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NUMERICAL TAXONOMY AND MORPHOLOGICAL VARIABILITY OF THE GENUS *ZIZIPHUS* IN KATSINA STATE, NORTHWEST NIGERIA

¹Gidado M. S., and ^{1,2*}Yaradua S. S.

¹ Department of Biology, Umaru Musa Yaradua University, Katsina, Nigeria

² Department of Geography, Environmental Management and Energy Studies, APK Campus, University of b Johannesburg, Johannesburg 2006, South Africa.

*Corresponding Author: dryaradua@gmail.com

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Abstract

The genus *Ziziphus* is a tropical genus that is native to the Northern hemisphere. The identification of the species in the genus is confusing due to their phenotypic diversity. In this study, numerical taxonomic approach was employed to identify the species in Katsina and to evaluate the useful characters to be use in delimiting the species. A total of 40 specimens were collected and subjected to morphological examination using both qualitative and quantitative characters. The result of the multivariate analysis revealed four major clusters at Euclidean distance of 0.58 and cophenetic correlation coefficient value of (r)=0.675. The result revealed that *Ziziphus* is represented by four species in Katsina, namely: *Z. spina-christi*, *Z. mauritiana*, *Z. mucronata* and *Z. abyssinica*. The petiole length, seed weight, leaf length are the important characters that were useful in delimiting the species with coefficient of variation of 41.06 %, 39.08 % and 33.67 %, respectively.

Keywords: Katsina State, Morphological variability, Numerical taxonomy, *Ziziphus*

INTRODUCTION

In the Old and New World's tropical and subtropical climates, the genus *Ziziphus* Mill., sometimes known as Ber, is classified as a member of the Rhamnaceae family (Liu and Cheng, 1995). The Rhamnaceae family includes ca. 57 genera (Hauenschild et al., 2016) and approximately 900 species, including trees, shrubs and climbers (Richardson et al., 2000). According to Medan and Schirarend (2004), the paraphyletic,

pantropical genus *Ziziphus* has over 100 species and is geographically divided into three different lineages; *Ziziphus* (old world *Ziziphus*), *Sarcomphalus* and *Condoliopsis* (New world *Ziziphus*) (Islam and Simmons, 2006; Islam and Guralnick, 2015; Hauenschild et al., 2016).

Ziziphus are cosmopolitan in distribution found mainly in the warm-temperate and subtropical regions of the world. This

genus is dominated by thorny shrubs or small trees with presence of pair of stipular spines, they are found in warm-temperate and subtropical regions throughout the world (Evreinoff, 1964; Bhansali, 1975; Liu and Qi, 2004). This genus has the most widely distributed members among all Rhamnaceae family.

The members of this genus are of economic and various medicinal importance which involve the uses of different plant parts for various purposes. Species of *Ziziphus* were in use for thousands of years in medicinal systems to treat various diseases, especially fever, diabetes and skin infections, and they have been reported to have antipyretic, antinociceptive, antioxidant, antibacterial and larvicidal activities (Hemmati *et al.*, 2015; Hamedi *et al.*, 2016; Verma, 2016; Verma *et al.*, 2018; Sobhani *et al.*, 2019). Based on the information from our local communities, the species are used as an immune system stimulant, anti-hypoglycemic, anti-inflammatory, anti-tumor, antioxidant, antimicrobial, anticancer, anti-ulcer, analgesic, sedative and antipyretic.

Numerical taxonomy which is also referred to as taxometrics in biological systematics, is a categorization scheme that deals with the numerical grouping of taxonomic entities according to their character states. Instead of relying on a subjective assessment of their qualities, it seeks to build a taxonomy using numerical procedures like cluster analysis (Sokal and Sneath, 1963).

Katsina is the major center for distribution of the genus *Ziziphus* in Nigeria, despite the great distribution, there is no publication that reports the number of species of the genus and their phenotypic variability. The number of *Ziziphus* species in Katsina and their morphological variabilities are not clear. The flora of West Tropical Africa by Hutchinson and

Dalziel which is the reliable document that report the diversity of Nigerian species, did not report any *Ziziphus* species in Katsina. These species play important roles in ecosystems as primary producers, hosts for many insect species, and as sources of food and medicine for humans. A better understanding of the relationships among species within the genus *Ziziphus* can help in conservation efforts and in the development of more effective management strategies for these important plant species. The taxonomic status of the genus is still complex due to high range of morphological variability among the species, hence the need for the present study.

Materials and Methods.

Sampling

Series of field trips were conducted across the three senatorial zones of Katsina State and several specimens belonging to *Ziziphus* were collected noting the diagnostic features of the genus and with aid of various floras. Typical specimens were collected, voucher specimens were prepared and deposited in the herbarium of Department of Biology, Umaru Musa Yaradua University, Katsina. Both the floral and vegetative parts were carefully examined and subjected to morphometric analyses.

Morphological characters

Character set, comprising of sixteen quantitative and qualitative morphological characters were recorded from the 40 individual's samples collected (Table 1). The abbreviation of the vegetative, fruit and floral characters of the studied plants used in the numerical analyses are presented in Table 1. The quantitative characters related to length and width of leaves (leaf length, leaf width, leaf size,) were measured using a 30 cm plastic ruler, the length and diameter of fruits and seeds was measured using a Vernier caliper, the weight of the fruits and that of seeds was

measured using a weighing balance (MXBAOHENG).

Data analyses

The multivariate analyses were carried out using Cluster Analysis (CA) and Principal Component Analysis (PCA) using the program PAST 3 (version 3.05). These analyses will evaluate and determine the morphological similarity among the studied *Ziziphus* species and the features that contribute in delimiting the taxon. For the purpose of standardizing the data matrix throughout all analyses, the data were initially log10 transformed. Pearson correlation was used to determine the coefficient of variation of the characters examined.

Cluster analyses

Specimens were grouped based on the quantitative and qualitative characters used in the cluster analysis, those that are too similar are grouped into one cluster revealing their relationships and variations in between and within the clusters by creating a similarity matrix among studied

specimens. Members found on same cluster are regarded as same species. In order to determine how well cluster analysis fits the distance matrix, the cophenetic correlation between the distance matrix and tree matrix was determined (Sokal & Rohlf 1998).

Principal component analyses (PCA)

The use of PCA is to determine linearly correlated variables (Hotelling 1933, 1936). According to Humphries et al. (1981), PCA makes it possible to identify group distinctions as well as the relative contributions of size-dependent and size-independent variation to species discrimination. As a result, it will display the traits that help to distinguish between the species. The morphological similarity between the species was determined using the Jaccards similarity coefficient. Each specimen was treated as an operational taxonomic unit (OUT) and all 16 morphological features, including both quantitative and qualitative characters, for all 40 specimens were employed in the study.

Table 1: Quantitative and qualitative characters used in the multivariate analysis

Quantitative characters			Qualitative characters	
Character	Unit	Abbreviation	Character	State
		n		
Leaf length	cm	LL	Leaf apex	Mucronate (1), Acute (0)
Leaf width	cm	LW	Leaf arrangement	Acute (2)
Leaf size	cm	LS	Leaf base	Rounded (0), Aequilateral (1), Acute (2)
Petiole length	cm	PL	Leaf margin	Entire (0), Serrate (1)
Fruit length	cm	FL	Leaf shape	Obovate (0), Ovate (1)
Fruit diameter	cm	FD		
Seed length	cm	SL		
Seed diameter	cm	SD		
Thorn length	cm	TL		
Fruit weight	g	FW		
Seed weight	g	SW		

Results

Phenetic variability

In this study, the morphological traits of the investigated individuals showed a wide

range of diversity (Table 2). The coefficient of variation CV was highest in petiole length 41.06%, whereas the fruits weight has the lowest CV with 7.86 %.

Most of the characters showed significant differences between various species, the characters with the highest variation were petiole length, seed weight, leaf length, leaf size, thorn length, fruit weight, and leaf width having CV of more than 20% (Table 2).

In *Ziziphus spina-christi*, the leaf length ranged from 3.1 to 4.1 cm, in *Ziziphus mauritiana* it ranged from 2.8 to 4.1 cm, in *Ziziphus mucronata* the leaf length is 4.5 to 6.6 cm, and 4.4 to 6.0 cm in *Ziziphus abyssinica*. The fruit length of *Z. spina-christi* ranged from 1.32 to 1.65 cm, 1.02 to 1.29 cm in *Z. mauritiana*, 1.23 to 1.32 cm in *Z. mucronata* and 0.63 to 0.99 cm in *Z. abyssinica*. The seed length also varied according to individual species, in *Z.*

spina-christi the size of the seed length is 1.02 to 1.22 cm, the seed length in *Z. mucronata* also ranged from 0.90 to 1.03 cm, and *Z. abyssinica* have a seed length of 0.81 to 0.91 cm. The weight of fruits ranged from 0.92 to 1.74 g in *Z. spina-christi*, 0.47 to 0.62 g in *Z. mauritiana*, 0.63 to 0.99 g in *Z. mucronata* and 0.63 to 0.89 g in *Z. abyssinica*. The seed weight in *Z. spina-christi* ranged from 0.36 to 0.76 g, 0.35 to 0.50g in *Z. mucronata*, 0.29 to 0.39 g in *Z. abyssinica*.

Ziziphus spina-christi has a thorn length ranging from 0.8 to 1.4 cm, *Z. mauritiana* have a thorn length of 0.8 to 1.6 cm, 0.7 to 1.0 cm is in *Z. mucronata*, and 0.6 to 1.1 cm in *Ziziphus abyssinica*

Table 2: The min, max, mean, standard deviation and coefficient of variation of characters in the studied *Ziziphus* species.

Character	Abbreviation	Unit	Minimum	Maximum	Mean	SD	CV(%)
leaf length	LL	cm	2.8	6.6	4.2	1.41	33.67
leaf width	LW	cm	1.7	4.7	2.45	0.49	20.2
leaf size	LS	cm	6.1	16	10.35	3.32	32.11
petiole length	PL	cm	0.5	1.7	1.55	0.64	41.06
fruit length	FL	cm	0.6	1.6	0.95	0.21	22.33
fruit diameter	FD	cm	1.04	1.65	1.65	0.13	8.14
seed length	SL	cm	0.84	1.91	1.39	0	0
seed diameter	SD	cm	0.39	1.7	1.11	0.24	21.66
thorn length	TL	cm	0.81	1.22	1.22	0.23	18.55
fruit weight	FW	g	0.78	1.1	0.99	0.077	7.86
Seed weight	SW	g	0.29	0.76	0.76	0.3	39.08

The coefficients of correlation of the characters examined were presented in Table 3. The length of the leaf (LL) is significantly correlated with leaf width ($r = 0.77$), leaf size ($r = 0.96$) and petiole length ($r = 0.49$). It is also negatively correlated with thorn length ($r = -0.59$), fruit weight ($r = -0.02$), seed length ($r = -0.58$), seed diameter ($r = -0.42$) and seed

weight ($r = -0.54$). Fruit weight showed positive correlation with leaf length ($r = 0.90$), leaf width ($r = 0.94$), leaf size ($r = 0.84$), petiole length ($r = 0.04$), thorn length ($r = 0.88$), seed length ($r = 0.51$), seed diameter ($r = 0.55$) and seed weight ($r = 0.69$). Seed weight was positively correlated with leaf weight ($r = 0.37$).

Table 3: Coefficients of correlations among the morphological characters in the studied individuals of *Ziziphus*.

	LL	LW	LS	PL	TL	FL	FD	FW	SL	SD	SW
LL		5E-09	1E-22	0.00	7E-05	8E-01	0.07	0.90	0.00	0.03	0.00
LW	0.77		2E-12	0.25	5E-04	8E-01	0.16	0.94	0.25	0.26	0.37
LS	0.96	0.86		0.01	6E-05	8E-01	0.07	0.84	0.02	0.09	0.01
PL	0.49	0.18	0.40		1E-02	6E-03	0.15	0.04	0.01	0.57	0.21
TL	-0.59	-0.52	-0.59	-0.38		9E-01	0.59	0.88	0.02	0.05	0.06
FL	0.05	-0.05	0.04	0.48	2E-02		0.00	0.00	0.02	0.00	0.08
FD	0.33	0.25	0.33	0.26	-1E-01	7E-01		0.00	0.14	0.06	0.20
FW	-0.02	0.01	-0.04	0.37	3E-02	8E-01	0.55		0.01	0.00	0.00
SL	-0.58	-0.23	-0.46	-0.50	4E-01	4E-01	0.29	0.51		0.00	0.00
SD	-0.42	-0.23	-0.33	-0.12	4E-01	6E-01	0.37	0.55	0.77		0.01
SW	-0.54	-0.18	-0.47	-0.25	4E-01	3E-01	0.25	0.69	0.70	0.52	

Table 4: Leaf architecture of the four *Ziziphus* species

Species name	leaf apex	leaf arrangement	leaf base	leaf margin	leaf shape
<i>Z.spina-christi</i>	1	2	0	0	0
<i>Z. mauritiana</i>	0	2	0	1	1
<i>Z. mucronata</i>	1	2	1	0	0
<i>Z. abyssinica</i>	0	2	2	0	0

KEYS: Leaf apex Mucronate 1 Acute 0 Leaf arrangement Acute 2 Leaf base Rounded 0 Aequilateral 1 Acute 2 Leaf margin Entire 0 Serrate 1 Leaf shape Obovate 0 Ovate 1

Cluster analysis

The cluster analysis clearly separated the collected *Ziziphus* species into four distinct groups at Euclidian distance of 0.58 (Figure 1) and the cophenetic correlation coefficient value of $r=0.675$). This indicates that the triangular distance matrix and the phenogram perfectly match each other (Sneath & Sokal 1973; Rohlf 1998). The results indicated that the collected individuals belong to four species of *Ziziphus* and were recognized as distinct groups because there is no mixture of operational taxonomic units between the clusters (Figure 2).

The cluster analysis further revealed the similarity and relationship among the

sampled *Ziziphus* species. The species were distributed in two major clusters in the dendrogram, the first cluster contained the samples labelled as Z4, Z3 and Z1 while the second cluster contained only samples labelled as Z2. This indicate that individuals in Z4 are more closely related to Z3 groups and these groups are sister to Z1 and these three clusters are sister to Z2 cluster indicating that Z2 is more morphologically different with the three groups. The clustering of this species together revealed certain similarities that are present among the members and shows the distance in which they are varied from the others.

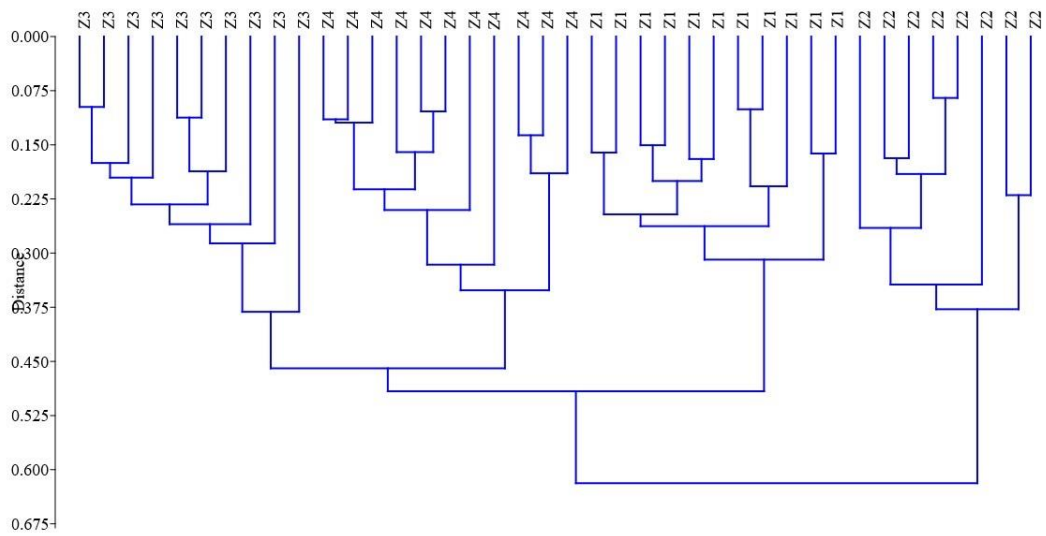


Figure 1: Unweighted Pair Group Method with Arithmetic mean (UPGMA) phenogram resulting from cluster analysis. Cophenetic correlation (r)=0.675

Ordination

The ordination analysis was analyzed using principal component analysis and the result clearly separated the sampled individuals into 4 major groups which correspond to that of the cluster analysis in Figure 2. Out of the 11 components, component 1 (PC1) and 2 (PC2) accounted for the majority of the variations with PC1 having 44.4% and PC2 with 25.28 %. The relationship between *Ziziphus* individuals in terms of phenotypic similarity and morphological features was depicted by a scatter plot created using the PC1 and PC2 data. The loadings of the principal components showed the contribution of each character in delimiting the samples. Petiole length have the highest value with 0.68, followed by leaf length with 0.40 and thorn length having the least value with -0.35 (Figure 3).

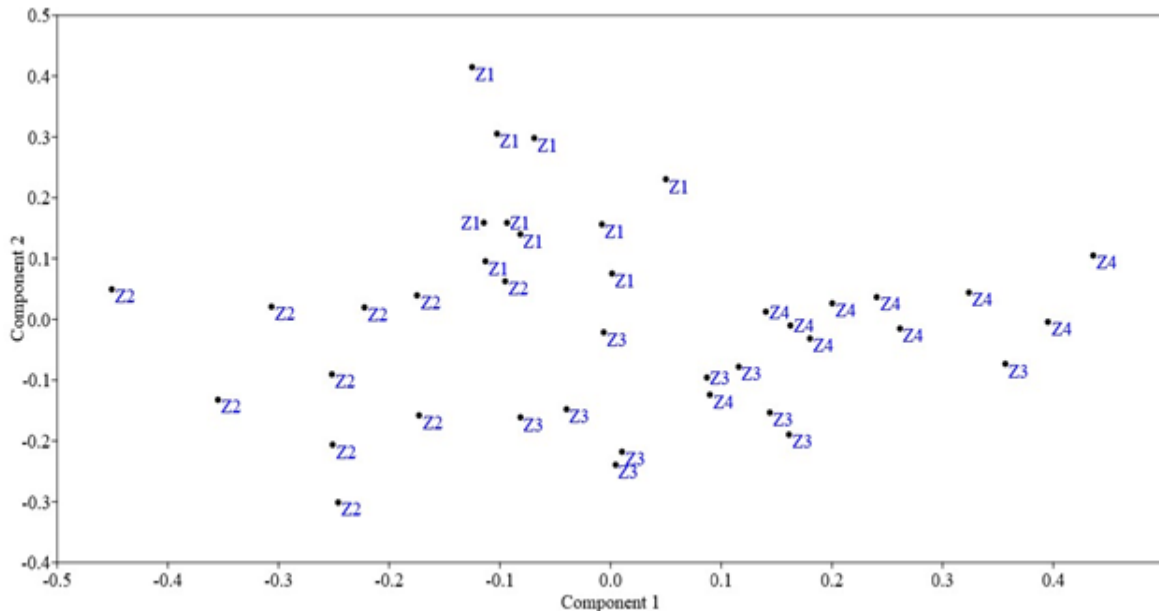


Figure 2: Plot of the first two principal component analyses (PCA) obtained from the analysis of the morphological data set for specimens of the studied species of *Ziziphus*

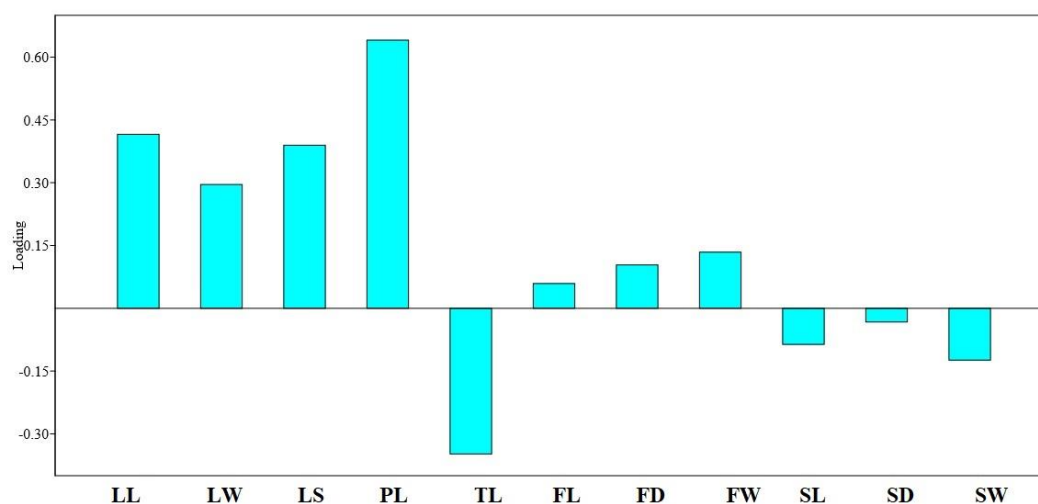


Figure 3: PCA loading of the characters showing contribution of each character to similarities among the species.

Discussion

Several morpho-types were identified in this study by describing the patterns of morphological variation across *Ziziphus* species in Katsina State. The findings of this study revealed that there were significant variations in morphological features of the *Ziziphus* in Katsina. During our field collection, we discovered that *Ziziphus* species are widely distributed and species with such type of distribution are likely to have ecotypes (Hjalmarsson and Ortiz, 2000). The result showed high degree of variation in fruit weight among the studied samples. The weight of fruits of the same taxon growing in the same environmental condition can differ due to genotypic effects (Karadeniz, 2002). The most crucial desirable fruit trait in breeding operations for *Ziziphus* spp. is a higher fruit weight. The cultivar and ecological conditions may affect the variation in fruit weight (Gao *et al.*, 2011). According to Markovski and Velkoska-Markovska (2015), *Ziziphus* fruits' seed weight and fruit weight both shown strong heritability estimations and significant genetic advancement.

In the present study, there was a positive correlation between leaf dimensions and

fruit attributes. The primary sites for photosynthetic sunlight absorption are on leaves. As a result, while leaf dimensions increase, leaf area become larger, which creates the ideal conditions for producing secondary metabolites as primary metabolites rise (Khadivi-Khub and Anjam, 2014). Findings of previous studies reported a significant positive association (correlation) between leaf width, fruit diameter, and fruit weight (Azam-Ali *et al.* 2001).

Four groups were formed from the *Ziziphus* individuals based on the results of the PCA and cluster analyses. The degree of population variability for the various traits under examination determines how diverse the phenotypes are. Additionally, the degree of phenotypic variability across the progenitor lines heavily influences how much heterosis the species were (Saran *et al.*, 2006). A frequent and distinctive trait of the genus *Ziziphus* is self- and cross-incompatibility (Weekley and Race, 2001; AzamAli *et al.*, 2001). In contrast to clonal and bud mutations, this results in greater genetic and phenotypic variety. The clustering procedure, as a whole, proved to be a useful tool for separating

the evaluated materials into groups according to their various phenotypes.

A cluster of various individuals into one is due to having similar phenotypic characters in both. As a result, a program of hybridization including phenotype from several clusters with high means for nearly all component traits may be started in order to collect favorable traits (Saran *et al.*, 2006). A high degree of phenotypic differentiation is typically brought about by elements including the breeding process, population isolation, and seed and pollen distribution distance. Habitat fragmentation, which resulted in population isolation, decreased size, and restricted gene flow among them, may cause a high degree of differentiation (Ferrazzini *et al.*, 2008). On the other hand, extinction may occur if aggressive action is not taken to re-create genetically diverse and sexually reproducing populations in remnant specimens, which is exacerbated by the effects of inbreeding, particularly in self-incompatible species (DeMauro, 1993, 1994; Godt *et al.*, 1995; Young *et al.*, 1999; Warburton *et al.*, 2000).

The percentages of the coefficient of variation in related to sample mean and standard deviations varies between 0 and 41.06%, this depends on the values of respective representatives, when the value of the sample mean is relatively too much higher than that of the standard deviation, the CV% will be larger and at same case when the values of same mean and standard deviation differ in small amount then the CV% will relatively be small. This shows the related variabilities in respect to various species of *Ziziphus* morphological characters.

Author Contributions

Research concept and design: SSY and MSG

Collection and/or assembly of data: SSY and MSG

Data analysis and interpretation: SSY

Writing the article: SSY

Critical revision of the article: SSY and MSG

Final approval of the article: SSY and MSG

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