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Short Communication

Comparative stomatal studies of some *Gossypium hirsutum* L. species in Nigeria

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Abstract

A comparative anatomical studies of six G. hirsutum species collected from Katsina and Zamfara states of Nigeria were carried out. Stomatal complex types identified in three of the species were diacytic in the abaxial leaf surfaces and tetracytic in the adaxial leaf surfaces. Others had diacytic stomatal complex at the abaxial leaf surface as well as the adaxial leaf surface. Stomata were present on both leaf surfaces, that is the leaves were amphistomatic. Trichomes were found in 4 species out of the whole collection made. The abaxial leaf surfaces of the accessions had higher Stomatal density (up to 352.67mm^{-2}) except in one (262.33mm^{-2}). Stomata were relatively large in all the species. The epidermal cell shapes of all the species collected were polygonal.

Keywords: Stomata, *Gossypium hirsutum.*

Introduction

Anatomical analysis of six *G. hirsutum* species were carried out in this work to understand the diversity and taxonomic relationship with regard to stomatal complex type, stomatal size and density as well as stomatal index in the these species. *G. hirsutum* is a cotton plant that belongs to the genus *Gossypium* and family Malvaceae, or mallow family. The plant is indigenous to the tropic and subtropic regions, including America, Africa, and India. It is the most widely grown species globally. It is cultivated on about 95% of the world cotton hectarage[1]. The English name is derived from the Arabic word *(al) qutn*[2]. Leaf epidermis, primary vascular system, wood, fruit structures are plant features that anatomical information could be obtained for Biosystematics purposes. The morphological and leaf epidermal features were discovered as essential elements to taxonomic studies[3]. Leaf epidermal features such as hairs, morphology and size of epidermal cell, size, arrangement and spread of stomata apparatus: rate of stomatal occurrences and percentage stomatal frequency to epidermal cell density are often useful at the exact and lower levels[4]. Researches on plant anatomy were successfully utilized to elucidate taxonomic position and aided in the classification of diverse species[5]. In recent past, studies to resolve taxonomic problems were carried out using anatomical and morphological features of monocots. The leaf of plants is mostly utilized in plant taxonomy[6]. The most vital feature in resolving taxonomic problems after cytology is the leaf epidermis[7]. Data obtained from anatomical studies are now known to provide facts on not only species delimitation but also for determination of the relationship and cause of evolution among related taxa. Hence, any biosystematic study without a serious regard to the anatomical features of the organisms that are being studied would be regarded as incomplete[8]. The purpose of this study is to assess the variations that could be used in the classification of the species and examine its importance in the plant taxonomy.

Materials and methods

Seeds Collection: The seeds of the *G. hirsutum* species used for this study were from parts of Katsina and Zamfara states, Nigeria where the plant is cultivated during harvest period from the field. The species were identified in the Department of Plant Biology Herbarium, University of Ilorin. The seeds were labeled appropriately to indicating where collections were made. The cotton seeds collected were brought for cultivation at the Botanical Garden of the University of Ilorin. Mature and healthy leaves were removed from the species growing in the Garden. Maceration with HNO_3 was found to provide good results as it was adopted in this research. A portion of fresh leaf from each accession was cut close to the midrib and HNO_3 to macerate it for microscopic studies. Each of the samples were placed on clean glass slides and stained with 1% safranin for 3-5 minutes. A quantity of glycerine was dropped to the sample, already stained with safranin, then cover slip was used to cover it. The epidermal cell type, epidermal size, stomatal complex type, stomatal size as well as the stomatal index and stomatal density were determined. These were profiled accordingly. Leaf epidermal cells were photographed using digital amscope microscope camera at X5 and X10 objectives. A sequential record of each photographed specimen was kept as it was taken.

Frequency of Stomatal Complex Types: Frequency of the stomatal complex type was determined by using a field of view at X40 objective as a quadrant, the number of subsidiary cells per stoma determines the frequency of a complex type in each population. Percentage occurrence of a complex type reveals the frequency of such complex type based on occurrences[9]. Terms used in expressing stomatal complex type was as described by Dilcher, Metcalfe and Chalk.

Determination of Stomatal Index and Stomatal Density: Stomatal index was calculated using the formula described by Salisbury;

Stomatal index (I) = S/E + S x 100

Where: S = number of stomata per square millimetre, E = number of epidermal cells per unit area.
The stomatal density (SD) was as described by Stace; that is the number of stomata per square millimetre.

Determination of Stomatal Size: Stomata Size (SS) of was calculated as the product of Length (L) and Breadth (B) determined from measuring guard cells, using micrometer eye piece, multiplied by Franco's constant (K) 0.78524. Measurements of 35 stomata were used. The formula is expressed as SS=L x B x K

Epidermal Size Measurement: Epidermal cell size was calculated by multiplying Length (L) by Breadth (B) of epidermal cell by the use micrometer eye piece. Size of the sample used was 35 cells.

Results and discussion

The results from the leaf anatomical study shows that the epidermal cell shapes are polygonal in all the species studied for both surfaces of the leaf. Epidermal cells of the periclinal walls are raised and convex. The abaxial and adaxial stomata were coplanar to the epidermal surface. The highest epidermal density (370.33mm^{-2}) on the abaxial side was observed in GS while the lowest abaxial epidermal density (249.33mm^{-2}) was observed in BK. The highest epidermal density (284.67mm^{-2}) for the adaxial surface was recorded in GS but the least epidermal density (201.33mm^{-2}) in the adaxial surface was obtained in KR. The Epidermal density on the abaxial surfaces was not significant different in all the species studied. However, the epidermal density on the adaxial leaf surface of GS was different significantly from that of YG. The indumentums consisted of trichomes. The shapes of the stomatal complex type were paracytic on both sides. The stomatal distribution was amphistomatic that is there was the presence of stoma on both abaxial and adaxial leaf surfaces except in one of the species. Oosterhuis and Jernstedt reported that stoma is present on the abaxial epidermal layer and adaxial epidermal layer, but is more numerous on the lower leaf blade surface this corroborate results obtained in this study. The highest stomatal index (56.03%) on the abaxial side was observed in GS1 while the lowest abaxial stomatal size (47.81%) was observed in KR. The highest stomatal index (52.27%) for the adaxial surface was recorded in YG but the least stomatal index (40.18%) in the adaxial surface was obtained in GS. The abaxial and adaxial stomatal indexes were not significantly different in all the species studied. Previous studies have shown that availability of water, CO_2 concentration, temperature and intensity of light affect stomatal frequency. Water stress results in a greater stomatal frequency. The highest stomatal density (352.67mm^{-2}) on the abaxial side was observed in GS1 while the lowest abaxial stomatal density (232mm^{-2}) was observed in KR. The highest stomatal density (265.33mm^{-2}) for the adaxial surface was recorded in GS1 but the least stomatal density (218.67mm^{-2}) in the adaxial surface was obtained in KR. Stomatal density at the abaxial leaf surface of GS1 was significantly different from YG. However, the stomatal density of BK, GS, KR, and FT were not significantly different from each other on the abaxial. GS1 stomatal density on the abaxial was significantly different from the abaxial surface of KR. It was observed that there was no difference significantly on the adaxial surfaces of all the species. The abaxial leaf surfaces of the species observed had a high stomatal density except in YG that had a higher adaxial stomatal density. There is variety of Stomatal frequency somewhat from one leaf to the other or same plant also even in different parts or same leaf[16]. There is diverse stomatal density on leaf surfaces in some plant species[17]. The highest stomatal size (53.07µm) on the abaxial side was observed in YG while the lowest abaxial stomatal size (42.91µm) was observed in BK. The highest stomatal size (57.77µm) for the adaxial surface was recorded in KR but the least stomatal size (43.9µm) in the adaxial surface was obtained in GS1. The stomatal size of the abaxial leaf surfaces of the species observed had no difference significantly. The GS and GS1 species' sizes of stomata on the abaxial leaf surface were different significantly from BK, KR, FT and YG. The size of KR adaxial stomata was different significantly from that of GS1and GS. Large stomatal sizes were recorded on the abaxial leaf surface and adaxial leaf surface of the species studied. Guard cells of stomata that are less than 15µm long are designated "small" while stomata that are greater than 38µm long are termed "large"[18]. The leaf anatomical characters revealed some significant similarities and differences with reference to their various sites of collection, showing that variations exist within the species. The highest stomatal lenght (9.26µm) on the abaxial side was observed in FT while the lowest abaxial stomatal lenght (8.29µm) was observed in GS. The highest stomatal lenght (9.51µm) for the adaxial surface was recorded in KR but the least stomatal lenght (8.13µm) in the adaxial surface was obtained in GS1. The stomatal length on all the abaxial surfaces of the species was not significantly different from each other. However, the stomatal length on the adaxial surface of KR, FT and YG were significantly different from that of GS, BK and GS1. The adaxial surfaces of KR, FT and YG had stomatal lengths that were not significantly different. The stomatal length of GS, BK and GS1 were also not significantly different. The highest stomatal width (7.71µm) on the abaxial side was observed in FT while the lowest abaxial

stomatal width (6.5µm) was observed in GS1. The highest stomatal width (7.88µm) for the adaxial surface was recorded in YG but the least stomatal size (6.44µm) in the adaxial surface was obtained in GS. No Significant difference was observed in between the population's abaxial stomatal width. There was also no difference between the species adaxial stomatal width significantly. The difference in guard cell area, stomatal density, stomatal index, and epidermal cell shape and walls depicts genetic diversity that could be utilized in distinguishing a plant species as reported in a study on three Nigerian species of *Aspilia*[19]. The features of a leaf epidermal cell, stomata and trichomes in *Ficus* were based on their genetics and can be used for identification and systematic purposes[20].

Table-1: Qualitative epidermal features.

Accession	Leaf Surface	Stomatal Complex Type	Trichome	Stomata Distribution	Epidermal Cell Shape
GS	ABAXIAL	Diacytic	Present	amphistomatic	polygonal
	ADAXIAL	Tetracytic	Nil	amphistomatic	polygonal
BK	ABAXIAL	Diacytic	Present	amphistomatic	polygonal
	ADAXIAL	Diacytic	Nil	amphistomatic	polygonal
KR	ABAXIAL	Diacytic	Present	amphistomatic	polygonal
	ADAXIAL	Diacytic	Nil	amphistomatic	polygonal
FT	ABAXIAL	Diacytic	Nil	amphistomatic	polygonal
	ADAXIAL	Diacytic	Nil	amphistomatic	polygonal
YG	ABAXIAL	Diacytic	Nil	amphistomatic	polygonal
	ADAXIAL	Tetracytic	Nil	amphistomatic	polygonal
GS1	ABAXIAL	Diacytic	Present	amphistomatic	polygonal
	ADAXIAL	Tetracytic	Nil	amphistomatic	polygonal

Table-2: Mean values of leaf epidermal features.

Accession	Surface	Stomatal Size (µm)	Stomatal Length (µm)	Stomatal Width (µm)	Stomatal Density (mm^{-2})	Epidermal Cell density (mm^{-2})	Stomatal Index
GS	Abaxial	43.22 ± 1.11^{ab}	8.29 ± 0.16^{a}	6.9 ± 0.15^{abc}	271.67 ± 20.93^{abc}	370.33 ± 9.60^{a}	48.81 ± 2.07^{a}
	Adaxial	44.57 ± 0.21^{cd}	8.43 ± 0.18^{cde}	6.44 ± 0.23^{bcd}	254 ± 41.62^{a}	284.67 ± 21.67^{a}	40.18 ± 4.27^{c}
BK	Abaxial	42.91 ± 0.11^{ab}	8.34 ± 0.56^{a}	7.55 ± 0.79^{abc}	289 ± 4.04^{abc}	249.33 ± 27.38^{a}	55.22 ± 2.71^{a}
	Adaxial	54.91 ± 3.86^{ab}	8.98 ± 0.33^{abcd}	7.16 ± 0.57^{abc}	254 ± 9.00^{a}	235.66 ± 30.33^{bcd}	50.73 ± 2.09^{abc}
KR	Abaxial	52.88 ± 1.05^{a}	8.89 ± 0.36^{a}	7.63 ± 0.29^{ab}	232 ± 21.78^{c}	252 ± 16.00^{a}	47.81 ± 2.49^{a}
	Adaxial	57.77 ± 4.13^{a}	9.51 ± 0.32^{ab}	7.45 ± 0.09^{ab}	218.67 ± 31.80^{a}	201.33 ± 9.37^{cd}	51.45 ± 4.10^{abc}
FT	Abaxial	44.25 ± 1.85^{ab}	9.26 ± 0.16^{a}	7.71 ± 0.08^{a}	308.32 ± 26.58^{abc}	269 ± 56.79^{a}	54.37 ± 5.44^{a}
	Adaxial	54.78 ± 2.92^{ab}	9.27 ± 0.17^{abc}	6.90 ± 0.38^{abcd}	237 ± 9.71^{a}	229.67 ± 17.64^{bcd}	50.89 ± 2.92^{abc}
YG	Abaxial	53.07 ± 1.25^{a}	8.76 ± 0.35^{a}	6.68 ± 0.15^{abcd}	262.33 ± 15.30^{bc}	263 ± 33.00^{a}	50.32 ± 2.2^{a}
	Adaxial	56.52 ± 3.14^{ab}	9.19 ± 0.35^{abc}	7.88 ± 0.8^{ab}	265 ± 24.34^{a}	243 ± 29.57^{bcd}	52.27 ± 2.53^{abc}
GS1	Abaxial	43.3 ± 1.28^{ab}	8.31 ± 0.32^{a}	6.54 ± 0.18^{abcd}	352.67 ± 18.94^{a}	278 ± 26.63^{a}	56.03 ± 2.77^{a}
	Adaxial	43.9 ± 0.76^{cd}	8.13 ± 0.35^{de}	6.68 ± 0.15^{abcd}	265.33 ± 24.34^{a}	263 ± 33.00^{bc}	50.39 ± 4.79^{abc}

Means with different letter (s) along a column were described as significantly different at probability level of 0.05.

Conclusion

Conclusively, results from this stomatal studies showed that variations exist within the species studied which could be of taxonomic importance. Similarities were also observed from the results that show relatedness of the species but may not be of taxonomic value in delimiting the species.

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