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Diagnostic Petiole Anatomical Characters and Their Systematic Importance in 18 Species of the Genus *Tephrosia* Pers. (Fabaceae) found in Nigeria.

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Abstract:
A comprehensive systematic anatomical investigation of diagnostic petiole characters of eighteen (18) species of *Tephrosia* found in Nigeria was carried out. *Tephrosia* Pers. belongs to the family Fabaceae, tribe Milletteae. It habits the fringes of the forests, abandoned wastelands. The eighteen (18) species include *T. bracteolata, T. candida, T. densiflora*, *T. elegans, T. flexuosa, T. hildebrandtii* and *T. hirsuta*. Others are *T. interupta, T. leptostachya, T. linearis, T. lupinifolia, T. mossiensis, T. nana, T. noctiflora, T. paniculata, T. pedicellata, T. purpurea and T. vogelii.* The study is aimed at using petiole characters to delimit, and establish any infrageneric groups based on the variations and affinities within the petiolar characters. A total of nine (9) diagnostic polymorphic characters were observed, photographed, assessed, scored and coded. These include Petiole Symmetry (PSY), Petiole Outline (POL), Petiole Vascular System (PVS), Petiole Perivascular Tissue (PPT), and Petiole Trichome Cell type (PTC) and Petiole Trichome Base (PTB). These characters are either multistate or binary. Principal Component Analysis and Cluster Analysis were carried out. A total of six (6) tables and three (3) figures were generated from the statistical treatment of the data matrix constructed. The Principal Component Analysis using Extraction method revealed four (4) principal components. High Level of dissimilarity among the species suggests distinct species. Highest number of similar species was three (3) which are *T. leptostachya, T. purpurea* and *T. mossiensis* while in morphology are much alike. The cluster analysis separated the group into twelve (12) hierarchies.

Keywords: *Tephrosia*, Petiole, Anatomy, Systematics, Identification.

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

The genus *Tephrosia* Pers. is a pan-tropical one belonging to the family Fabaceae. It has about 400 species worldwide, of which only 5% are represented in Nigeria [1]. The genus members are majorly found in the Sub-Sahara region of the country. They are growing on the fringes of the forests, and on abandoned or waste lands. The use of both living and dead plant materials to obtain useful diagnostic information has been established in various works of [2] and [3]. The taxonomic challenges of the genus dates back to the work of [4] and [5] who first worked on the members and named the genus *Cracca* L. The present accepted name *Tephrosia* Pers. was coined by Persoon, C.H. (1807) nom.cons. Before and after, these different names had been coined but rejected (nom rej). The genus *Tephrosia* Pers. had been classified into subgenera and sections by various authors as shown in Table I.

Despite the taxonomic treatments, *Tephrosia* species had been plagued with the problem of synonyms. The genus *Tephrosia* has shown to be of many uses to man in areas of agriculture, horticulture, fish farming, and medicinal. Species like *T. candida, T. densiflora* and *T. vogelii* are frequently used for piscicidal activities to stupefying fish in the water which is due to the presence of the substance called rotenone [6]. *T. vogelii* has horticultural value because of its dense inflorescence and flowering nature. Generally, *Tephrosia* have been shown to contain the active principles that have molluscidal activities by various authors like [7], [8] and [9]. Pesticidal activities in storage of maize and beans against borers and weevil. [10] established the anti-helminthic activity of the methanolic leaf extract of *T. purpurea*, expelling intestinal parasitic worms.

The use of petiolar characters to solve taxonomic problems has been established for several genera as up to the work of [11], [12], [13] and [14].

This study therefore aimed at elucidating the petiolar anatomical variations and the possibility of utilizing this to classify at the sub-generic level for the genus *Tephrosia.*

2. METHODOLOGY

2.1 Plant Materials

Both dry herbarium and fresh leaf specimens were collected for the 18 species of *Tephrosia* in Nigeria and used for the petiole studies. The herbaria include the Forest Reserved Herbarium (FHI), Lagos University Herbarium (LUH) and University of Ibadan Herbarium (UIH), while fresh samples were collected from various locations of Badagry Division (6.4316°N, 2.8876°E) of Lagos State, Nigeria.

2.2 Rehydration and Sectioning

The whole petiole was cut off from the leaf using a new sharp blade. The species petiole sectioned include those of *T. bracteolata, T. candida, T. densiflora, T. elegans, T. flexuosa, T. hildebrandtii, T. hirstuta, T. interupta, T. leptostachya, T. linearis, T. lupinifolia, T. mossiensis, T. nana, T. moctiflora, T. paniculata, T. pedicellata, T. purpurea* and *T. vogelii*. The cut petiole length for each was subjected to serial rehydration of grade 70%, 50%, 30%, 10% and distilled water. Three (3) rehydrated petiole samples were then thinly hand-sectioned using a sharp blade, at the mid (median) portion, as described in work of [2]. In cases of dry petiole specimens, petioles were briefly boiled at 60°C water-bath to loosen and soften the tissues before sections were made. In fresh leaf, samples were collected under sunny weather between 13:00hr – 15:00hr GMT from matured healthy and fully developed leaves. Petioles were exercised off and fixed in the preservative FAA (3:1 acetic acid alcohol) for few days before use in line with the methods of [15].

2.3 Staining

Sections were stained using 2 or 3 drops of Safranin stain on clean slides. They were quickly flipped over a low flame burner 3-5 times. Second round 2 to 3 drops of Safranin stain was applied to the cut samples on the slide and then covered with a cover-slip carefully to expel any air trapped. The stained preparations were again dried by smearing it over a low flamed burner in line with [16]

2.4 Light Microscopy

Three (3) samples of prepared petiole slide per species were observed under Olympus compound light microscope at both objective lens of ×4 and ×10. Photomicrograph were taken with a digital camera in a HP laptop system. From the images taken, polymorphic characters were observed, studied and recorded and analyzed.

2.5 Statistical Treatment

Petiolar characters were scored and coded from the qualitative characters tables to generate a data matrix as shown in Table 4 for the purpose of determining the closeness or similarities. A total of nine (9) petiole characters were assessed. These are Petiole Symmetry (PSY), Petiole Outline (POL), Petiole Vascular System (PVS), Petiole Perivascular T issue (PPT), Petiole Trichome Existence (PTC), Petiole Trichome Abundance (PTA), Petiole Trichome Occurrence (PTO), Petiole Trichome Cell type (PTC) and Petiole Trichome Base (PTB). Both multistate and binary characters were included. From the character data matrix, SPSS 20.0 Statistical Package was used to deduce the Principal Component Analysis table, Proximity Matrix table, Correlation Matrix for the nine (9) characters, Correlation Matrix for the components extracted and Rotated Component Matrix for the extracted components. Scree plot of Eigenvalue against Component number was plotted as shown is Figure 2. And lastly, a dendrogram using the average linkage was generated.

3. RESULTS AND DISCUSSION

The use of petiole characters to resolve some systematic problems in the groups cannot be over-emphasized. Several works in this aspect include those of [13] in petiole anatomy in Cinnamomum, [15] in the tribe Dipterocarpeae (Diptorecarpaceae), [17] on Asteraceae among others. Nine (9) anatomical characters were assessed from the photograph images of the eighteen (18) species of *Tephrosia* studied as shown in Figure 1(a-r). The descriptive diagnostic characters were qualitatively described in Table II. The character state was assigned numeric Figures 1, 2, 3, etc. (Table III), dictated in parenthesis for the purpose of generating quantitative matrix data (Table IV).

The polymorphic petiole diagnostic characters used in this study are nine (9) in all (Table III). To have a more accurate and appropriate interpretation of the results obtained from the petiolar studies, Component analysis and Cluster analysis were generated out to produce a framework of the similarity or affinity degree among the species of the *Tephrosia,* using the un-weighted pair group clustering method. The result from the Principal Component Analysis (PCA) (Table V) showed that only four (4) components were extracted, and played major role in the establishment of degree of similarity and delimitation of the species (Table VI). Petiole Symmetry (PSY), Petiole Vascular System (PVS), Petiole Perivascular Tissue (PPT) and Petiole Trichome Abundance (PTA) are the characters that constitute Principal Component Analysis (PCA-1), Petiole Trichome Occurrence (PTO) and Petiole Trichome Base (PTB) formed PCA-2, Petiole Trichome Existence (PTE) formed PCA-3 while Petiole Outline (POL) and Petiole Trichome Cell type (PTC) formed PCA-4. Proximity matrix generated showed the degree of dissimilarity within the species (Table VI). Results showed that four (4) Principal Component consisting 22.618%, 21.554%, 15.18% and 13.3% variance were extracted in the qualitative characters of the species. The PCA revealed the accumulative contribution rate of the four principal components to be 72.604% (Table V).

From the similarity matrix based on the correlation coefficient of the species, the highest correlation exists between Petiole Vascular System (PVS) and Petiole Symmetry (PSY) with the highest value of 0.496. The lowest correlation was shown by Petiole Trichome Cell type (PTC) and Petiole Trichome Occurrence (PTO) with the value 0.055. The values are low in general. Hence, they are not related. Since, they are not related but they can jointly give a useful information concerning the similarity and in the delimitation of the genus *Tephrosia*. However, there exist some high negative correlations between some characters. These include Petiole Trichome Occurrence (PTO) and Petiole Vascular System (PVS) with the value -0.399 also Petiole Trichome Base (PTB) and Petiole Vascular System (PVS) with the value -0.340. This simply means a lesser or insignificant role in taxa delimitation.

In addition, the phenogram (Figure 3) showed relationship between qualitative petiolar characters of the 18 species of *Tephrosia* in Nigeria. Results of cluster analysis on the species revealed that based on characters studied, various levels of phenotypic relationships were generated. It was observed that a total number of twelve (12) hierarchies (clusters) were established. The twelve clusters are represented using numeric figure 1-12. It was observed that *T. bracteolata* and *T. hirsuta* showed certain level of similarities hence constituted cluster 1, *T. candida* and *T. linearis* constituted cluster 2, *T. densiflora* and *T. pedecillata* formed the 3rd clusters, *T. elegans* constituted the 4 cluster while *T. flexuosa* and *T. vogelli* formed the 5 clusters which indicates certain levels of similarities based on the characters studied. It was also noticed that *T. hildebrantii* formed 6, *T. interupta* formed 7, *T. leptostachya, T. mossiensis* and *T. purpurea* made up of 8 cluster, *T. lupinifolia* formed the 9 and *T. nana* formed the 10 while *T. noctiflora* and *T. paniculata* constituted the 11 and 12 clusters in that order. This simply means that the petiolar anatomical characters cannot be used alone but in conjunction with other sources of taxonomic characters for the purpose of delimitating the genus *Tephrosia* in Nigeria.

4. CONCLUSION

This study has not only revealed the petiolar polymorphism but equally shown that some petiole anatomical characters can be of high diagnostic and systematic values to delimit species of *Tephrosia.* However, the use of petiole anatomical characters in systematics of genus *Tephrosia* must be taken with caution, as there is integrating pattern or continuous variation shown among the species. Also, potential influence of various factors like plant habit, age, edaphic and other environmental factors on the petiole anatomical characters must not be ignored in applying obtained results to the infra-generic systematic treatment. It is more reliable to use both quantitative and qualitative petiole anatomical characters for taxonomic purposes in combination with the phytography data.

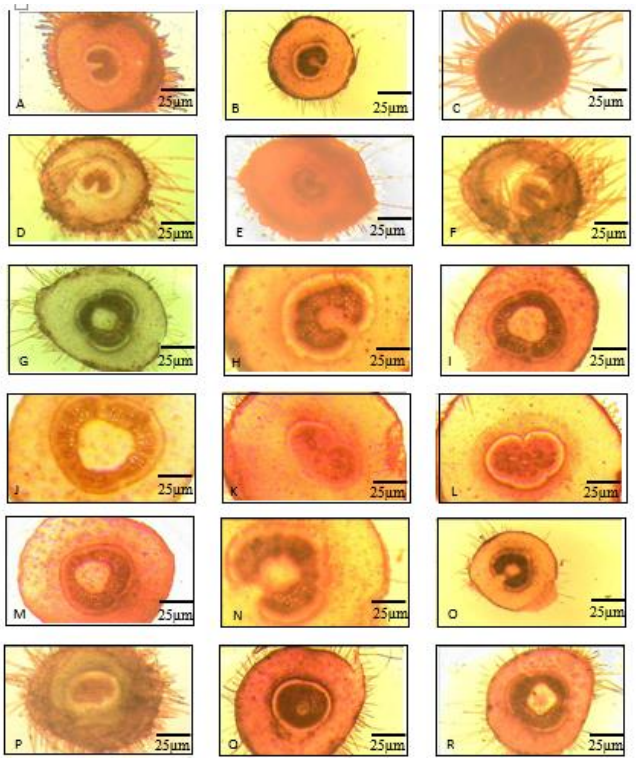


Fig 1 (A – R): Petiole anatomy of Tephrisia spp in Nigeria. A – *T. bracteolata*, B – *T. candida*, C – *T. densiflora*, D – *T. elegans*, E – *T. flexuosa*, F – *T. hildebrandtii*, G – *T. hirsuta*, H – *T. interupta*, I – *T. leptostachya*, J – *T. linearis*, K – *T. lupinifolia*, L – *T. mossiensis*, M – *T. nana*, N – *T. noctiflora*, O – *T. panicluata*, P – *T. pedecillata*, Q – *T. purpurea*, R – *T. vogelii* Scale bar = 25µm

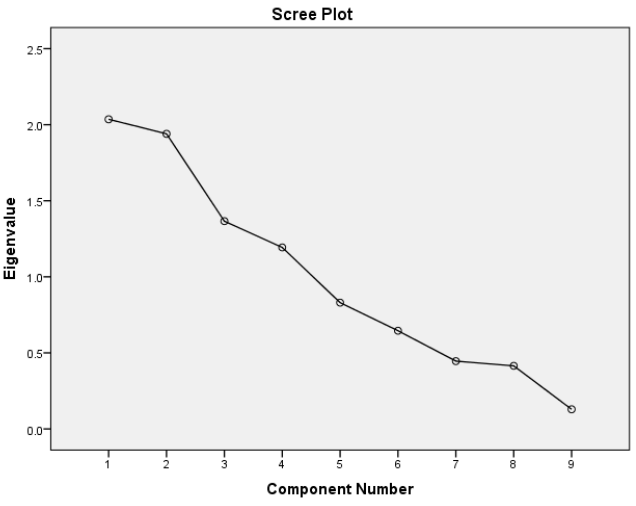


Fig. 2: Scree plot graph showing the four (4) principal components among the nine (9) petiolar characters used in this study.

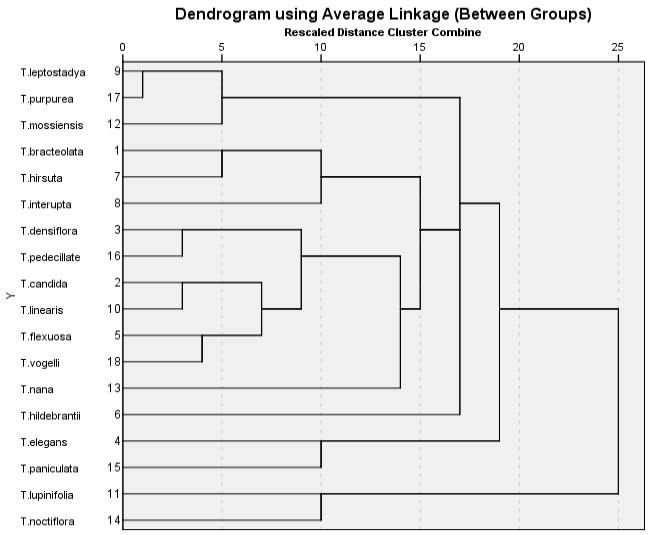


Fig. 3: Cluster analysis of eighteen (18) species of *Tephrosia* based on combined nine (9) petiolar characters

Table I: Comparison of classification systems in *Tephrosia*

S/N	AUTHORS	YEAR	CLASSIFICATION SYSTEM
1	De Candle, Alphonse	1825	Grouped into four (4) sections based on Style Indumentum which are: • Mundulea • Brissonia • Croccoides • Reineria
2	Bentham, George Baker, John Gilbert	1865 1871	Grouped into three (3) subgenera based on Style Indumentum which are: • Macronyx • Brissonia • Reinera
3	Wood, C. E. Jr.	1949	Grouped into two (2) groups based on Style Indumentum which are: • Glabristyled • Barbistyled
4	Brummit, Richard Kenneth	1981	Grouped into two (2) subgenera based on Style Indumentum which are: • Glabrous style • Trichiferous style

Table II: Tabular summary of qualitative petiole anatomical diagnostic characters

Species	Petiole Symmetry (PST)	Outline (POL)	Vascular bundleshape (PVS)	Perivascular tissue (PPT)	Trichome Existe	Petiolar Trichomes characters (PTX)			
						Abundance (PTA)	Occurrence (PTO)	Cell (PTC)	Base (PTB)
T. bracteolata	Sym.	Dorsi-vent flat	Open-circular	Sclerenchyma	Present	High	Tufted	Uniseriate	Unmodified
T. candida	Sym.	Sub-circular	Open-circular	Sclerenchyma	Present	High	Singly	Uniseriate	Unmodified
T. densiflora	Sym.	Sub-circular	Open-circular	Collenchyma	Present	High	Tufted	Uniseriate	Unmodified
T. elegans	Sym.	Circular	Open-circular	Collenchyma	Present	Few	Tufted	Multiseriate	Unmodified
T. flexuosa	Sym.	Sub-circular	Open-circular	Collenchyma	Present	Few	Singly	Uniseriate	Unmodified
T. hildebrandtii	Non-sym.	Sub-circular	Open-circular	Sclerenchyma	Present	High	Tufted	Uniseriate	Unmodified
T. hirsute	Sym.	Dorsi-vent flat	Open-circular	Sclerenchyma	Present	High	Singly	Uniseriate	Unmodified
T. interupta	Sym.	Dorsi-vent flat	Open-circular	Sclerenchyma	Present	High	Singly	Multiseriate	Unmodified
T. leptostachya	Sym.	Sub-circular	Crescentiform	Sclerenchyma	Present	High	Singly	Uniseriate	Modified
T. linearis	Sym.	Circular	Closed-circular	Sclerenchyma	Present	High	Singly	Uniseriate	Unmodified
T. lupinifolia	Non-sym.	Dorsi-vent-circular	Semi-circular	Collenchyma	Present	Sparce	Singly	Uniseriate	Unmodified
T. mossiensis	Sym.	Subcircular	Open-circular	Sclerenchyma	Present	High	Singly	Uniseriate	Modified
T. nana	Sym.	Dorsi-vent-circular	Closed-circular	Sclerenchyma	Present	Sparce	Singly	Uniseriate	Unmodified
T. noctiflora	Non-sym.	Dorsi-vent-circular	Crescentiform	Collenchyma	Present	High	Singly	Multiseriate	Unmodified
T. paniculata	Sym.	Circular	Open-circular	Sclerenchyma	Present	Few	Singly	Uniseriate	Unmodified
T. pedicillata	Sym.	Subcircular	Open circular	Collenchyma	Present	Few	Tufted	Uniseriate	Unmodified
T. purpurea	Sym.	Subcircular	Crescentiform	Sclerenchyma	Present	High	Singly	Uniseriate	Modified
T. vogelii	Sym.	Circular	Open-circular	Collenchyma	Present	High	Singly	Uniseriate	Unmodified

Table III: The coded qualitative petiole anatomical diagnostic characters of the eighteen (18) species studied.

CN	DIAGNOSTIC CHARACTERS	CODE	CHARACTER STATE (VALUE)
1	Petiole Symmetry	PSY	Symmetrical (1); Non-symmetrical (2)
2	Petiole Outline	POL	Circular (1); Sub-circular (2); Dorsi-ventral circular (3); Dorsi-ventral flat (4)
3	Petiole Vascular Bundle Shape	PVS	Open-circular (1); Closed circular (2); Crescentiform (3); Semi-circular (4)
4	Petiole Perivascular Tissue	PPT	Sclerenchyma (1); Collenchyma (2)
5	Petiole Trichome Existence	PTE	Present (1); Absent (2)
6	Petiole Trichome Abundance	PTA	High (1); Few (2); Sparce (3)
7	Petiole Trichome Occurrence	PTO	Singly (1); Tufted (2)
8	Petiole Trichome Cell Type	PTC	Uniseriate (1); Multiseriate (2)
9	Petiole Trichome Base	PTB	Modified (1); Unmodified (2)

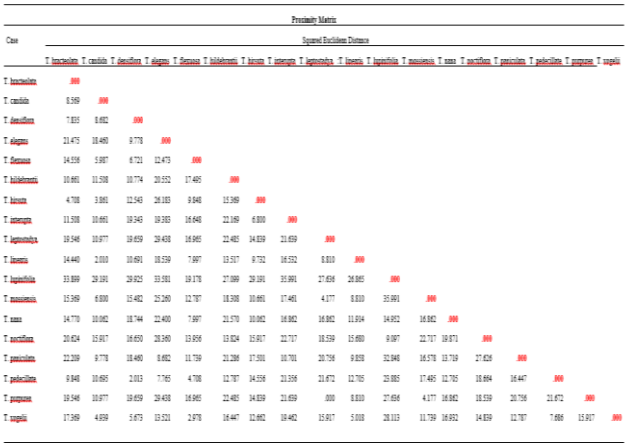
Table IV: Data matrix generated for determination of similarity index or affinity among the 18 *Tephrosia* species using nine (9) petiolar diagnostic characters.

Species	Petiole Symmetry (PSY)	Outline (POL)	Vascular bundle shape (PVS)	Perivascular tissue (PPT)	Trichome Existence (PTE)	Petiolar Trichomes characters			
						Abundance (PTA)	Occurrence (PTO)	Cell (PTC)	Base (PTB)
T. bracteolata	1	4	1	1	1	1	2	1	2
T. candida	1	2	1	1	1	1	1	1	2
T. densiflora	1	2	1	2	1	1	2	1	2
T. elegans	1	1	1	2	1	2	2	2	2
T. flexuosa	1	2	1	2	1	2	1	1	2
T. hildebrandtii	2	2	1	1	1	1	2	1	2
T. hirsute	1	4	1	1	1	1	1	1	2
T. interupta	1	4	1	1	1	1	1	2	2
T. laptostachya	1	2	3	1	1	1	1	1	1
T. linearis	1	1	2	1	1	1	1	1	2
T. lupinifolia	2	3	4	2	1	3	1	1	2
T. mossiensis	1	2	1	1	1	1	1	1	1
T. nana	1	3	2	1	1	3	1	1	2
T. noctiflora	2	3	3	2	1	1	1	2	2
T. paniculate	1	1	1	1	1	2	1	1	2
T. pedicillata	1	2	2	2	1	2	2	1	2
T. purpurea	1	2	3	1	1	1	1	1	1
T. vogelii	1	1	1	2	1	1	1	1	2

Table V: Principal component analysis of the nine (9) petiolar characters (Total Variance Explained) used in the study.

Total Variance Explained									
Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	**2.036**	**22.618**	**22.618**	**2.036**	**22.618**	**22.618**	**1.849**	**20.545**	**20.545**
2	**1.940**	**21.554**	**44.172**	**1.940**	**21.554**	**44.172**	**1.741**	**19.345**	**39.889**
3	**1.366**	**15.175**	**59.347**	**1.366**	**15.175**	**59.347**	**1.600**	**17.775**	**57.665**
4	**1.193**	**13.257**	**72.604**	**1.193**	**13.257**	**72.604**	**1.345**	**14.939**	**72.604**
5	.830	9.226	81.830						
6	.645	7.172	89.002						
7	.446	4.954	93.957						
8	.415	4.609	98.565						
9	.129	1.435	100.000						

Table VI: Proximity matrix of the 18 species of *Tephrosia* studied based on nine (9) petiolar characters.



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