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Comparative Leaf Epidermal Characteristics of Selected Species of the Family Brassicaceae in Kano, Nigeria

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Abstract

Leaf epidermal features play an important role in plant identification and adaptation. In this study, the leaf epidermal characteristics of three members of the Brassicaceae family; *Brassica oleracea var. capitata*, *Brassica oleracea var. botrytis*, and *Raphanus sativus*, were examined, focusing on stomatal type, distribution, and some quantitative parameters. Epidermal peels were obtained from both adaxial and abaxial leaf surfaces and observed under light microscope. All species showed anisocytic stomata on both leaf surfaces and were amphistomatic, with higher stomatal frequency on the abaxial surface. There were noticeable differences among the species in stomatal number, pore length, epidermal cell number, and stomatal index. The highest stomatal index on the lower surface was recorded in *Raphanus sativus* with 20.1%, and the least was recorded in *Brassica oleracea var. botrytis* with 12.28%. While the highest stomatal index on the upper surface was recorded in *Raphanus sativus* with 17.9%, and the least was recorded in *Brassica oleracea var. botrytis* with 8.18%. These results showed that epidermal features, especially quantitative ones, can be useful in distinguishing species and understanding their physiological behavior.

Introduction

The family *Brassicaceae* consists of mainly herbaceous plants that may be annual, biennial, or perennial, with few shrubs, dwarf shrubs, and rare aquatic species such as *Subularia aquatica* (Al-Shehbaz, 2012). Most members of the family are terrestrial and show a wide range of structural features. Their root systems are commonly taproots, although some species may develop modified forms such as woody bases or rhizomes. The leaves are usually simple and lack stipules, and they may appear either as basal rosettes or along the stem. Trichomes in this family are typically unicellular and show considerable morphological diversity, including simple, forked, stellate, dendritic, and T-shaped forms, and are characteristically non-glandular (Al-Shehbaz, 2012; Beilstein et al., 2006). The stems of *Brassicaceae* may be erect, ascending, or prostrate and are generally

herbaceous. Leaves are exstipulate, though paired glands may occur at the base of petioles or pedicels. Leaves are simple, entire or lobed, rarely compound, and may form basal rosettes. Cauline leaves are typically arranged alternately (Al-Shehbaz, 2012; Raza et al., 2020). One important anatomical feature in *Brassicaceae* is the presence of anisocytic stomata. This stomatal type, which consists of three unequal subsidiary cells, is widely regarded as a characteristic feature of the family (Carpenter, 2005; Frank et al., 2017). Genome sizes within *Brassicaceae* are generally small to moderate, ranging from approximately 135 Mbp in *Arabidopsis thaliana* to over 2400 Mbp in *Bunias orientalis* (Lysak, 2018). Chromosome numbers vary widely due to polyploidy, and hybridization is common in genera such as *Cardamine*, *Arabis*, *Rorippa*, and *Boechera*, contributing to extensive morphological and genetic diversity (Lysak, 2016; Lysak, 2018; Zhang, 2025).

Brassicaceae is distinguished from closely related families by the presence of bisymmetrical corollas, fruits divided by a persistent septum, exstipulate leaves, and predominantly simple leaf blades. The sister family Cleomaceae differs by having bilaterally symmetrical flowers, stipules, palmately compound leaves, and fruits that often lack a septum. Capparaceae is further separated by the frequent presence of a gynophore or androgynophore and a variable number of stamens (Al-Shehbaz, 2012; Raza et al., 2020).

Brassica oleracea var. capitata (Cabbage), is an economically important vegetable crop within the Brassicaceae family. The species comprises several cultivated forms, including cabbage, broccoli, cauliflower, kale, Brussels sprouts, collards, and kohlrabi (Cheng et al., 2016). Cabbage is a herbaceous plant characterized by compact heads formed from overlapping leaves. The species is predominantly outcrossing and exhibits sporophytic self-incompatibility, requiring insect-mediated pollination for effective seed production. Cross-pollination contributes to genetic variability within cultivated populations (Sehgal & Singh 2018; Murase et al., 2024).

Brassica oleracea var. botrytis (Cauliflower), is a biennial vegetable crop characterized by a compact curd composed of arrested floral meristems. Like other members of the family, its flowers possess four petals arranged in a cruciform pattern (Cheng et al., 2016). Cauliflower is self-incompatible and requires cross-pollination for seed set. Vernalization is essential for floral induction, and population size influences seed yield and genetic diversity (Sehgal & Singh 2018, Yadava 2022, Murase et al. 2024).

Raphanus sativus (Radish), is an annual or biennial root vegetable widely cultivated worldwide. Molecular and morphological evidence suggests that the species originated in the eastern Mediterranean and Near Eastern regions rather than East Asia (Shirasawa & Kitashiba 2017, Li et al., 2025). The edible portion consists primarily of the hypocotyl and upper taproot.

Information on leaf epidermal features in Brassicaceae species is still limited. This makes it difficult to clearly distinguish closely related species using microscopic characters (Chater et al., 2017). Leaf epidermal traits can help in accurate plant identification. They also provide useful information on gas exchange and water regulation in plants (Sack & Buckley, 2016). The aim of this study was to investigate the leaf epidermal characteristics of three members of the Brassicaceae family; *Brassica oleracea var. capitata*, *Brassica oleracea var. botrytis*, and *Raphanus sativus*, focusing on stomatal type, distribution, and some quantitative parameters.

Materials and Methods

Study area

The study was conducted at the Plant Physiology Laboratory, Department of Plant Biology, Faculty of Life Sciences, Bayero University, Kano, Nigeria. The laboratory is situated at the university's Old Campus (11°58'51.2'' N}, 8°28'57.4'' E).

Sample Size Determination

To ensure adequate statistical power for detecting differences in quantitative stomatal parameters among the three species, a sample size calculation was performed for a One-Way ANOVA ($\alpha = 0.05$, target power $1 - \beta = 0.80$). Assuming a large effect size ($f = 0.65$) based on historical variation in

Brassicaceae epidermal indices, a minimum of n = 8 microscopic fields per group was determined necessary. To accommodate potential field-of-view artifacts or slide preparation flaws, sample size was adjusted to n = 10 randomly selected microscopic fields per surface for each species (derived from 5 biological plant replicates, 2 optical fields per replicate), yielding a total of N = 30 fields per surface across all taxa.

Sample Collection

Fresh, healthy, and fully expanded leaves *of Brassica oleracea var. capitata, Brassica oleracea var. botrytis,* and *Raphanus sativus* were collected from cultivated plants. Only leaves of similar size and maturity were selected to ensure consistency. The samples were placed in clean, labeled bags and taken to the laboratory. They were processed shortly after collection to maintain their freshness and prevent deterioration.

Epidermal Preparation

Epidermal peels were obtained from the middle portion of each leaf using the direct peeling and scraping methods. Both adaxial and abaxial surfaces were prepared following standard plant anatomical procedures (Yuan, 2020). The peels were stained with 1% aqueous safranin, mounted in glycerol, and covered with a coverslip for examination.

Microscopy and Measurements

The prepared slides were examined under a light microscope at ×400 magnification. Stomatal type was identified based on the arrangement of subsidiary cells. Stomatal number, epidermal cell number, stomatal length and breadth, and pore length and breadth were measured from five randomly selected microscopic fields using an ocular micrometer.

Stomatal Index

Stomatal index (SI) was calculated as: $SI = \frac{S}{S+E} X\ 100$ (Salisbury, 1927; Esau, 1977).

Where S is the number of stomata and E is the number of epidermal cells per field.

Data Analysis

Quantitative leaf epidermal data—including stomatal frequency, epidermal cell count, stomatal dimensions (length and breadth), pore dimensions, and stomatal index (SI)—were subjected to descriptive statistics and expressed as mean values. Data distributions were first evaluated for normality using the Shapiro-Wilk test, and homogeneity of variances was assessed using Levene's test to ensure compliance with the assumptions of parametric analysis. Differences among the three target taxa *(Brassica oleracea var. capitata, Brassica oleracea var. botrytis,* and *Raphanus sativus*) were evaluated separately for adaxial and abaxial surfaces using a One-Way Analysis of Variance (ANOVA). Where significant F-statistics were obtained ($p < 0.05$), mean separation was performed using Fisher's Least Significant Difference (LSD) post hoc test at a 5% probability level to pinpoint specific pairwise morphological differences. All statistical analyses were computed using SPSS version 26.0.

Results

Microscopic examination showed that all three species possessed anisocytic stomata on both leaf surfaces. The leaves were amphistomatic, although more stomata were found on the abaxial surface. As seen in Table 1 and 2, there were clear differences among the species. *Raphanus sativus* consistently recorded the highest stomatal number and stomatal index, while *Brassica oleraceae var. botrytis* generally showed lower values.

Discussion

All the species studied showed that all three species exhibited anisocytic stomata, which agrees with the earlier reports that this stomatal type is typical of the Brassicaceae family (Lysak, 2018, Raza et al., 2020). The fact that all the three species shared this feature suggests that stomatal type is relatively stable within the family and may not be very useful for distinguishing closely related species. The leaves were amphistomatic with stomata present on both surfaces. However, more stomata were seen on the lower surface. This pattern is common in many plants and helps reduce water loss since the lower surface is less exposed to sunlight and

wind. At the same time, having stomata on both surfaces may improve gas exchange, especially in fast-growing plants (Franks et al., 2017, Raza et al., 2020).

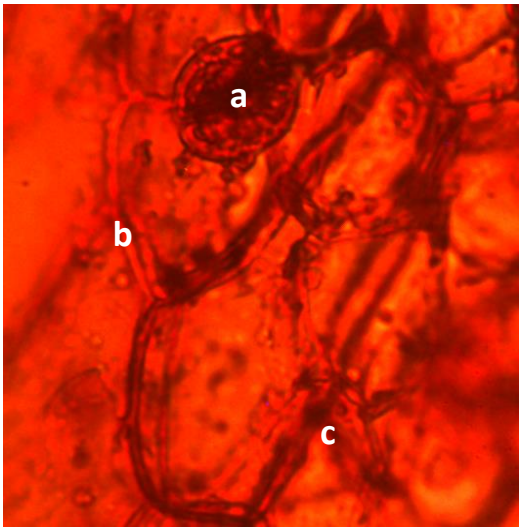

Figure 1: Anisocytic stomata found in upper surface of *Brassica oleracea* var *capitata* (x400)

Key: a= Stomata, b= Subsidiary Cell, c= Epidermal Cell,

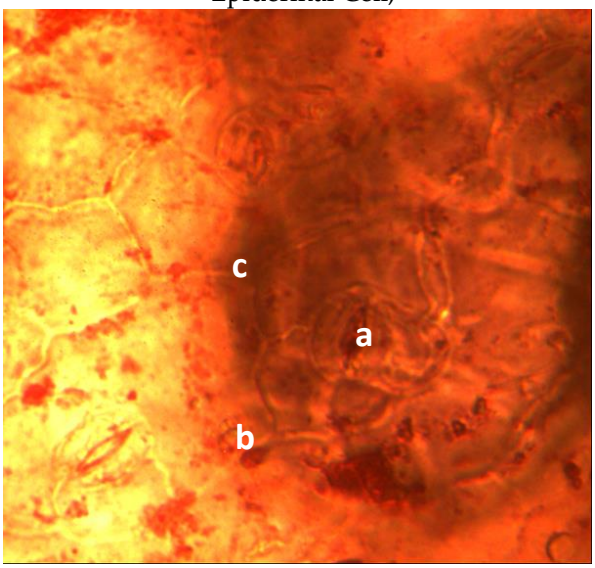

Figure 2: Anisocytic stomata found in upper surface of *Raphanus sativus* (x400)

key: a= Stomata, b= Subsidiary Cell, c= Epidermal Cell

There were noticeable differences in stomatal number, epidermal cell number, and stomatal index among the species. *R. sativus* had the highest stomatal index, which suggests that it may have a greater capacity for gas exchange. This could be related to its rapid growth and high metabolic activity (Lawson & Blatt, 2014). On the other hand, *B. var. botrytis* showed lower stomatal values, which may indicate a more conservative water-use strategy. These differences suggest that even closely related species can vary in how they regulate water loss and gas exchange.

The lack of significant differences in stomatal length and breadth among the species suggests that these traits are more conserved structurally, while stomatal density and pore size are more responsive to environmental and physiological demands. This pattern aligns with findings that stomatal size and density often adjust to help plants perform better under different environmental conditions (Drake et al., 2013).

Stomatal index (SI) also varied among the species with *R. sativus* having the highest values on both leaf surfaces. Compared to stomatal number, SI is considered more reliable because it takes into account both stomata and epidermal cells, and is less affected by leaf expansion (Sack & Buckley, 2016). Higher SI values are usually linked to better gas exchange and photosynthetic activity (Dow & Bergmann, 2014). This may explain why *R. sativus* appears more adaptable and widely cultivated. On the other hand, the lower SI values in *B. var. botrytis* may suggest a reduced rate of water loss. The findings of this study showed that combining both qualitative and quantitative epidermal features can be useful in plant taxonomy.

Overall, the results showed that while some features such as stomatal type are similar across species, quantitative traits provide better insight into their differences and adaptations (Sack & Buckley, 2016). Furthermore, these anatomical features have significant ecological implications. Variations in stomatal characteristics are closely linked to plant responses to environmental stress, including drought and temperature fluctuations (Lawson & Vialet-Chabrand, 2019). As such, epidermal traits can serve as useful indicators in studies of plant adaptation, climate change responses, and crop improvement.

Table 1: Abaxial Stomatal Characteristics of the Sampled Brassicaceae

Species	Types of Stomata	No. of Stomata Per Field	No. of E.C. Per Field	L.S(μm)	B.S(μm)	L.P(μm)	B.P(μm)	S.I
Brassica oleracea var. capitata	Anisocytic	18.8[b]	96.2[a]	85.68	71.4[a]	48.55[b]	17.85	16.3[b]
Brassica oleracea var. botrytis	Anisocytic	9.1[c]	67[b]	91.39	75.68[a]	51.41[b]	17.85	12.28[c]
Raphanus sativus	Anisocytic	24.7[a]	97.8[a]	94.24	78.54[a]	59.9[a]	18.42	20.1[a]
LSD 5%		1.25	3.5	9.01	8.21	6.38	5.76	1.12

Means followed by different superscript letters within a column are significantly different at $p < 0.05$ (LSD test).

Key: No. E.C = No. of Epidermal Cells, L.S. = Length of Stomata, B.S. = Breadth of Stomata, L.P. = Length of Pore, B.P. = Breadth of Pore, S.I. = Stomatal Index, a = Highest value, b = Moderate value, c = Lowest value

Table 2: Adaxial Stomatal Characteristics of the Sampled Brassicaceae

Species	Types of stomata	No.of stomata per field	No.of E.C per field	L.S(μm)	B.S(μm)	L.P(μm)	B.P(μm)	S.I
Brassica oleracea var. capitata	Anisocytic	12.8[b]	82.7[b]	81.39	67.12[a]	42.84[b]	16.42	13.55[b]
Brassica oleracea var. botrytis	Anisocytic	5.9[c]	77.4[c]	79.96	65.68[a]	51.41[a]	17.85	8.18[c]
Raphanus sativus	Anisocytic	21.3[a]	97.4[a]	79.96	65.68[a]	51.41[a]	18.27	17.9[a]
LSD 5%		1.23	3.18	6.63	6.63	5.52	5.36	2.24

Means followed by different superscript letters within a column are significantly different at $p < 0.05$ (LSD test)

Key: No. E.C=No. of Epidermal Cells, L.S= Length of Stomata, B.S= Breadth of Stomata, L.P= Length of Pore, B.P= Breadth of Pore, S.I= Stomatal Index, a = Highest value, b = Moderate value, c = Lowest value

The observed amphistomatic distribution—coupled with significantly greater abaxial stomatal density—is a functional adaptation to the environmental conditions of Kano, Nigeria. Situated in the semi-arid Sudan Savanna, Kano experiences elevated solar radiation, high ambient temperatures, and low relative humidity during the dry-season farming cycle. Concentrating stomata on the shaded abaxial surface reduces direct thermal exposure and minimizes transpiration rates while maintaining atmospheric carbon dioxide assimilation capacity (Franks et al., 2017; Lawson & Vialet-Chabrand, 2019).

The high Stomatal Index recorded in *Raphanus sativus* (20.1% abaxial) correlates with its rapid vegetative growth cycle and elevated metabolic requirements (Lawson & Blatt, 2014). In contrast, the lower Stomatal Index and stomatal frequency observed in *Brassica oleracea var. botrytis* (12.28% abaxial) reflect a more conservative water-use strategy designed to limit transpirational water loss under high evaporative atmospheric demand.

These findings align with anatomical trends documented across West African and Nigerian flora. AbdulRahaman and Oladele (2003) demonstrated that stomatal density and index in Nigerian vegetable species display plastic adjustments tailored to localized microclimatic stress. Similar amphistomatic patterns and abaxial stomatal predominance were reported by Omolokun et al. (2024) across tropical dicotyledons in northern Nigeria. Furthermore, AbdulRahaman

et al. (2013) noted that in semi-arid agrarian regimes, crops with lower stomatal indices on the adaxial surface exhibit superior resistance to atmospheric desiccation, corroborating the conservative anatomical profile observed here for *Brassica oleracea var. botrytis.*

Ecological and Agricultural Implications

Water-Use Efficiency (WUE): *Brassica oleracea var. botrytis* exhibits foliar traits indicative of higher Water-Use Efficiency, making it anatomically better suited to withstand moderate water stress during periods of restricted irrigation.

Irrigation Management: Due to its high Stomatal Index and potential for rapid transpirational loss, *Raphanus sativus* requires consistent moisture management when cultivated during dry, windy harmattan periods in northern Nigeria to prevent wilting.

Crop Breeding: Quantitative foliar metrics (such as lower adaxial stomatal density) can serve as physiological selection criteria when screening *Brassicaceae* cultivars for drought tolerance in dryland farming systems.

Limitations

This study evaluated anatomical responses under field conditions within a single region (Kano) during one cropping season. Environmental variables such as precise soil moisture deficits, ambient vapor pressure deficits, and light intensity were not manipulated independently.

Recommendations:

Future investigations should incorporate scanning electron microscopy (SEM) to evaluate cuticular wax deposition and micro-morphology of anticlinal cell walls.

Multi-locational trials across different agro-ecological zones (e.g., Rainforest, Guinea Savanna, and Sudan Savanna) should be conducted to measure environmental plasticity.

Physiological studies combining foliar anatomy with real-time gas exchange measurements (A/C curves and stomatal conductance) are recommended to confirm the functional performance of these crops under heat and drought stress.

Conclusion

The study showed that leaf epidermal characteristics are useful in understanding both the taxonomy and physiology of *Brassicaceae*. Although the species shared anisocytic stomata and amphistomatic leaves, they differed significantly in stomatal number, pore size, and stomatal index. *R. sativus* showed the highest stomatal index, suggesting a greater gas exchange capacity. These differences highlight the importance of quantitative epidermal traits in species identification and studying their ecological adaptations.

Conflicts of Interest

The authors declare no conflicts of interest.

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Author contributions

AmM, ZM, AiM, AK, and UAM conceived and designed the study. AmM, ZM, AiM, AK, and AA did data collection and analysis. AmM, ZM, AA, and UAM contributed to writing of the manuscript. All the authors have read and agreed to the final manuscript.

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