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Electron Micrograph of the Leaf Epidermal Structures and Stomata Ontogeny in Some Members of the Leguminosae Family

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Abstract

Our goal was to find morphological features that can help us identify or describe the stomata ontogeny and epidermal structures. Our team got seeds of two types of leguminous plants—*Arachis hypogaea L. groundnuts* and *Vigna subterranean* L. bambaranuts—from the Kano State Agricultural and Rural Development Authority and the Yankaba market in Nassarawa, which is in Kano State. The study specifically evaluated the leaf epidermal studies, stomata development (ontogeny), types of stomata, stomata size, pore length, pore width, and stomata index in both the surfaces of the leaves. We conducted ontogenetic studies using safety razor blades on the epidermal layers. We determined the stomata index by studying the type of stomata, the number of stomata, and the number of epidermal cells. We subjected the obtained data to a one-way analysis of variance. All the studied varieties/species exhibited amphistomatic stomata on both surfaces, with a higher density on the lower surface. In SAMNUT 23, we found the paracytic type of stomata, which is perigynous and has an irregular anticlinal cell wall. In Dandakar, we found the tetracyclic type, which is mesogynous and has a pentagonal epidermal cell shape and a slightly straight anticlinal cell wall. Finally, in Bambara nuts, we found the endocytic type, which is polygonal with a curved anticlinal wall and a mesoperigynous ontogeny.

Keywords: Epiderma structure,ontogeny,mesogenous, mesoperigenous, meristemoid

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Introduction

Electron micrography is a system that involves the use of an electron microscope to observe or study the feature of the structure of a biological specimen or chemical regiment using images taken in the form of micrographs or photomacrographs(slightly magnified <10×) (Shibata et al., 2019); (Ali et al., 2022); (Grover et al., 2022); (Cheville & Stasko, 2014). A micrograph or photomicrograph is a photograph or digital image taken through a microscope or any other similar device to show a magnified image of an object. In the same vein but opposing to a micrograph is the photomacrograph which is also taken on a microscope but only slightly magnified, usually less than 10 times. Micrography is the practice or art of using a microscope to make photographs.

An electron micrograph is a micrograph prepared using an electron microscope. An electron microscope is a microscope that uses a beam of electrons as a source of illumination (Kalinin et al., 2023). They use electron optics that are analogous to the glass lenses of an optical light microscope to control the electron beam, for instance focusing them to produce magnified images or electron diffraction patterns (Sheridan et al., 2020); (Ansari et al., 2024). As the wavelength of an electron can be up to 100,000 times smaller than that of visible light, electron microscopes have a much higher resolution of about 0.1nm, which compares to about 900nm for a light microscope (El-Gomati, 2016); (Lelek et al., 2021). Examples of electron microscopes are Transmission electron microscope (TEM), Scanning Electron microscope (SEM), Electron microprobe, Ultrafast Scanning Electron microscopy, Low-energy electron microscopy (LEEM), and Photoemission electron microscopy (PEEM) (Mankos et al., 2014).

To study the epidermal cell type and describe stomata variations, development ontogeny and stomata index among species of Arachis hypogaea.L.{SAMNUT-23 and DANDAKAR} and bambaranuts {Vigna subteraneana.L.} to introduce further morphological features that can evaluate species delimitation and establishment of new taxa for distinctions among the Nigerian species of legumes.

Methodology

The research was conducted in the Botanical garden at Bayero University Kano which is located between latitude 12.002179 and longitude8.591956 with the GPS coordinates of 12°0'7.8444'' N and 8°35'31.0416''E in the Sahelian geographic region,south of the Sahara in Nigeria. The area is characterized by distinct wet and dry seasons with an elevation of 469.376m above sea level. An average annual temperature range of 38.2°C and receives an average of 696mm of precipitation annually. Leaves of plant species collected from sowed local varieties of Bambara nuts brought from Yankaba market Nassarawa local government of Kano State and the two varieties of SAMNUT-23 improved groundnuts from Kano State Agricultural and Rural Development Authority (KNARDA)

Results and Discussion

Three species of Leguminosae were studied and measurements were tabulated as represented in Tables 3.1 and 3.2 below the findings were as follows. In SAMNUT-23, matured stomata are paracytic on both surfaces while in Dandakar stomata types are trachytic and bambaranuts are both 12% paracytic in the adaxial and about 88% anisocytic in the abaxial surfaces.

Leaf Epidermal Cell, Stomata Types and Ontogenical Development

According to Ibrahim R.et al 2021, a mesogenous ontogenically development refers to a situation where subsidiary cells have a common origin with the guard cell as the protoderm divide symmetrically or equally i.e the subsidiary cell and the two guard cell mother cell(GCMC) arise from the same origin or loci of the meristemoid and asymmetric or unequal division of the protoderm to form a stomatal complex having at least one subsidiary cells from the same origin as the meristemoid in a mesoperigynous ontogenic situations. Meristemoids are observed to be a characteristic conspicuous nucleus with a dense cytoplasm identified by their small sizes and doubled walls as they are deeply stained in features. Based on the study of the nature of stomatal complex,Metcalfe and Chalk (1988) have classified stomata into different types and our terminologies in describing them are based on the assertions though new development emerges in characteristic feature ontogeny and phylogeny. The varieties of leguminous species studied have distinctive features as revealed from their images and electron photomacrograph as follows.

PEA NUT(SAMNUT-23)-Arachis Hypogaea:

In the stomata development pattern of SAM 23 mesoperigynous development of stomata was indicated and paracytic stomata features were observed in the photomacrograph of the matured layers as the two indistinct surrounding cells maintain their long axes p integral part of the stomata complex. parallel to the guard cells. Meanwhile, the activities of growth in the apex make the subsidiary appear as an equal integral part of the stomata complex.

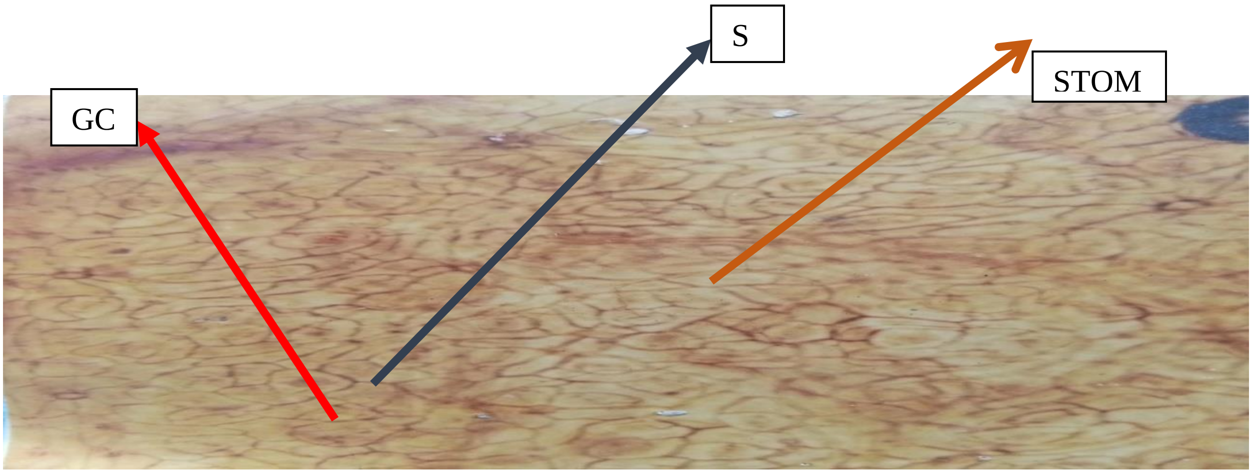

Figure 1. The Mesoperigynous development of stomata in the abaxial(lower) surface of the leaf epidermal peel of Arachis hypogaea (SAM-23) showing the paracytic type of stomata and sinuous/irregular cell wall using the photomacrographs MG (×400)

Information : STOMA: stoma singular, stomata plural; stomata complex, GC; guard cell.

Bambaranuts (vigna subteraneana.L.): Mesogenous feature of stomata development was observed in the bambaranuts(vigna subteraneana. L) electron photomacrographs MG (×400) and Anisocytic stomata types were indicated in the matured epidermal layer as one cell is considerably smaller or larger than the other two of the three unequal subsidiary cells surrounding the stomata.

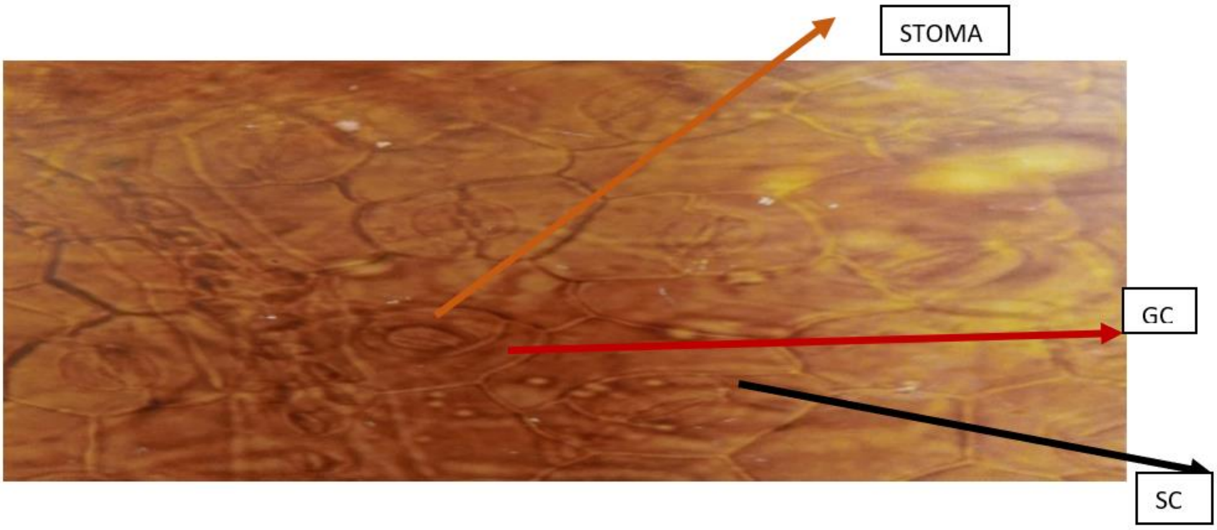

Figure 2.The mesogenous development of stomata in the lower surface of the leaf epidermal peel of bambaranuts (vigna subteraneana.L) photomacrographs MG (×400) showing anisocytic type of stomata and polygonal epidermal cell wall

Information :STOMA: stoma singular, stomata plural; stomata complex, GC; guard cell

GROUNDNUTS (DANDAKAR). Arachis. hypogaea.L: The development stage of Dandakar groundnut species' local varieties was found to be mesoperigynous in nature. A tetracytic type of stomatal complex was seen in the matured epidermal photomicrograph layer of Dandakar as four subsidiary cells surrounding the guard cell maintain their position at the polar and lateral view of the guard cells.

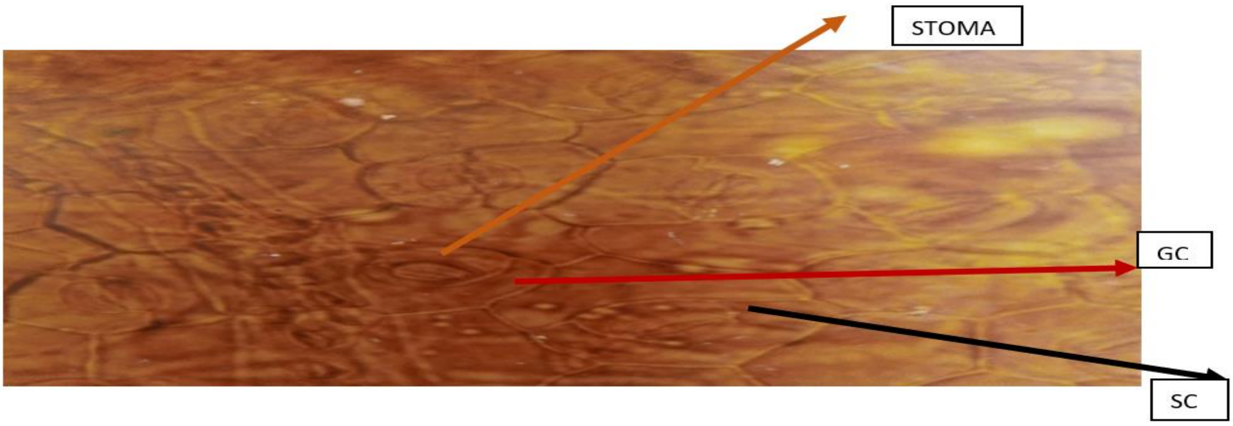

Figure 3.The mesoperigynous development of stomata in the lower surface of the leaf epidermal peel of Arachis hypogaea. L {Dandakar} photomacrograph (MG×400) showing tetracytic type of stomata pentagonal cell wall.

Information : STOMA:stoma singular,stomata plural; stomata complex, GC; guard cellThe results obtained indicate that several anatomical traits are variable to all taxa TABLE and thus of diagnostic value down to the generic or specific level. The outline of epidermal cells in all species is sinuous or thin-walled on the abaxial and irregular on the adaxial portion of the leaflet's blade.

Stomatal Ontogeny

The stomata measurement was reviewed while the difference between the mean and the least significance were compared at 5% levels of significance and the following results were obtained.

Table 1. Mean Stomata Measurement (MG ×400) and stomata features on the abaxial surface leaves of three legume plants.

Varieties/ Species	Stomata features	Cell Shape	Length of Stomata μm	Width of Stomata	Pore Length of Stomata	Pore Width of Stomata	No of Stomata	No Epidermal cell	Stomata Index
A.hypogaea.L {Dandakar}	Tetracytic	Pentagonal	2.38	1.78	2.12	0.50	1.72	6.14	5.76
A.hypogaea.L	Paracytic	Irregular	2.54	1.96	2.12	0.50	3.18	4.22	11.62

{SAM 23}									
V.subteraneana.L {Bambara nuts}	Anomocytic	Polygonal	2.32	1.90	2.00	0.90	2.92	4.48	10.66
L.S.D.5%			0.268	0.24	0.328	0.753	0.28	0.311	0.507

Table 2. Mean Stomata Measurement (MG ×400) and stomata features on the adaxial surface leaves of three legume plants.

Varieties/ Species	Stomata feature	Cell shape	Length of Stomata μm	Width of stomata	Pore Length of Stomata	Pore Width of Stomata	No of Stomata	No of Epidermal cell	Stomata Index
A.hypogaea.L {Dandakar}	Tetracytic	Pentagonal	2.38	1.72	2.12	0.5	1.48	5.98	5.28
A.hypogaea.L {SAM 23}	Paracytic	Irregular	2.38	1.96	2.06	0.46	2.88	4.82	9.94
V.subteraneana.L {Bambara nuts}	Anomocytic	Polygonal	2.08	1.52	2.02	0.48	2.26	5.58	7.8
L.S.D.5%			0.253	0.153	0.375	0.065	0.282	0.393	0.88

Lower Epidermal Layer: There is no significance difference in comparing the width of stomata,pore length of stomata and pore width of stomata between the three legumes. However significance difference was found between Arachis hypogaea {SAM 23) with the other two varieties in length of stomata,width of stomata,number of stomata and stomata index.Meanwhile in terms of number of epidermal cell and pore lengths it has shown significant difference exist between Arachis hypogaea{Dandakar} and the other two varieties which are {SAM 23} and bambaranuts.

Upper Epidermal Layer:Significance difference was observed between Arachis hypogaea{SAM 23} and the other two varieties in mean lenght,number of stomata and stomata index.In terms of width of stomata,pore length and pore width of stomata there is no significant difference between the three legumes varieties.The significance difference was found between Arachis hypogaea {Dandakar} and the other two varieties A.hypogaea {SAM 23} and bambaranuts in number of epidermal cell while SAM 23 and V.subteraneana.L {bambaranuts} are significant in stomata index. NOTE:The table of mean measurement of stomata in lower and upper surface are shown in Table 3.1 and Table 3.2 respectively.

Dichotomous Key

To delimit the varying species by observing their shapes and features represented in genetic variabilities and conformities which could be use to establish relationship among the taxa as describe by the dichotomous keys as follows;

1a. Leaflets shape is elliptical lanceolate……………………………………………………………2a

1b. Leaflets shape is Obtuse Lanceolate/Obovate…………………………………………………2b

1c. Leaflets shape is Linear Lanceolate ……………………………………………….2c

2a. Trichomes are less numerous in the adaxial surface…………………………………………3a

2b. Trichomes are more on the abaxial………………………………………………………….3c

2c. Trichomes are glabrous/ absent on all surfaces……………………………………………….3b

3a. Epidermal cell shape is Irregular…………………………………………………………….4a

3b. Epidermal cell shape is Polygonal..…………………………………………………………4b

3c. Epidermal cell shape is pentagonal…………………………………………………………4c

4a. Stomata features is Paracytic,Mesoperigynous develpment………………………………SAMNUT-23

4b. Stomata features is Anomocytic,Mesogenious development……………………………V.subterraneana

4c. Stomata features is Tetracytic,Mesoperigynous development……………………………Dandakar.

5a. Stomata development pattern is Mesoperigynous…………………………………………A.Hypogaea.

5b. Stomata development pattern is Mesogenous……………………………………………V.subtraneana

5c. Stomata development pattern is Mesoperigynous……………………………………….A.hypogaea

The characteristic features of the paracytic A. Hypogaeae SAMNUT-23 and tetracytic types of stomata in A.hypogaeae Dandakar clearly indicates how closely related the both species in similarity having Mesoperigynous developmental patterns which differs from the anomocytic V.subterraneana bambaranuts having a mesogenous development. High length of stomata which is more in abaxial surfaces of SAMNUT-23 indicates that the two varieties of the A. hypogaea have more efficient gaseous exchange mechanism to promote photosynthesis and ensures high sustainability compare to V.subterraneana bambaranuts.

Significant differences was observed in the number of stomata as they are more numerous on the abaxial leaves surfaces for A.Hypogaea in SAMNUT-23 Table 3.4.1 and in the number of epidermal cells on the abaxial of A.hypogaea in Dandakar which are functional for efficient gaseous exchange and effective photosynthesis of the plants specie as a need for promoting adaptability to new environment in accordance to the report of (Le et al., 2011); (Hasanuzzaman et al., 2023); (Ali et al., 2022) being a characteristic features and mechanisms to conserve water losses by transpiration in the event of harsh environmental conditions such as relative humidity, high light intensity and wind speed in accordance to (Wu et al., 2024); (Richter et al., 2022); (Koutouleas et al., 2022) for SAMNUT-23 and Dandakar respectively.

Furthermore the epidermal cell shapes of the legumes studied is observed to be sinuous and/or irregular in SAMNUT-23,polygonal in bambaranuts and pentagonal in Dandakar as a result of the genetic make-up,climatic conditions and environmental factors which can be described to enhance cell turgidity as reported by (Zhao et al., 2021); (Pacheco-Hernández et al., 2021); (Fischer et al., 2014).

The stomatal ontogeny in the species of legumes as viewed from the electron micrographs shows mesogenous which reveals anomocytic type of stomata in the bambaranuts while the A.Hypogaea varieties shows mesoperigynous development in a similar circumstances.The stomatal index of the leguminous species from the table 3.1 and 3.2 above shows variations on the adaxial and considerable increases on the abaxial surfaces of the leaves because of exposure to high light intensity which promotes the photosynthetic activities in accord with Adedeji and (Ahmed et al., 2020) but its has a highest value in the SAMNUT -23 among the variety of the legumes studied. This study has succeeded in the classification of plant species and varieties using diagnostic characters shown in the dichotomous key above in the identifications and nomenclature in line with (Martino et al., 2023).

Conclusion

The leaf epidermal features of the legumes and stomata ontogeny are taxonomic evidence that can be used in determining the differences and uniformity as exhibited in the number of epidermal cell lengths and features of the stomata complex. Species of groundnut and bambaranuts show two types of development which are Mesogenious and Mesoperigynous while similarity was observed among them in stomata types. The research agrees with the works of Ibrahim. R.et al. 2023 as the studied structures were considered to be useful in the separation of SAMNUT-23 from Dandakar as well as separating them from the bambaranuts species.

Conflicts of Interest

The authors declare no conflict of interest.

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