Morphometric Studies of *Clausena anisata* (Willd.) Hook. f. ex. Benth. in Coastal Savanna Zone of Ghana

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Abstract

Numerical taxonomic study was conducted on five populations of *C. anisata* in the coastal savanna zone of Ghana to determine their patterns of taxonomic variation and identify diagnostic characters among the populations studied. The results showed that the five populations of *C. anisata* exhibited complex patterns of morphological variation. The study also revealed that the populations studied could be phenetically classified into two main clusters. The phenetic groupings identified could not be considered as botanical varieties because of the complex patterns of morphological variation. From the numerical character analysis, morphological characters like peduncle length, ratio of sepal length to width, number of floral branches per inflorescence, anther length, style diameter, filament length, style length, plant height, petiole diameter, sepal length and petal length were identified as diagnostic morphological characters. Even though the phenetic groups identified within *C. anisata* could not be classified as morphological varieties, they could still be described and documented for general purposes such as communication, management and conservation but not for traditional taxonomic purposes.

Introduction

Clausena anisata (Wild.) Hook. f. ex. Benth. belongs to the family Rutaceae and is the only species, out of the 15 species of the genus, found on the African continent (Molino, 1994) . Several morphological characters have been used in the description of the family and one diagnostic feature of this family is the presence of oil glands in its foliage organ. The presence of the aromatic or essential oils in this plant species has led to a number of phytochemical, ethnomedical and biological investigations (Napralet Database, 1999).

As part of a comprehensive assessment of the essential oils of a number of oil-bearing plants in three West African countries, Addae-Mensah *et al.* (1996) investigated the essential oil constituents of *C. anisata* in the coastal savanna zone of Ghana for industrial purposes. From their assessment, they identified three chemovarieties, namely estragole (methyl chavicol), trans-anethole and low-oil yield chemovarieties. The estragole and the low-oil yield varieties were widespread in the areas sampled but the trans-anethole was restricted to a particular locality, the Ofankor plains of the Greater Accra Region of Ghana. From their chemovariation studies, they concluded that there are three chemical varieties of *C. anisata* in Ghana. Despite the taxonomic significance of this discovery, current taxonomic treatment of *C. anisata* within the genus *Clausena* contradicts this chemical classification. According to Molino (1994), there are only two recognised morphological varieties of *C. anisata* on the African continent.

The main aim of any biological classification is to place different organisms with similar biological characteristics into one natural group. These biological characteristics are based on different sets of taxonomic characters such morphology, phenology, chemistry, genetics and many

more. Such taxonomic characters may also exhibit different patterns of variation. However, for the purposes of biological classifications, such taxonomic characters are expected to exhibit positive correlation within a particular plant group under study (Prentice, 1986).

The purpose of this study, therefore, was to investigate the patterns of morphological variation among the populations of *C. anisata* within the coastal savanna zone of Ghana and determine whether the different patterns of morphological variation correlated with the patterns of variation of the essential oil constituents of *C. anisata,* using numerical taxonomic techniques. The study also sought to identify diagnostic characters, using numerical methods that could be used to differentiate between the various phenetic groups recognized.

Materials and methods

Description of the study area

The morphological studies of *C. anisata* populations were conducted within the distributional range of the species along the coastal savanna zone of Ghana, based on the agro-ecological zones of the Meteorological Services Department (1990) (Fig. 1). Plant samples were specifically collected from Legon, Ofankor, Pokuase, Ayikuma and Winneba. These sites were selected so as to coincide with the localities of an earlier chemovariation study by Osei-Safo (1999) and Addae-Mensah *et al.* (1996).

Sampling of populations of C. anisata.

Populations of *C. anisata* were sampled from different localities within the coastal savanna zone of Ghana for morphological study, from October to December 1998. Seven individual plants were randomly sampled from each of the five localities of *C. anisata* previously used by Osei-Safo (1999) for her chemovariation studies, for the numerical taxonomic study. The sample size of seven individual plants per population selected for the study was considered as adequate representation of the diversity within a particular population, as suggested by Goodman (1973), Sneath (1976), and Jardine & Sibson (1971).

Measurement of taxonomic characters

Twenty-seven morphological characters (Table 1), largely based on personal observations and literature survey, were selected and measured. Plant height was measured with the measuring tape. Length and breadth of a leaf were measured by using a pair of dividers and millimetre ruler; three of such readings were taken for each plant and the mean value was used as measurement for an individual plant. Vernier callipers were used to measure stem diameter and micrometer screw gauge was used to measure the diameter of the peduncle, pedicel, style and anther. Other quantitative variables of the plant populations were either counted or measured with both vernier callipers and micrometer screw gauge. Materials like inflorescences, leaves and twigs, which could not be measured on the field, were preserved in 50% alcohol in sample tubes for detailed laboratory work.

Numerical analysis

Population variables of *C. anisata* were analysed using multivariate statistical methods. Ordination plots and cluster analysis were used to detect the patterns of morphological variation among the samples

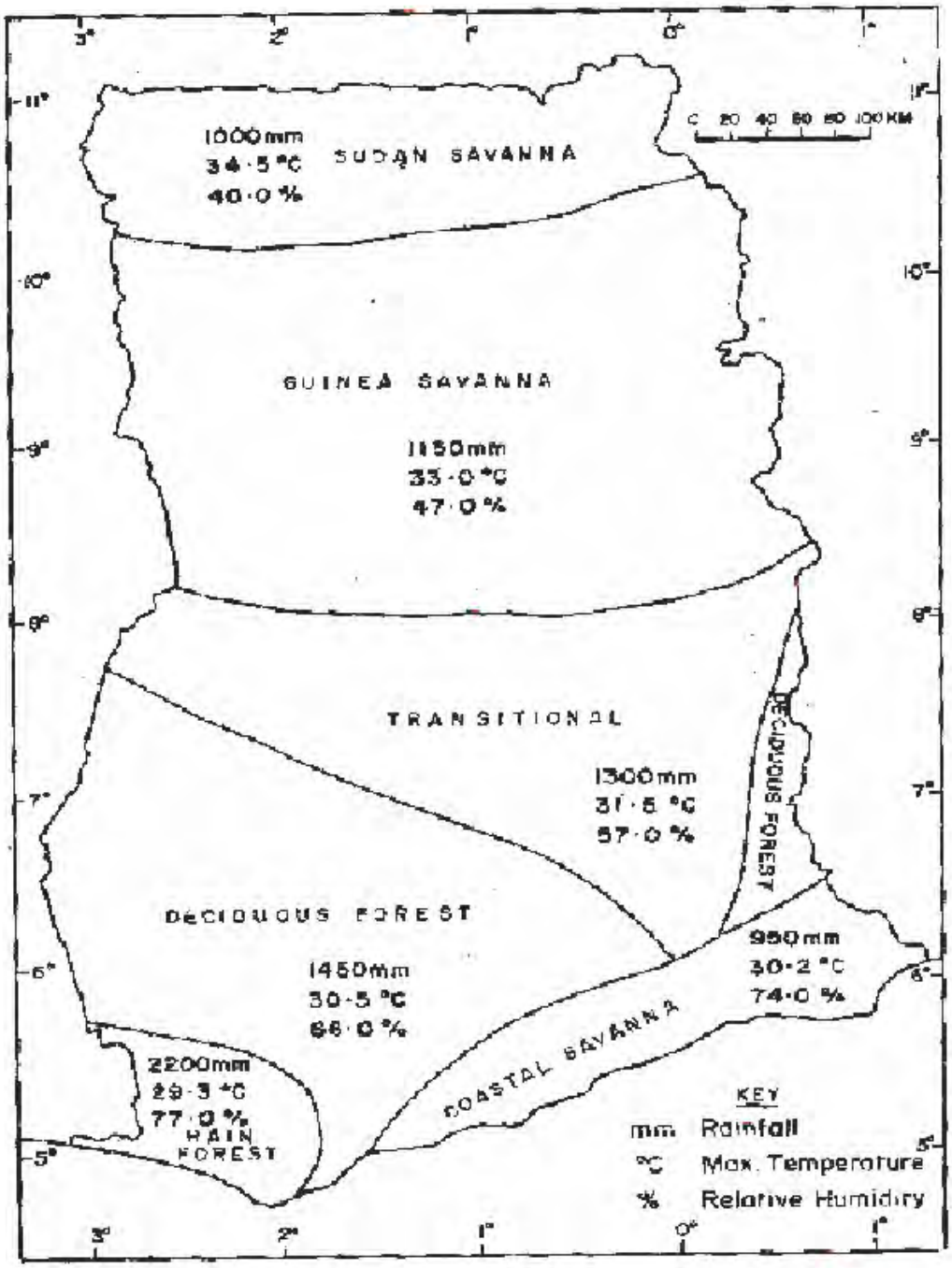

Fig. 1. Agroecological zones of Ghana
Source: Metrological Service Department, Accra (1990)

of *C. anisata* at both the individual and at the population levels. From Table 1, the 27 morphological characters were first assessed on their degree of redundancy by calculating the Pearson's correlation coefficient for all pairs of characters and, subsequently, deleting from the data matrix one member of a highly correlated set (Pearson's correlation $\geq$ 0.90), as suggested by Goodman (1973), Zaharof (1988), and Alvarez (1996). This is because such highly correlated characters might be epistastically or chromsomally linked (Table 1). In order to minimize the effects of the measurements of several plant sizes during numerical analyses, some morphological characters were also studied as ratios of two variables, such as suggested by Zaharof (1988).

From the assessment of the degree of redundancy among the 27 morphological traits, 17 quantitative morphological characters, namely plant height, internode length, leaf length, petiole length, petiole diameter, number of floral branches per inflorescence, peduncle length, sepal length, ratio of sepal length/width, petal length, petal width, ratio of petal length/width, filament length, anther length, style length, style diameter and ovary diameter were then selected for the estimation of similarity and dissimilarity coefficients.

Similarity coefficient among the 35 individual plants of *C. anisata* was then estimated by calculating their Pearson's correlation coefficients. The correlation coefficients were then used to perform the principal component analysis (PCA), using the regression factor method of SPSS version 6 for Windows (SPSS for Window 1993). Also, the quantitative measurements were standardized by ranging before performing the cluster analysis based on Ward's methods (Prentice, 1986).

Dissimilarity coefficient was estimated by calculating Euclidean distances for all pairs of Operational Taxonomic Units (OTUs) before plotting their dendrograms using Ward's method. Identification of diagnostic characters of *C. anisata* was based on high factor loadings of rotated principal matrix (Dunn & Everett, 1982) of the 17 morphological characters. Scatter plots were used as a guide in the selection of diagnostic characters.

Results

The patterns of population variation of C. anisata *based on morphological characters*

Correlation matrix of the morphological characters after the elimination of one member of highly correlated sets of characters is presented in Table 2. The cluster analysis of the 35 individual samples of *C. anisata* (Table 2) in the coastal savanna zone of Ghana has been captured in Fig. 2. From the graph and at 20 rescaled similarity distance, the 35 individuals were clustered into two groups, Group 1 and Group 2. Cluster one consisted of 19 individuals of which seven were from Winneba, four from Ayikuma, five from Legon, two from Ofankor and one from Pokuase. The other cluster consisted of 16 individuals of which five were from Ofankor, five Pokuase, three from Ayikuma and two from Legon (Fig. 2).

To obtain meaningful patterns of morphological variation of *C. anisata* within the coastal savanna zone of Ghana, the pattern of variation exhibited by the ordination plot was superimposed on the ordination plot. A combination of the ordination plot and cluster analysis at 20 rescaled distance of the 35 individual samples is indicated in Fig. 2. From the

TABLE 1
List of morphological characters measured for numerical analysis

Item	*Character*	*Measurement units*
Q1	Plant height	m
Q2	Stem diameter (at base)	cm
Q3	Internode length (of a stem branch)	cm
Q4	Lateral leaf length, mean	cm
Q5	Lateral leaf width mean,	cm
Q6	Ratio of lateral leaf length/width	
Q7	Number of leaflets per leaf (mean)	
Q8	Petiole length, mean	cm
Q9	Petiole diameter, mean	cm
Q10	Number of inflorences per stem branch	
Q11	Number of floral branches per inflorescence	
Q12	Inflorenscence length	cm
Q13	Peduncle length	cm
Q14	Peduncle diameter	cm
Q15	Pedicel diameter x 10	mm
Q16	Sepal length x 10	mm
Q17	Sepal width x 10	mm
Q18	Ratio of sepal length/width	
Q19	Petal length	mm
Q20	Petal width	mm
Q21	Ratio of petal length/width	
Q22	Filament length x 10	mm
Q23	Filament diameter x 10	mm
Q24	Anther length x 10	mm
Q25	Style length x 10	mm
Q26	Style diameter x 10	mm
Q27	Ovary diameter x 10	mm

ordination plot, the 35 individual samples of *C. anisata* could still be divided into two phenetic groupings, Group 3 and Group 4. Group 3 consisted predominantly of 12 individuals from both Pokuase and Ofankor. Group 4 was made up of 23 individuals from Legon, Winneba, Ayikuma and Ofankor. Out of the 23 individuals, only two were from Ofankor. Careful examination of both ordination plot and cluster analysis showed that the 35 individuals of *C. anisata* within the coastal savanna zone of Ghana exhibited similar pattern of variation in each plot (Fig. 3).

In Fig. 4, a cluster analysis of the populations of *C. anisata,* based on the morphological characters, is shown. From the dendrogram, two main phenetic groupings could also be identified. Cluster one consisted of populations of Legon, Ayikuma and Winneba whereas the other cluster consisted of Ofankor and Pokuase populations.

An ordination plot of regression factor score 3 against regression factor score 1 based on population mean scores is

Table 2
Correlation matrix of morphological characters

	Q1	Q3	Q4	Q8	Q9	Q11	Q13
Q1	1.00000						
Q3	.32205	1.00000					
Q4	.57617	.81972	1.00000				
Q8	.76256	.58014	.47764	1.00000			
Q9	-.70824	.08786	-.06899	-.33374	1.00000		
Q11	-.66187	.48295	.16524	-.32393	.69168	1.00000	
Q13	-.36598	.65682	.39379	-.18513	.31849	.89589	1.00000
Q16	.36539	.27062	.55787	.37658	.35591	-.13204	-.26461
Q18	-.25788	-.83800	-.73351	-.25191	.19511	-.47587	-.79926
Q19	.33758	.85422	.83972	.58127	.34288	.34442	.35913
Q20	-.10146	.66107	.32409	.49300	.62024	.52289	.33936
Q21	.59081	.08205	.55706	-.02264	-.58533	-.35859	-.02272
Q22	.14940	-.23781	-.47351	.51683	-.07968	-.45825	-.62923
Q24	-.27647	-.37233	-.03507	-.81112	-.07259	.09603	.19356
Q25	-.87936	-.54690	-.69578	-.63626	.74904	.34215	-.07144
Q26	-.05745	-.75050	-.29720	-.65621	-.26883	-.43900	-.40796
Q27	.31210	-.67688	-.14832	-.27384	-.41358	-.75013	-.70256

	Q16	Q18	Q19	Q20	Q21	Q22	Q24
Q16	1.00000						
Q18	.12866	1.00000					
Q19	.72176	-.50152	1.00000				
Q20	.44433	-.19529	.76916	1.00000			
Q21	.13957	-.39418	.04358	-.60181	1.00000		
Q22	.03637	.58002	-.13075	.28859	-.62845	1.00000	
Q24	-.24806	-.08309	-.42237	-.72719	.59969	-.80783	1.00000
Q25	-.07508	.65592	-.34368	.13036	-.68256	.16994	.09314
Q26	-.11605	.39928	-.63799	-.89096	.55455	-.36744	.81573
Q27	.17615	.48986	-.45001	-.78208	.60561	-.10099	.52469

	Q25	Q26	Q27
Q25	1.00000		
Q26	.13774	1.00000	
Q27	-.07221	.89383	1.00000

displayed in Fig. 5. Cluster analysis of Fig. 4 is superimposed on the ordination plot of Fig. 5. From the ordination plot, two phenetic groupings were identified. Group 5 consisted of Ofankor-Pokuase populations, while Group 6 consisted of Ayikuma, Legon, and Winneba. Ayikuma and Winneba populations formed a sub-cluster within Group 6.

Identification of diagnostic morphological (quantitative) characters of C. anisata

A summary of the first four factor scores with eigenvalue greater than one is presented in Table 3. These extracted factor scores cumulatively accounted for 100% of the total variation under consideration (Table 3). Therefore, the variables of each extracted factor score with the highest factor loadings

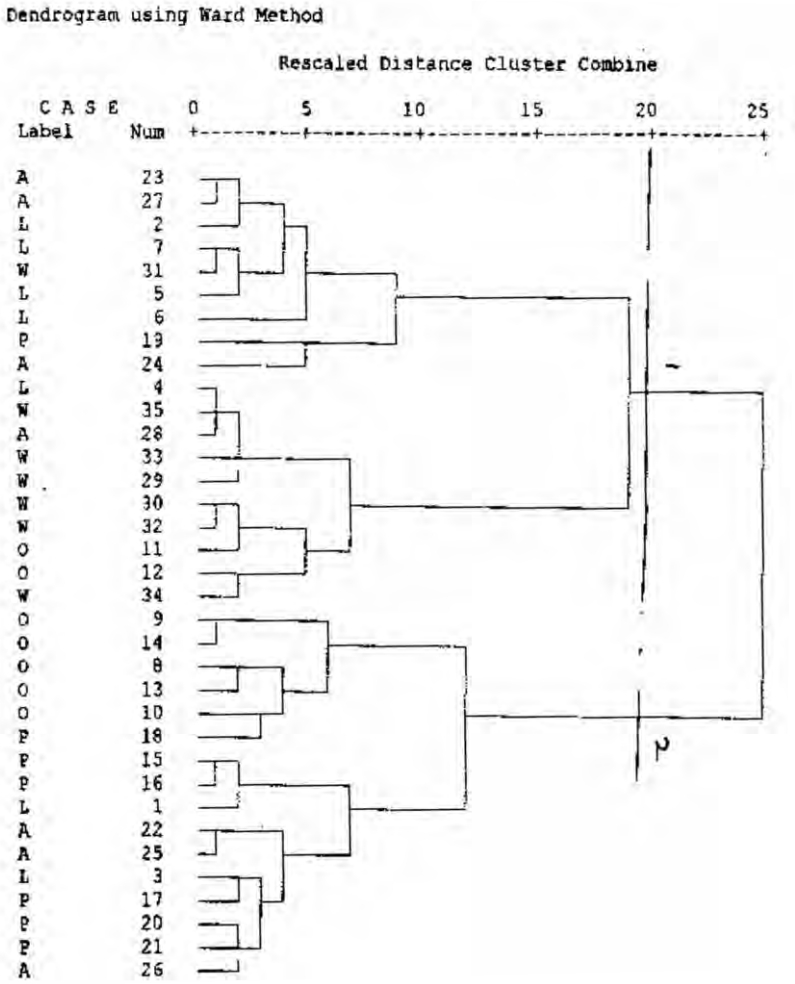

Fig. 2. Dendrogram of Ward's method using Euclidean distances based on 17 morphological characters of 35 individuals of *C. anisata*

(in circles) could be considered as the most useful or diagnostic characters of the populations of *C. anisata* under the study. From the factor scores extracted, the first principal component was highly correlated with peduncle length (0.979), the ratio of sepal length to width (0.897) and the number of floral branches per inflorescence (0.808) (Table 3). The second factor score was predominantly associated with anther length (0.974), style diameter (0.855) and filament length (0.795). The third principal component was associated with style length (0.956), plant height (0.924) and petiole diameter (0.887). Lastly, the fourth component score was correlated with only two characters; sepal length (0.971) and petal length (0.828 (Table 3).

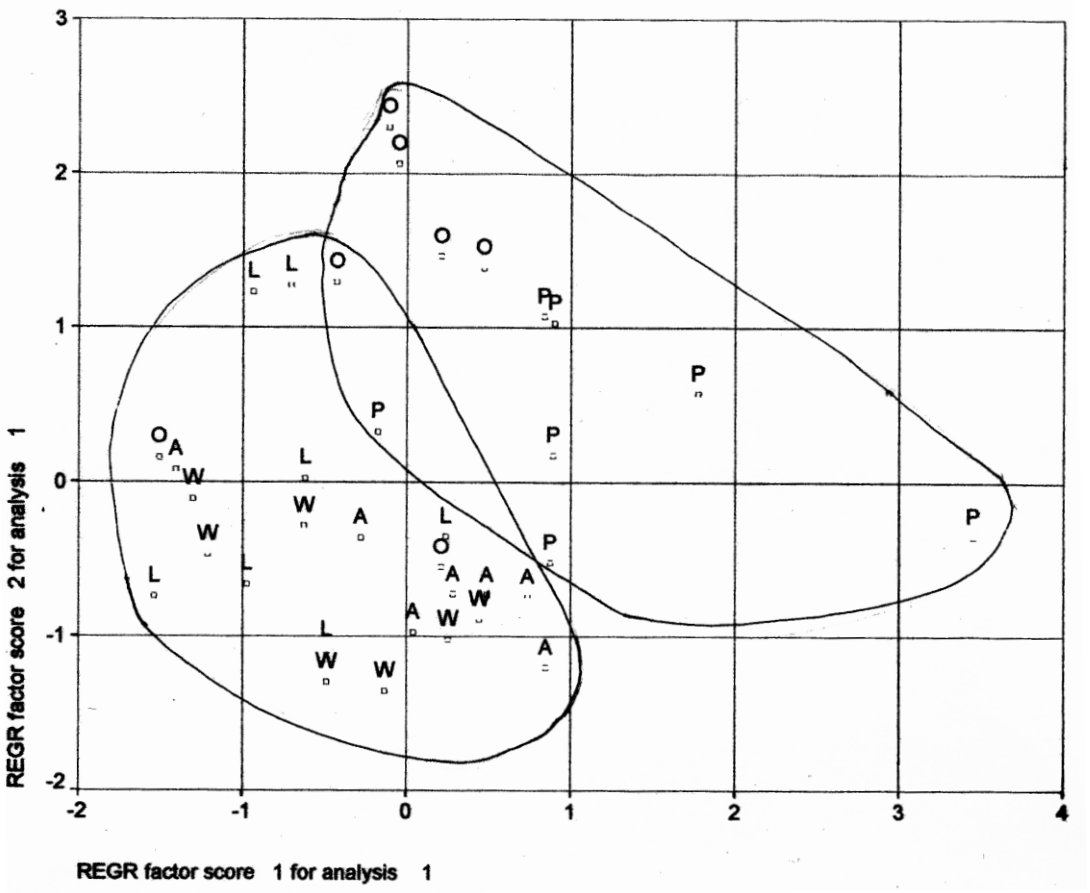

Fig. 3. An ordination plot based on 17 morphological characters of individuals of *C. anisata* along the first two factor scores. Clusters at 20 rescaled distance from Fig. 2 are superimposed the 35 OTUs

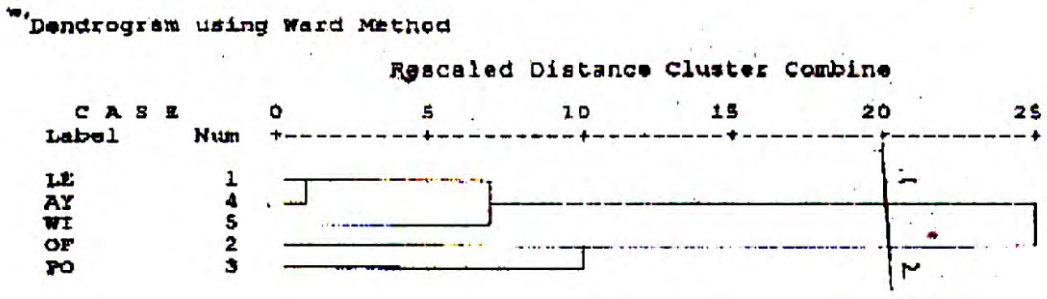

Fig. 4. Dendrogram of Ward's method using Euclidean distances based on 17 morphological character means of 5 *C. anisata* populations

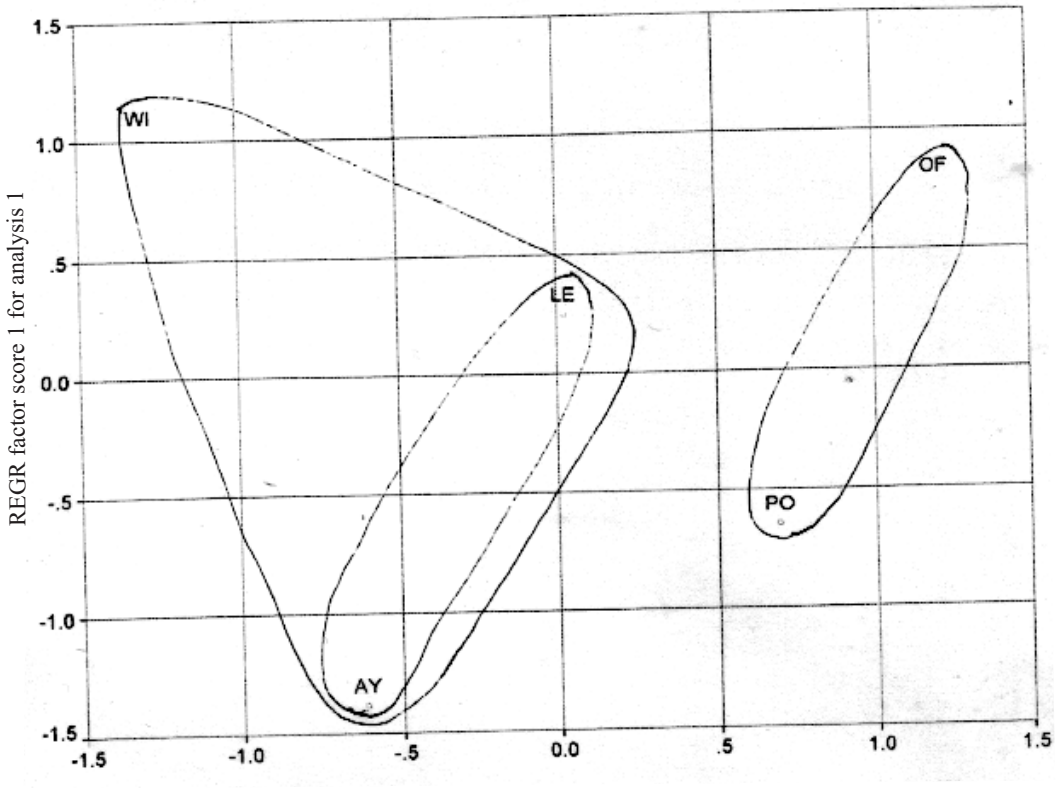

Fig. 5. An ordination plot based on 17 morphological characters of 5 *C. anisata* populations along the first and third factor scores. Clusters at 20 rescaled similarity distance from Fig 4 are superimposed of the OTUs

Also, from Fig. 2 and 3, the 17 morphological characters used in the ordination plot could be described as highly variable because the five populations of *C. anisata,* within the coastal savanna zone of Ghana, were widely scattered.

Discussion

Patterns of morphological variation within C. anisata *populations*

Numerical analyses of the populations of *C. anisata* within the coastal savanna zone of Ghana, in terms of their morphological features, revealed complex patterns of morphological variation. Morphologically, the populations of *C. anisata* at both Ofankor and Pokuase were found in the same cluster, suggesting that they were phenetically similar. However, when the same populations were examined at their individual levels, they were observed to be morphologically very variable. For example, phenetic analyses showed that the individual plants of *C. anisata* at both Ofankor and Pokuase, within a cluster, were widely distributed in terms of the morphological features studied, suggesting some form of morphological heterogeneity. This pattern of

Table 4: Rotated principal factor matrix of 17 morphological character means of populations of *C.anisata*.

Variables	Factor (Component)			
	1	2	3	4
1	-0.179	-0.104	-0.924	0.322
3	0.770	-0.348	-0.306	0.439
4	0.512	0.091	-0.489	0.700
8	-0.006	-0.701	-0.628	0.339
9	0.179	- .123	0.887	0.406
11	0.808	- .040	0.585	0.058
13	0.979	0.730	0.187	-0.033
16	-0.232	-0.061	-0.005	0.971
18	-0.897	-0.059	0.430	-0.083
19	0.443	-0.319	-0.127	0.828
20	0.361	0.730	0.296	0.500
21	0.056	0.721	-0.662	0.199
22	- 0.586	-0.795	-0.012	-0.153
24	0.098	0.974	0.112	-0.170
25	-0.259	0.012	0.956	-0.135
26	-0.486	0.855	-0.003	-0.182
27	-0.716	0.649	-0.255	0.050
Eigenvalue	6.44	4.77	3.89	1.90
% of Variance	37.9	28.1	22.9	11.2
Cumulative % of variance	37.9	65.9	88.8	100.0

variability could be attributed to the phenotypic plasticity of the morphological characters studied. Generally, morphological features of plant species are significantly influenced by both intrinsic and extrinsic factors and, therefore, the wide morphological differences observed among the individual plants could be attributed to either environmental or genetic differences. In a related study, Osei-Safo (1999) reported that these two populations of *C. anisata* are chemically related in terms of their essential oil constituents, even though some differences are also observed.

Phenetic studies of the remaining three populations of *C. anisata* at Ayikuma, Legon and Winneba also showed phenotypic relatedness in terms of their morphological characteristics. The phenotypic relatedness was observed at two levels. One level was between the populations at both Ayikuma and Legon while the other level of similarity involved only the individual plants at Winneba. This pattern of morphological

variation was also confirmed by Osei-Safo(1999) when she reported similar patterns of chemical variation among these populations. She observed that the populations of *C. anisata* at both Ayikuma and Legon typically produce low-yield essential oil constituents but those at Winneba are basically Estragole (essential oil) producing variety.

Davis & Heywood (1963) described a taxonomic variety as a group of plants with distinct morphological features and occupies a restricted geographical area. Such group of plants should also be correlated in several of their taxonomic characters. Despite the correlation between the patterns of morphological and chemical variation among the populations of *C. anisata* within the coastal savanna zone of Ghana, the phenetic groupings identified in this study could not be considered as taxonomic varieties in terms of their geographical and ecological distributions. The current taxonomic treatment of *C. anisata* within the genus *Clausena* recognizes two taxonomic or boatanical varieties, namely var. *anisata* and var. *paucijuga (*Molino, 1994). The two varieties are differentiated in terms of their vegetative phenology. The variety *anisata* is evergreen and is found in both Africa and southeast Asia whereas the variety *paucijuga* is deciduous and is restricted only to southeast Asia (Molino, 1994). The morphological variety as described by Molino (1994) is taxonomically different from the phenetic similarity identified in this study or the chemical varieties described and reported by both Addae-Mensah *et al.* (1996) and Osei-Safo (1999).

Biological classification of plant species of high predictive value is based on two or more correlated characters (Prentice, 1986). But in situations where such phenetic groupings exhibited complex patterns of variation and, therefore, could not be classified, as reported in this numerical taxonomic study, the complex patterns of morphological variation could still be described and recognized for the purposes of communication and other management strategies as suggested by Prentice (1986).

Numerical analysis of morphological characters

In taxonomic studies, diagnostic characters are characters that are constant within a group but vary between groups. Such characters could be used to identify natural plant groups from several others of similar ranking (Kent & Coke, 1992; Dunn & Everett, 1982; Crovello, 1974; Davis & Heywood, 1963). In a numerical analysis, diagnostic characters exhibit high and absolute factor scores and are also capable of separating OTUs under study into distinctive groups. From the principal component analysis of the rotated factor score (Table 3), characters such as peduncle length, ratio of sepal length to width, number of floral branches per inflorescence, anther length, style diameter, filament length, style length, plant height, petiole diameter, sepal length and petal length were identified as diagnostic.

It was also observed that most of these diagnostic characters were quantitative in nature and could easily be influenced by environmental factors. The quantitative diagnostic characters identified in this study could be described as bad taxonomic characters (Davis & Heywood, 1963) because they are easily modified by environmental factors. Nevertheless, such

bad taxonomic characters could still be utilized in any taxonomic studies or considerations provided their genetic bases have been ascertained through a series of transplant experiments. In fact, according to Snaydon (1973) and Waddington (1953), there are some instances where quantitative morphological characters have been proven to have genetic bases. In a related work, Gill, Lawrence and Morton (1973) also observed that the North American populations of *Mentha arvensis* L. are morphologically very variable but much of its variation is genetic.

Conclusions and recommendations

From the numerical taxonomic analysis, the populations of *C. anisata* within the coastal savanna zone of Ghana consisted of two phenetic groupings. The various populations studied exhibited complex patterns of morphological variation which did not support the current taxonomic treatment of the species. Therefore, the various morphological types identified in this study could be described and documented for the purposes of communication and future research work. Also, because of the wide morphological variation exhibited by the populations studied, the species could be used in a wide range of habitats for conservation and restoration programmes in degraded mining environments. Taxonomically, the various diagnostic characters identified could not be considered as suitable taxonomic characters for the identification of the various morphological types unless their genetic bases have been ascertained.

Acknowledgement

The authors would like to thank the Technical staff of the Department of Botany and the Ecological Laboratory Unit, University of Ghana, for their technical support during the collection and preparation of sample materials.

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