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A comparative foliar epidermal and morphological study of five species of the genus *Amaranthus*

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ABSTRACT

A comparative foliar epidermal and morphological study of five members of the genus Amaranthus was carried out with a view to elucidating their taxonomic significance in the proper identification of five different species studied. Seeds of five species of Amaranthus namely; A. hybridus, A. caudatus, A.viridis, A. spinosus and A. dubius were harvested from different part of Anyigba and grown under the same environmental condition at the research garden of the Biological Sciences Department, Kogi State University, Anyigba, Kogi State, Nigeria. Strips of epidermal layers were gotten from the adaxial and abaxial surfaces of the leaves and viewed under the microscope according to method outlined by [13]. The number, length and breadth of the stomata and epidermal cells were taken for both adaxial and abaxial surfaces. Seven qualitative morphological attributes and twelve leaf epidermal attributes were considered. The data obtained were subjected to Analysis of Variance (ANOVA) while leaf epidermal traits with significant difference were separated using Duncan Multiple Range Test (DMRT). Six out of the seven qualitative morphological attributes differentiated the five studied plants while, eleven out of the twelve leaf epidermal attributes studied showed significant difference. The important morphological and leaf epidermal traits were then used to construct indented dichotomous keys for easy identification of the studied plants species. Further studies on the other members of the genus are therefore recommended.

Key words: *Amaranthaceae,* Epidermal, qualitative, adaxial, abaxial.

INTRODUCTION

The family Amaranthaceae are classified into two subfamilies which according to [6] include, Amaranthoideae and Gomphrenoideae. The Amaranthoideae and some genera of Gomphrenoideae were found to be polyphyletic, so taxonomical changes are needed [11].

Plant classification has been a subject of discussion among plant taxonomist and systematists over the years [1]. Plants are classified and re-classified as soon as new evidence arises and this is going to be a continuous exercise over some years to come. [2] reported that most plants are classified base on external morphological structure such as flowers and fruits. These structures are not always available in plants because they are seasonal in production. Due to these reasons other methods of identification needs to be developed so that plant could be easily identified at any period of the year. These methods among others include the use of anatomical studies especially that of the leaves since it occurs at every season of the year mostly among the evergreen plants.

Striking similarities has been observed among a sizable number of *Amaranthus* species. This is revealed by their morphological appearance, which no doubt posed problems to the proper identification of the plant taxa. *Amaranthus* (pigweed) according to [10], exhibit difficulties, especially in the early state of seedling growth as

many species look identical, once matured, identification is less difficult but not all together straight forward. However, some *Amaranthus* species may cross to produce hybrids. These hybrid plants may exhibit the characteristics of both parents. Some species of the *Amaranthus* also have been reported to be poisonous to human and livestock. [5] reported that some weedy amaranths tend to accumulate excess nitrates (when soil fertility is very high) in their tissues that become toxic to human infants and some animals. It is therefore very necessary to design easier way of identifying each species.

The focus of this study are to identify the qualitative morphological and leaf epidermal attributes of some members of the genus *Amaranthus* that are species specific and to construct taxonomic keys for the purpose of easy identification of the five species of this genus.

MATERIALS AND METHODS

Sample Collection

The seeds of five different species of *Amaranthus* were gotten from different parts of Anyigba, in Kogi State, Nigeria. The seeds of each sample were identified and planted in perforated polythene bags under the same environmental conditions at research garden of the Biological Sciences Department in Kogi State University, Anyigba. The bags were labelled as followed:

A – *Amaranthus hybridus*
B – *Amaranthus caudatus*
C – *Amaranthus viridis*
D – *Amaranthus spinosus*
E – *Amaranthus dubius*

Qualitative Morphological Attributes

The seven qualitative morphological characteristics studied are colour of the inflorescences, leave, stems and roots, shape of the leaves, arrangement of the leaves on the stem and presence of spines.

Leaf Epidermal Studies

Fresh leaves were collected from each of the five different plant samples. Each leaf was painted with finger nail polish on both the adaxial and abaxial surfaces and allowed to dry. After drying, short clear cellophane tape was firmly pressed over the dried nail polish on the surfaces according to the method of [13]. Epidermal strips were taken from the median portion of matured leaves, stained in alcoholic safranin and mounted in 50% glycerine jelly for microscopic examination. Epidermal strips from both the adaxial and abaxial surfaces were prepared and mounted separately. Photographs of good preparations were taken at a magnification of X400 objective for photomicrograph. The length and width of epidermal cells and stomata apparatus were measured with micrometer eyepiece graticule. The number of stomata and epidermal cells were observed and recorded. Ten peelings were mounted for each leaf surface, while observations and measurements were made from 30 microscope fields of focus at × 40 objectives.

Data Analysis

Data obtained from each leaf epidermal attributes on both the abaxial and adaxial surfaces were subjected to Analysis of Variance (ANOVA) and means with significant difference separated using Duncan Multiple Range Test (DMRT).

The Stomata Index (SI) was estimated for the leaf surfaces using the following formulae as described by [17].

Stomata index (SI)

$$SI = \frac{S}{S + E} \times 100$$

Where:
SI = Stomata Index
S = Number of Stomata per unit area and
E = Number of Epidermal Cells in the same unit area.

RESULTS

(a)

(b)

(a) = Photograph of the Five (5) speceis of *Amaranthus* studied (b) = Photograph of *Amaranthus spinosus* showing the spines(S)

Plate 1: The Photograph Showing the Morphology of the Five Studied Plants

The five sample species are shown in plate 1a and 1b. The plates show clearly some of the morphological appearances of the five members of the genus *Amaranthus* studied. *Amaranthus spinosus* could be distingused from the other members due to the presence of spines (plate 1b). *Amaranthus viridis* and *Amaranthus dubius* are similar morphologically but the possession of purple stem by the latter differentiate it from the former. *Amaranthus hybridus* and *Amaranthus caudatus* are morphologically similar but the possession of purple leaves and infloresence in *Amaranthus caudatus* differentiate it from *Amaranthus hybridus*.

Table 1: The Qualitative Morphological Features of the Five Studied Species of *Amaranthus*

Plant Species	Colour of Stem	Colour of Leaves	Colour of the Root	Colour of Inflorescence	Shape of Leave	Presence of Spines	Arrangement of Leave on Stem
A.hybridus	Green	Green	Brown	Green	Ovate	Absent	Alternate
A.caudatus	Purple & Green	Purple & Green	Purple	Purple	Ovate	Absent	Alternate
A. viridis	Green	Green	Brown	Green	Obovate	Absent	Alternate
A.spinosus	Green	Green	Brown	Green	Obovate	Present	Alternate
A. dubius	Purple	Green	Purple	Green	Obovate	Absent	Alternate

The qualitative morphological features of five species of *Amaranthus* are shown in table 1. It could be observed that only *Amaranthus spinosus* among the studied species has spines while the remaining four species lack spines. *A.dubius* and *A. caudatus* are the only members among the studied species with purple stem. *Amaranthus caudatus* distinguished itself by having a purple inflorescence while the remaining four species have green inflorescence. Also from the table it could be observed that only *Amaranthus hybridus* and *Amaranthus caudatus* have ovate leave shape while the remaining 4 species have obovate leave. All the studied plant taxa considered in this study have alternate arrangement of leaves on the stem.

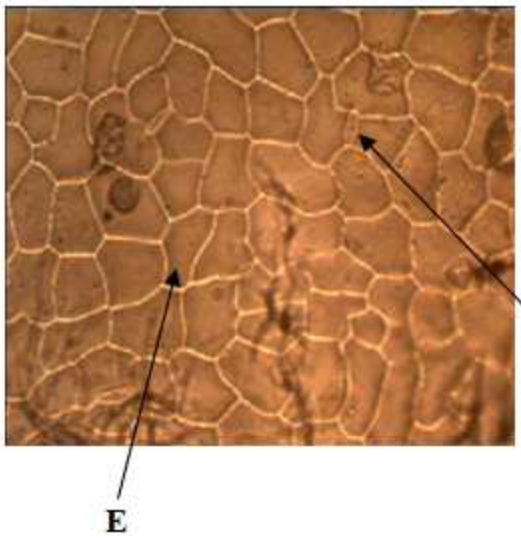

Plate 2a : Adaxial leaf surface for *A. hybidus*

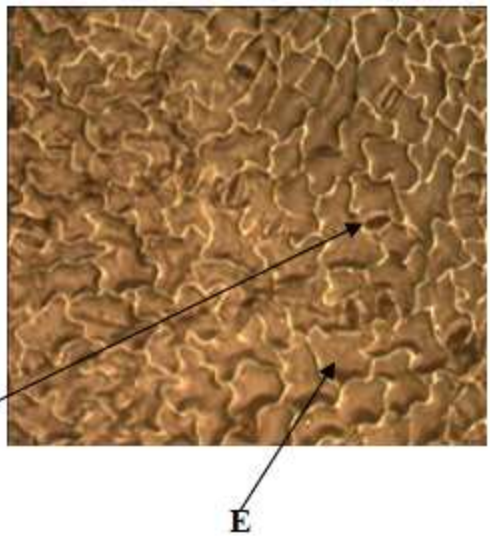

Plate 2b: Abaxial surface leaf for *A.hybridus*

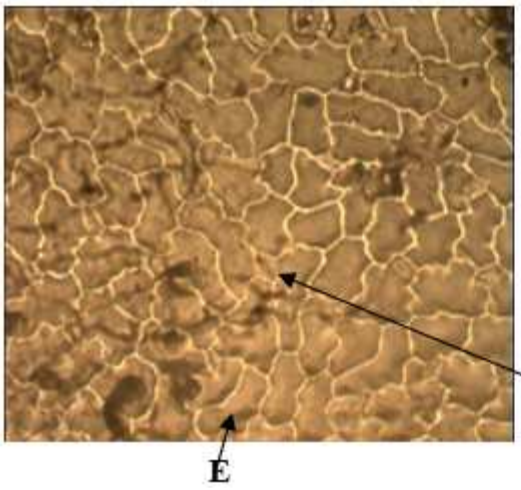

Plate 3a: Adaxial leaf surface for *A. viridis*

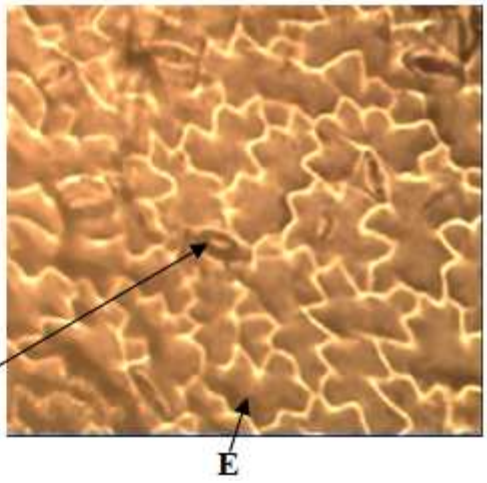

Plate 3b: Abaxial leaf surface for *A. viridis*

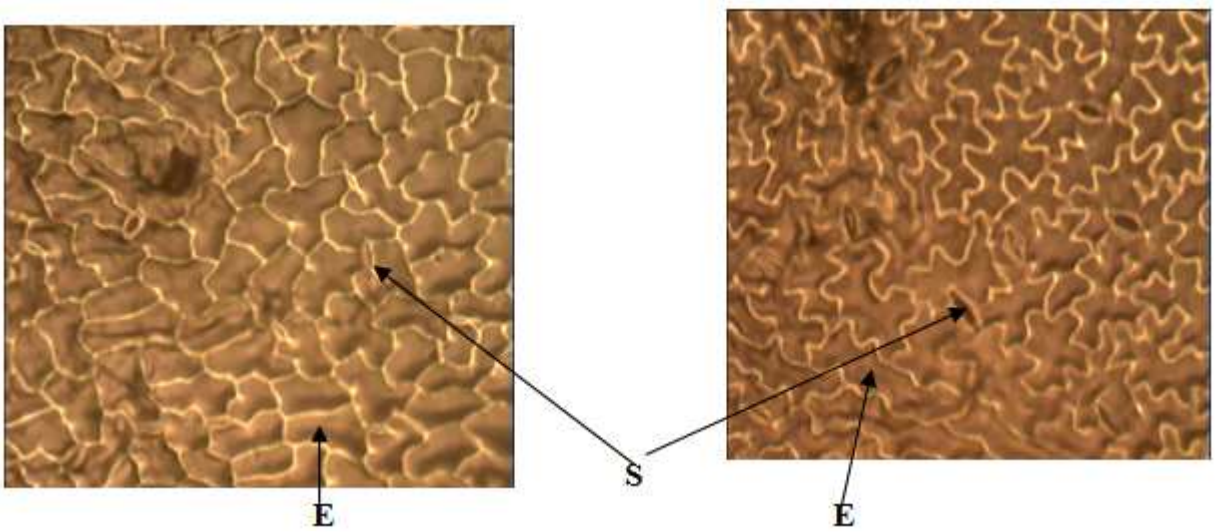

Plate 4a: Adaxial leaf surface for *A.dubius* **Plate 4b: Abaxial leaf surface for *A.dubius***

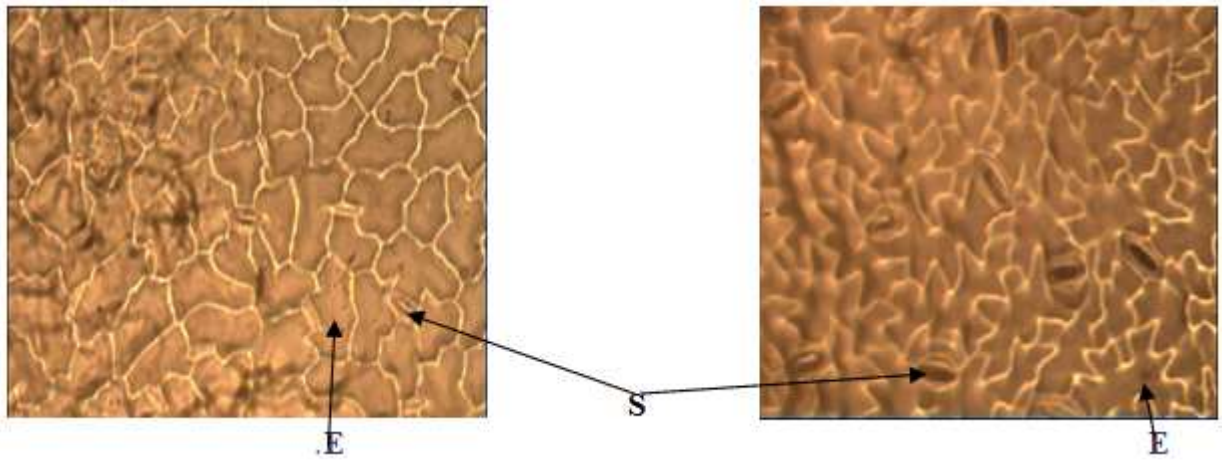

Plate 5a: Adaxial leaf surface for *A. spinosus* **Plate 5b: Abaxial leaf surface for *A. spinosus***

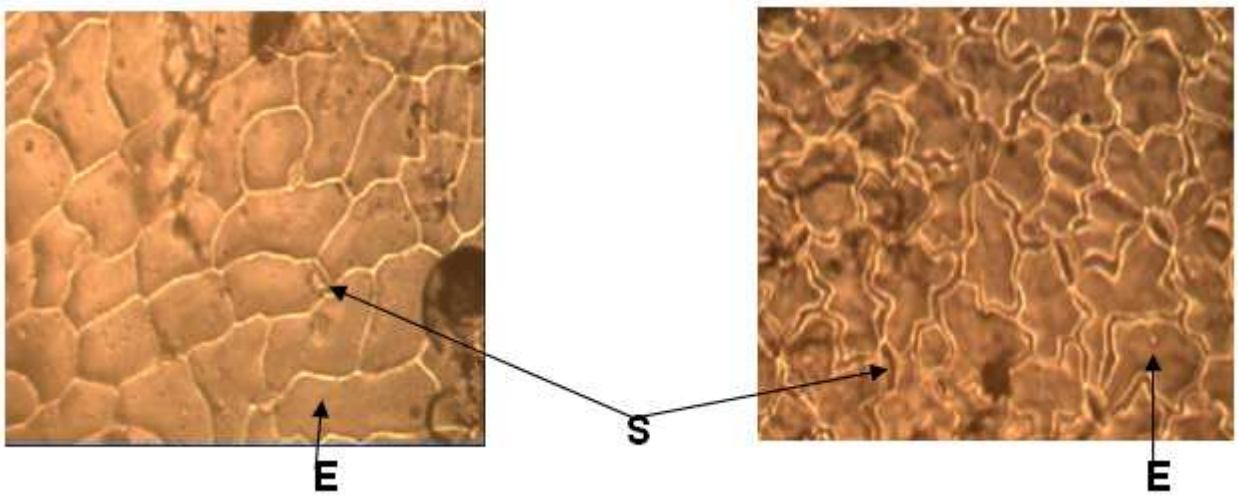

Plate 6a: Adaxial leaf surface for *A. caudatus* **Plate 6b: Abaxial leaf surface for *A. caudatus***

Magnification ×400

Plate 2- 6: Photomicrographs of the adaxial and abaxial leaf surfaces for the five *Amaranthus* species studied
Key: E-Epidermal cell, S-Stoma

Table 2: Summaries of Some Foliar Epidermal Attributes found both on the Adaxial and Abaxial surfaces of the Five Studied Plant Species

Plant species	Surface	Shape of Epidermal Cells	Epidermal Wall Pattern	Stomata Type (percentage occurrence)	Leave Conditions
A.hybridus	Adaxial	P,I	S,C	Anisocytic(55%), paracytic(40%), anomocytic(10%)	Hypoamphistomatic
	Abaxial	I	U	Anisocytic(53%), paracytic(39%), anomocytic(8%)	
A.caudatus	Adaxial	P,I	S,C	Anisocytic(21%), paracytic(72%), anomocytic(7%)	Hypoamphistomatic
	Abaxial	I,Si	U	Anisocytic(55%), paracytic, (45%)	
A. viridis	Adaxial	P,I	S,C	Anisocytic(51%), paracytic(41%), anomocytic(8%)	Hypoamphistomatic
	Abaxial	I	U	Anisocytic(42%), paracytic(58%).	
A.spinosus	Adaxial	P,I	S,C	Anisocytic(64%), paracytic. (36%)	Hypoamphistomatic
	Abaxial	I	U	Anisocytic(40%), paracytic(25%), anomocytic(30%)	
A. dubius	Adaxial	P,I	S,C	Anisocytic(61%), paracytic(33%), anomocytic(6%)	Hypoamphistomatic
	Abaxial	I	U	Anisocytic(20%), paracytic(68%), anomocytic(12%)	

Key: P-Polygonal; I-Irregular; Si-Sinus; S-Straight; C-Curve; U- Undulating.

From the observed recorded in table 2, *Amaranthus caudatus* is the only species with sinuous wall around the epidermal wall on the abaxial surface (plate 6a and b). The abaxial and adaxial surface of all the studied species show irregular shape of epidermal cell (plate 2-6). All the five studied species possessed straight and curve epidermal walls on the adaxial surface while undulating stomata walls on the adaxial surface. They are also hypoamphistomatic (more stomata on the abaxial surfaces than the adaxial leaf surfaces) in the nature. It was also observed that *Amaranthus caudatus*, has the highest numbers of paracytic stomata type on the adaxial surface (75%) while, *Amaranthus spinosus* (64%) has the highest percentage of anisocytic stomata type and *Amaranthus spinosus* (30%) has the highest anomocytic stomata type. All the other species of *Amaranthus* studied possess higher percentage of anisocytic stomata types on both the adaxial and abaxial surface. It could also be observed that all the five studied species possessed anisocytic and paracytic on both the adaxial and abaxial surfaces.

Table 3: The Mean Measurement for the Leaf Epidermal Attribute on the Adaxial Surface for the five Species of *Amaranthus* Studied

Plant species	Length of Epidermal cells	Breadth of epidermal cells.	Numbers of Epidermal cells	Length of stomata	Breadth of stomata	Number of stomata	Stomata Index (%)
A.hybridus	2.7100 ^{b}	1.3270	90.8670 ^{a}	1.3270 ^{a}	0.3900 ^{b}	14.6000 ^{a}	13.8431
A.caudatus	2.6630 ^{b}	1.2670	80.8330 ^{b}	0.8600 $^{c\,d}$	0.4300 ^{b}	12.0000 ^{b}	12.9264
A. viridis	2.9130 ^{b}	1.3770	78.4670 ^{b}	0.9900 ^{c}	0.4533 ^{a}	8.8000 ^{c}	10.0839
A.spinosus	2.7270 ^{b}	1.4770	73.6670 ^{c}	0.9200 ^{c}	0.4933 ^{a}	11.8670 ^{b}	13.8740
A. dubius	3.0170 ^{a}	1.3900	58.3000 ^{d}	1.1300 ^{b}	0.5000 ^{a}	10.077 $^{b\,c}$	14.7374
Significant	S	NS	S	S	S	S	

The mean measurements for the leaf epidermal attributes on the adaxial surface for the five species of *Amaranthus* studied are shown in table 3. It was observed also that five of the attributes studied show significant difference, while the breadth of epidermal cells of the adaxial surface is the only traits that do not show significant difference among the studied species.

Table 4: The Mean Measurement for the Leaf Epidermal Attribute on the Abaxial Surface for the five (5) Members of *Amaranthus* studied

Plant species	Length of Epidermal cells	Breadth of epidermal cells	Numbers of Epidermal cells	Length of stomata	Breadth of stomata	Number of stomata	Stomata Index (%)
A.hybridus	2.7400 ^{c}	1.1200 ^{c}	84.4000 ^{a}	0.9667 ^{c}	0.5133 ^{c}	22.3330 ^{a}	20.9242
A.caudatus	2.9730 ^{c}	1.9030 ^{a}	69.2670 ^{c}	1.0100 $^{b\,c}$	0.6200 $^{a\,b}$	20.0330 ^{b}	22.4334
A. viridis	3.7570 ^{a}	2.1270 ^{a}	53.1670 ^{d}	1.4100 ^{a}	0.7167 ^{a}	10.8330 ^{d}	16.9266
A.spinosus	2.8430 ^{c}	1.7130 $^{a\,b}$	75. 8000 ^{b}	1.0530 ^{b}	0.6467 ^{a}	15. 1330 ^{c}	16.6419
A. dubius	3.3030 ^{b}	1.2000 ^{c}	70.8000 ^{c}	1.1300 ^{b}	0.6500 ^{a}	18.9330 ^{b}	21.0993
Significant	S	S	S	S	S	S	

Table 4 shows the mean measurement of six leaves epidermal attributes on the abaxial surface for the five species. It was observed that all the six attributes studied show significant difference.

DISCUSSION

The significance of leaf epidermal attribute in taxa delimitation has been reported by [12], [7], [14], [3], [9], [18] and [19].

Excellent reports on the application of morphological attributes in plant systematics were reported by [16], [8] and [15].

The quantitative characteristics result shows that there are differences among the five species of *Amaranthus* studied (table 1). However, alternate pattern of leave arrangements on the stem is common to the five studied plant species. The fact that all the five species studied shared this attribute in common is an indication that they have a common ancestor.

Qualitative morphological attributes like colour of stem, colour of leaf, colour of root, colour of inflorescence, shape of the leaf and presence of spines varies among the five species which indicates that despite the fact that these five plant species originated from a common ancestor, each species is of distinct genotype. The presence of spines on the stem of only *Amaranthus spinosus* is a divergence from the basic plan and an adaptive feature which could be transferred to edible *Amaranthus* species for protection against insect and herbivorous animals.

Ovate leaf shape separated the edible *Amaranthus* from the non edible *Amaranthus* which indicated that the attributes can be used for easy identification of edible *Amaranthus* among different species of the genus. These findings supported the earlier report of [15] that the use of morphological features has been found to be of immense importance to plant taxonomy.

The qualitative morphological attributes considered in this study did not show much variation among the five studied *Amaranthus* species because qualitative traits are known to be controlled by single or very few genes with little or no environmental influence on their expression.

All the plant species considered in this study possess hypoamphistomatic condition (having more stomata on the abaxial surface than the adaxial surface), polygonal and irregular shape of epidermal cells on the adaxial surface except *Amaranthus caudatus* that has sinous epidermal cell wall on the adaxial surface (table 3). Also the possession of straight and curved epidermal cell wall pattern on the adaxial surface and undulating epidermal cell wall pattern on the abaxial surface is an indication that these common attributes are fixed in the genus *Amaranthus*, though the possession of sinous wall in *Amaranthus caudatus* is a species specific trait that can be used to delimit the taxon from the others.

This study also revealed that anisocytic and paracytic stomata types are the most common types of stomata on both the adaxial and abaxial surfaces of the genus *Amaranthus*, while anomocytic type is found occasionally at low frequency among the studied species, this suggested that anomocytic is a recent stomata type that can be used for the delimitation of the genus *Amaranthus*.

The high degree of similarity displayed for the leaf epidermal characteristics in table 2, suggests that the five plant species studied are of close genetic relationship which therefore points to their common evolution.

Since the plant used for this study were subjected to the same treatment and environmental conditions, any significant variation observed in the leaf epidermal features among the member of the *Amaranthus* studied will therefore reflect genetic variations, that could be used for their delimitation.

All the twelve analysed epidermal characteristics on the abaxial and adaxial leaf surfaces for the five studied species of *Amaranthus* showed significant difference except the breath of epidermal cell on the abaxial surface (Table 3 and 4). The fact that these epidermal attributes show significant difference among the five species is an indication that all the traits are good taxonomic indicators. Despite the fact that they have common origin, there is indication that their evolution is along different trend. [4] reported that the foliar epidermis is one of the most noteworthy taxonomic characters from the biosystematics point of view and the taxonomy of a number of families has been made on the basis of leaf epidermis.

The number of epidermal cells and the number of stomata on both the adaxial and abaxial surfaces respectively (Table 3 and 4) put the five species in four different taxonomic groups which indicate that the number of epidermal cells and stomata are the best tools for the delimitation of member of the genus *Amaranthus*.

In this study, the highest Stomata Index (SI) values were recorded on the abaxial surface (Table 4) than the adaxial surface (Table 3). This observation is in agreement with Stomata Index value reported by [14]. The variations observed in Stomata Index among the study plant species is also a valuable tool for their delimitation since no two species have the same value. This agrees with the report of [9] that the role of Stomata Index in plant systematics cannot be over emphasized.

Indented Dichotomous Key

Base on the most reliable qualitative morphological and leaf epidermal features observed in the study, artificial indented dichotomous keys for the delimitation of the five species are presented as follows:

A. Quantitative Morphological Attributes.

1a.*Amaranthus* without spines……. *A. caudatus, A. hybridus, A. dubius, A. viridis,*
1b. *Amaranthus* with spines……. *A. spinosus*
2a.*Amaranthus* with purple inflorescence…… *A. caudatus*
2b.*Amaranthus* with green inflorescence……. *A. hybridus, A. dubius, A.viridis*
3a. *Amaranthus* with ovate leaf shape…….*A.hybridus*
3b. *Amaranthus* with obovate leaf shape ……. *A. dubius, A. viridis*
4a. *Amarannthus* with purple stem and purple root……... *A. dubius*
4b. *Amaranthus* with green stem and brown roots……. *A. viridis*

B. Epidermal Attributes

1a. *Amaranthus* without sinus epidermal walls…….., *A. spinosus, A. hybridus, A.dubius, A. viridis*
1b. *Amaranthus* without sinuous epidermal wall…….*A. caudatus*
2a. *Amaranthus* lacking anomocytic stomata type on the adaxial surface…… *A. spinosus,*
2b. *Amaranthus* having anomocytic stomata type on the adaxial surface……. .*A. hybridus, A. dubius, A. viridis*
3a. *Amaranthus* having number of epidermal cell up to 80 on both surface……..*A. hybridus.*
3b. *Amaranthus* having number of epidermal cell less than 80 on both surface.... *A. dubius, A. viridis.*
4a. *Amaranthus* with length of epidermal cell up to 3.0*u*m on both surface…….*A. dubius*
4b. *Amaranthus* with length of epidermal cell less than 3*u*m on both surface and stomata less than 10 on the adaxial surface ……*A. viridis*

CONCLUSION

The qualitative morphological and leaf epidermal traits investigated are therefore relevant to the identification and taxonomy of the five species of *Amaranthus* considered in this study. Despite the fact that the five species studied had a common origin each species evolved along different evolutionary trends. The most reliable qualitative morphological and leaf epidermal attributes have been used to construct a dichotomous key for easy and quick identification of each of the studied species.

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