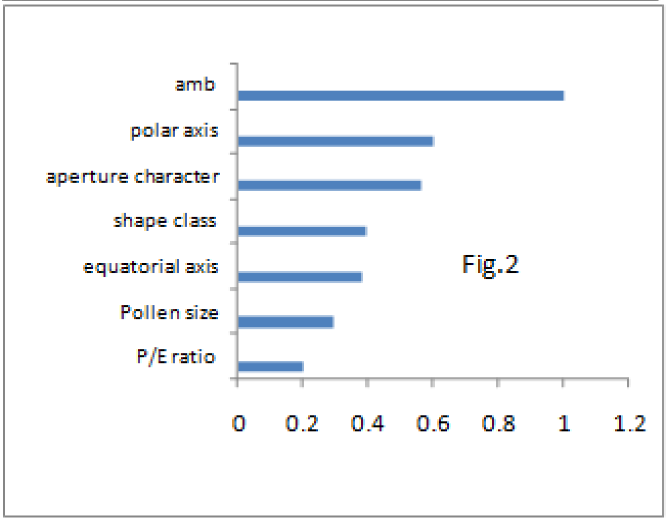
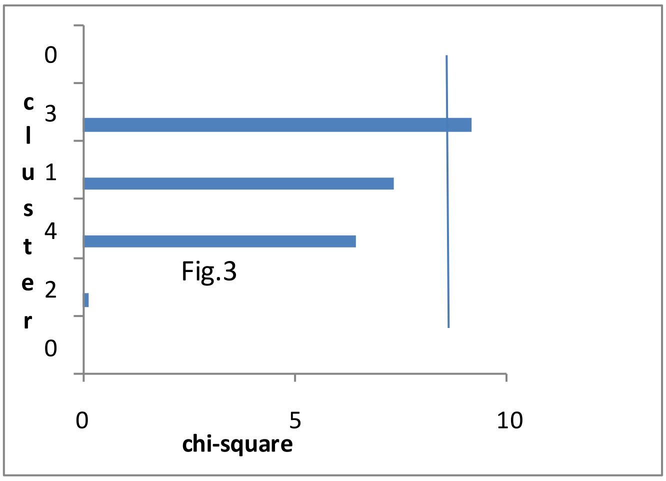
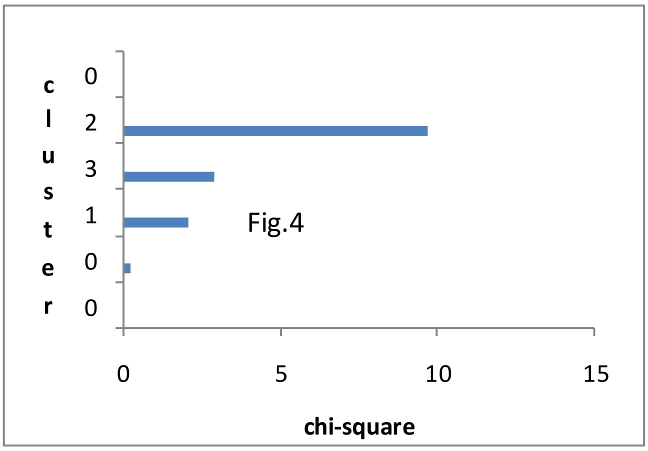


Fig.2





Figs. 3. and 4. Chi-Square cluster wise variable importance of characters in two Different four– cluster solutions.

Fig.1 and 2, The amb and the polar axis were the most important pollen characteristics in both the three and four cluster solution (Fig 1. and Fig.2). The polar axis importance is higher, 0.75 in a three cluster solution than 0.59 in a four cluster solution Fig. 3. Chi square cluster wise variable importance of amb in four–cluster solution.

Fig 4 Chi-Square cluster wise variable importance of aperture character in four–cluster solution.
Fig. 3, 4. The aperture character was critically important in cluster 2 of the four cluster solution using mixed variables (Fig 3) while the amb shape was critically significant in cluster 3 of the four – cluster solution using mixed variables (Fig. 4).

Two step cluster 3 Bonferroni Adjustment Applied

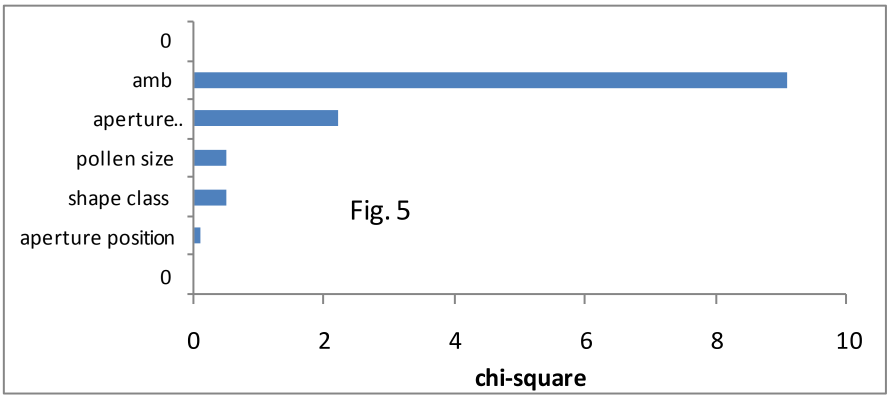


Fig. 5. Significant importance of amb shape in four–cluster solution mixed variables in cluster three.

*Corresponding Author: Sikolia[1]

Two step cluster 2 Bonferroni Adjustment Applied

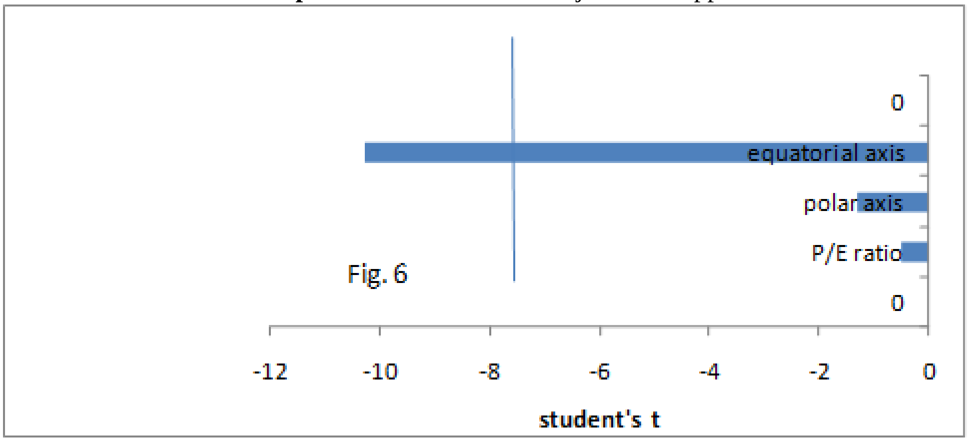


Fig. 6.Significant importance of equatorial axis in cluster two of four–cluster solution mixed variables.

Fig. 5 and 6 Figure 5 and 6 show a variable wise assessment of various pollen characteristics. The amb shape is the most important character in cluster three of four cluster solution (Fig. 5) while equatorial axis is the only quantitative character critically important in the characterization of the Indigofera in four – cluster solution using mixed variables (Fig. 6). Equatorial axis is the only critically important parameter in the characterization of cluster 2-4CS members (Fig.6.). The Amb is just critically important feature in the characterization of the cluster 3-4CS species (Fig.5.)

In the determination of the optimal number of clusters, precaution was taken to ensure the relative number of clusters were meaningful as advised by Steinbach, Tan, and Kumar (2004) [20]. The three and four cluster points also occur at 1.261 and 1.105 distance changes respectively (Table, 4). More than four clusters could have been of low resolution, limited distinction, and reduced weights (significances). The quantitative and qualitative values of t and chi square statistics.

IV. DISCUSSION

Quite often most taxonomic studies in the real world have both quantitative as well as qualitative variables [7]; the challenge however is the integration of both the different types of variables in the development of cluster solutions. It was therefore necessary to use the two step cluster analysis to come up with a cluster solution but at the same time compare the results with the others. The qualitative characteristics coded (Table 3) so as to numerically analyze by the algorithm. The integration of both qualitative and quantitative data would show effects as well as expose the dynamism in the distribution of alternative character states among the pollen characteristics in the 17 *Indigofera* species studied.

Examination of the variable importance the algorithm used exposed the cluster wise (Figs. 3 and 4) and variable wise (Figs.5 and 6) variable importance. The aperture position was the least important variable in distinguishing the phenons since all pollen has the zonoapertuarate distribution. In figures 1 and 2, the importance of various pollen characteristics varies between the three and four cluster solution except for the aperture position. This variation can be attributed to the shifting in the membership between the three and four cluster solutions [9]. The shifting of membership changes the distribution of various character states of the pollen characters. While the predictor importance charts have put the importance of various variables in a scale of 0to1; the cluster wise importance of the variables was used to determine which of the variables was significant in the characterization of the *Indigofera* species studied. The cluster 2 of the four–cluster solution based on mixed variables, show a critically important aperture character (Fig.4), and amb for the characterization of the cluster 3 (Fig 3). The critical importance is based on the chi square statistics (IBM SPSS, 2011) [17] and the t statistics of equatorial axis-a continuous variable. Apart from the aperture character and amb shape importance there is no other qualitative variable critically important in cluster two and three respectively in the four–cluster solutions using mixed variables. In cluster two (Fig.6) equatorial axis is the only quantitative variable that is critically important in the characterization of the cluster, however the importance value is negative. This could be attributed to the fact that the mean of cluster two for the equatorial axis is less than those of the other clusters as well as the overall equatorial axis mean for the four clusters.

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 [5]. With few exceptions, ten pollen types recognized in the Papilionoideae [5] correspond to Polhill and Raven's tribal classification Polhil (1981) [21] 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 [3][5]. Advancement in the microscopy should refine the studies of palynology and its application in science, especially taxonomy.

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