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Research Article

Investigating Leaf Epidermal Traits and Their Impact on Fruit Yield of Watermelons

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Abstract

Background and Objective: Investigating leaf epidermal traits and their impact on fruit yield in watermelons is an important area of agricultural research. The epidermis or outer layer of leaves, plays a critical role in photosynthesis, water regulation and overall plant health. Limited research has been conducted to explore the impact of leaf epidermal traits on the fruit yield of watermelons. Therefore, this study investigated the relationship between leaf epidermal characteristics and fruit yield of watermelon plants.

Materials and Methods: This study was conducted in 2018 on four varieties of watermelon (sugar baby, Kaolack, Grey belle and Collos F1) under controlled conditions. Their leaf epidermal features such as stomatal density, stomatal size, shapes and fruit yield were examined. Paradermal sections of the adaxial and abaxial were made on the 32 leaves of the four varieties of watermelon. Data collected were analyzed using ANOVA and means were compared using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. **Results:** Significant variations in leaf epidermal traits were observed among watermelon varieties. Epidermal cells were polygonal and irregular, with anticlinal cell walls varying from straight to wavy. Stomatal complexes included tetracytic, anomocytic and anisocytic types, with shapes such as round, elliptic and oval. Key measurements for the Kaolack variety included a guard cell area of $506.55 \mu\text{m}^2$, stomatal density of 32.30 mm^{-2} and a stomatal index of 19.86, correlating with the highest fruit yield of 61.83 ton/ha, compared to 51.99 ton/ha for Collos F1 and 31.05 ton/ha for sugar baby. Higher stomatal density and smaller size are linked to increased yields. **Conclusion:** The investigation into the relationship between leaf epidermal traits and fruit yield in watermelons revealed a complex interplay of factors influencing plant performance.

Key words: Coordinating cell, epithelial cell, leaf pores, ventral, watermelon, fruit yield

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Watermelon (*Citrullus lanatus*) is an economically important fruit crop widely cultivated worldwide, valued for its sweet and refreshing taste¹. Various factors, including genetic traits environmental conditions and cultural practices influence the productivity and quality of watermelon fruits². Leaf epidermal traits, such as stomatal density, stomatal size and trichome density, have been recognized as important indicators of plant physiological processes and adaptability to different growing conditions³. Understanding the impact of leaf epidermal traits on fruit yield can provide valuable insights for overall crop improvement and management strategies.

Leaf epidermal traits play a crucial role in the overall photosynthetic performance and water relations of plants. Stomata, microscopic openings on the leaf surface, regulate gas exchange, including carbon dioxide uptake for photosynthesis and water vapor release through transpiration⁴. Stomatal density and size influence the efficiency of gas exchange and water use efficiency. Trichomes, on the other hand, are epidermal hairs that can affect leaf surface temperature, reduce water loss and provide protection against pests and diseases⁵.

The genetic variation among different watermelon varieties gives rise to variations in leaf epidermal traits, which may impact the plant's ability to cope with environmental stresses and influence fruit yield. By investigating the relationship between leaf epidermal traits and fruit yield, researchers and growers can gain insights into the physiological mechanisms underlying high-yielding watermelon plants. Several studies have explored the association between leaf epidermal traits and fruit yield in various crop species. A study by Shahinnia *et al.*⁶ and Mokhesur Rahman *et al.*⁷, revealed that leaf stomatal density was found to be positively correlated with grain yield in wheat, indicating the potential of this trait as a selection criterion for high-yielding varieties. Studies on other fruit crops, such as grapes and apples, have demonstrated the influence of leaf epidermal traits on photosynthesis, transpiration and overall fruit quality^{8,9}. Research on tomato plants has revealed that stomatal density and trichome density are correlated with plant water use efficiency and resistance to insect pests¹⁰.

The objective of this study was to investigate leaf epidermal traits and their impact on fruit yield in four varieties of watermelons. By analyzing stomatal density and size, guard cell area in leaves of different watermelon varieties.

This study identifies any associations between these traits and fruit yield.

MATERIALS AND METHODS

Collection of plant materials: Field experiments were conducted between March and June, 2018 at the Teaching and Research Farm of Ladoke Akintola University of Technology Ogbomoso on four varieties of watermelon. The four watermelon varieties selected (sugar baby, Kaolack, Grey belly and Collo F1) were grown under controlled conditions, these seeds were obtained at The Seed Project Co. Ltd., Khadejia Road, Kano, Nigeria and harvesting, matured leaves of four varieties of watermelon used for this analysis were collected.

Tissue sectioning: Paradermal sections of the adaxial and abaxial leaves were made in accordance with the technique described by Eminagaoglu and Ozean¹¹ by bending the leaf over an index finger and carefully cutting thin slices off the curved epidermal surface with a sharp razor blade into a few drops of water on a microscope slide.

Treatment, clearing and mounting of sections: The cut sections were placed in a few drops of water on a microscope slide. Staining was done with 1% aqueous Safranin for 5-10 min and rinsed carefully in water to remove excess stain. The rinsing was done continuously until no excess stain bled out. The sections were then serially dehydrated in 30, 50, 70 and 100% (absolute ethanol) for 2 min each. The dehydrated sections were cleared in pure xylene for 2 min and mounting was done with diluted glycerin.

Microscopic examination and data collection: The prepared slides were examined using an Olympus binocular microscope CX23, Hachioji, Tokyo, Japan. Both qualitative and quantitative studies were carried out on the slides. The qualitative characteristics examined include the shape of epidermal cells, anticlinal walls, stomata shape and stomata occurrence. Quantitative features include epidermal cell density, epidermal cell width, stomata length, stomata breadth, guard cell area, stomata density and stomata index. Also, the density of stomata was estimated from an average of thirty samples by counting the number of stomata present per unit area of the leaf. The length and width of the stomata were measured at X40 magnification using a calibrated ocular micro-meter. The guard cell area and

stomata index (SI) were estimated using the following formulas as defined by Franco¹²:

$$\text{Guard cell area (GCA)} = \text{Length} \times \text{Breadth} \times K$$

Where:

K = Franco's constant = 0.78524

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

Where:

SI = Number of stomata index per unit area

E = Number of epidermal cells in the same unit area

S = Number of stomata per unit area

Statistical analysis: Data collected were subjected to Analysis of Variance (ANOVA) according to Statistical Analysis System¹³ and means were compared using Duncan's Multiple Range Test (DMRT) at a 5% probability level.

RESULTS

Leaf epidermal characteristics and stomata complex of four varieties of watermelon studied: Table 1 and Plate 1-4 show some qualitative features of epidermal and leaf stomata of four varieties of watermelon. The epidermal cells on the adaxial and abaxial surfaces are polygonal and irregular

shapes and vary in an anticlinal wall (Plate 1). On the adaxial, sugar baby and Kaolack have the highest polygonal (71 and 57 %) than irregular shapes (29 and 43%) while Grey belle and Collos F1 showed the same percentage on both shapes (37 and 63%) respectively. In the case of abaxial, sugar baby, Grey belle and Collos F1 showed more polygonal (63, 67 and 56%) than irregular shapes (37, 33 and 44%) while Kaolack shared the same percentage for both polygonal and irregular shapes (50%). The anticlinal walls of the adaxial and abaxial cells vary from straight to curved, wavy and sinuous.

The leaf stomata shape, sugar baby shape was round, elliptic with the same percentage (22%), oval (66%), Kaolack was elliptic (29%) and oval (71%) while Grey belle and Collos F1 were only oval on the adaxial surface. On the abaxial surface, Kaolack, Grey belle and Collos F1 were elliptic (29, 37 and 17%) and oval (71, 63 and 83%) in shape, respectively while sugar baby shape was oval. The stomata complex types were anomocytic (Plate 1a, b and d) and anisocytic (Plate 2a, 3b and 4b) tetracytic (Plate 3d and 4d), on the adaxial surface of Kaolack and Collos F1 while sugar baby and Grey belle were tetracytic (Plate 4d and anomocytic (Plate 4d)). On the abaxial surface, all four varieties were tetracytic (Plate 1b, 2d, 3c and 4d) and anomocytic (Plate 1a, 2b, 3c and 4d) except Grey belle include anisocytic (Table 1 and Plate 1-4).

Epidermal cell and stomata dimensions of quantitative attributes of the leaf of watermelon: A significant difference exists among the leaf epidermal cells of the four varieties of

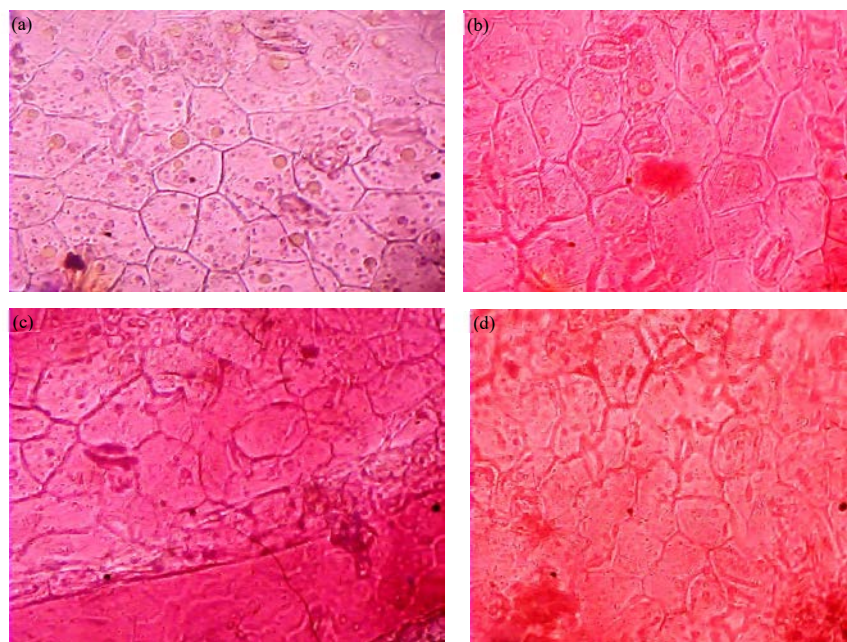


Plate 1(a-d): Photomicrographs of adaxial and abaxial leaf epidermal, (a) Adaxial (X100), (b) Adaxial (X400), (c) Abaxial (X100) and (d) Abaxial (X400) of *Citrullus lanatus* (sugar baby)

Table 1: Qualitative features of epidermis cell and stomata attributes of leaf of four varieties of watermelon

Epidermal cells			Anticlinal wall	
Stomata		Shape	Shape	Stomata complex types
Adaxial				
Sugar baby	Polygonal (71%); Irregular (29%)	Straight (71%); Curve (29%)	Round (22%); Elliptic (22%); Oval (66%)	Tetracytic (75%); Anomocytic (25%)
Kaolack	Polygonal (57%); Irregular (43%)	Straight (38%); Curve (31%); Wave (31%)	Elliptic (29%); Oval (71%)	Tetracytic (54%); Anomocytic (35%); Anisocytic (11%)
Grey belle	Polygonal (37%); Irregular (63%)	Straight (38%); Curve (38%); Wave (24%)	Oval	Tetracytic (64%); Anomocytic (36%)
Collos F1	Polygonal (37%); Irregular (63%)	Straight (31%); Curve (25%); Sinuous (19%)	Oval	Tetracytic (51%); Anomocytic (44%); Anisocytic (5%)
Abaxial				
Sugar baby	Polygonal (63%); Irregular (37%)	Straight (45%); Curve (45%); Wave (10%)	Oval	Tetracytic (88%); Anomocytic (12%)
Kaolack	Polygonal (50%); Irregular (50%)	Straight (36%); Curve (36%); Wave (28%)	Elliptic (29%); Oval (71%)	Tetracytic (53%); Anomocytic (47%)
Grey belle	Polygonal (67%); Irregular (33%)	Straight (42%); Curve (42%); Sinuous (16%)	Elliptic (37%); Oval (63%)	Tetracytic (61%); Anomocytic (37%); Anisocytic (2%)
Collos F1	Polygonal (56%); Irregular (44%)	Straight (38%); Curve (31%); Wave (31%)	Elliptic (17%); Oval (83%)	Tetracytic (54%); Anomocytic (46%)

Table 2: Epidermal cell and stomata dimensions of quantitative attributes of leaf of four varieties of watermelon

Epidermal cells			Adaxial				Abaxial			
Parameters	Sugar baby	Kaolack	Grey belle	Collos F1	Sugar baby	Kaolack	Grey belle	Collos F1	Sugar baby	Collos F1
Epidermal cells										
Width (μm)	49.15 $\pm$ 1.36 ^b	50.18 $\pm$ 1.77 ^{ab}	53.68 $\pm$ 1.66 ^{ab}	54.10 $\pm$ 1.45 ^a	44.5 $\pm$ 1.494 ^b	48.46 $\pm$ 1.64 ^b	67.67 $\pm$ 2.12 ^a	47.79 $\pm$ 1.81 ^b	169.02 $\pm$ 2.92 ^a	165.84 $\pm$ 1.79 ^a
Density (mm^{-2})	161.26 $\pm$ 2.38 ^b	203.49 $\pm$ 3.01 ^a	105.94 $\pm$ 1.86 ^d	125.57 $\pm$ 1.98 ^c	89.00 ^a	80.00 ^c	110.65 $\pm$ 2.21 ^b	80.00 ^c	89.00 ^a	80.00 ^c
Rel/abund. (%)	88.00 ^b	92.00 ^a	86.00 ^c	80.00 ^d	89.00 ^a	80.00 ^c	85.00 ^b	80.00 ^c	89.00 ^a	80.00 ^c
Stomata										
Length (μm)	28.07 $\pm$ 0.39 ^c	30.46 $\pm$ 0.27 ^b	27.90 $\pm$ 0.33 ^c	34.48 $\pm$ 0.45 ^a	24.41 $\pm$ 0.34 ^d	30.81 $\pm$ 0.45 ^b	27.82 $\pm$ 0.38 ^c	32.51 $\pm$ 0.56 ^a	24.41 $\pm$ 0.34 ^d	32.51 $\pm$ 0.56 ^a
Breadth (μm)	15.96 $\pm$ 0.36 ^c	16.81 $\pm$ 0.26 ^b	14.85 $\pm$ 0.19 ^d	18.77 $\pm$ 0.30 ^a	11.44 $\pm$ 0.31 ^d	17.15 $\pm$ 0.35 ^b	15.02 $\pm$ 0.34 ^c	18.35 $\pm$ 0.30 ^a	11.44 $\pm$ 0.31 ^d	18.35 $\pm$ 0.30 ^a
Guard cell area (μm^2)	388.77 $\pm$ 13.36 ^b	506.55 $\pm$ 7.08 ^a	325.58 $\pm$ 6.14 ^c	351.65 $\pm$ 9.03 ^c	220.08 $\pm$ 7.98 ^c	439.31 $\pm$ 13.62 ^a	328.84 $\pm$ 9.57 ^b	444.63 $\pm$ 11.02 ^a	220.08 $\pm$ 7.98 ^c	444.63 $\pm$ 11.02 ^a
Density (mm^{-2})	26.45 $\pm$ 0.91 ^b	32.30 $\pm$ 0.85 ^a	16.92 $\pm$ 0.65 ^c	28.62 $\pm$ 0.93 ^b	19.56 $\pm$ 0.60 ^b	42.86 $\pm$ 1.05 ^a	19.20 $\pm$ 0.53 ^b	40.95 $\pm$ 0.63 ^a	19.56 $\pm$ 0.60 ^b	40.95 $\pm$ 0.63 ^a
Rel/abund. (%)	12.00 ^c	20.00 ^a	8.00 ^d	14.00 ^b	11.00 ^c	20.00 ^a	15.00 ^b	20.00 ^a	11.00 ^c	20.00 ^a
Stomata index	12.36 $\pm$ 0.39 ^c	19.86 $\pm$ 0.72 ^a	13.69 $\pm$ 0.37 ^{bc}	14.09 $\pm$ 0.43 ^b	10.46 $\pm$ 0.36 ^c	20.23 $\pm$ 0.54 ^a	14.89 $\pm$ 0.45 ^b	19.8 $\pm$ 0.34 ^a	10.46 $\pm$ 0.36 ^c	19.8 $\pm$ 0.34 ^a

Means along the column with the same letter are not significant at p<0.05 test at a 5% probability level and $\pm$: Standard deviation

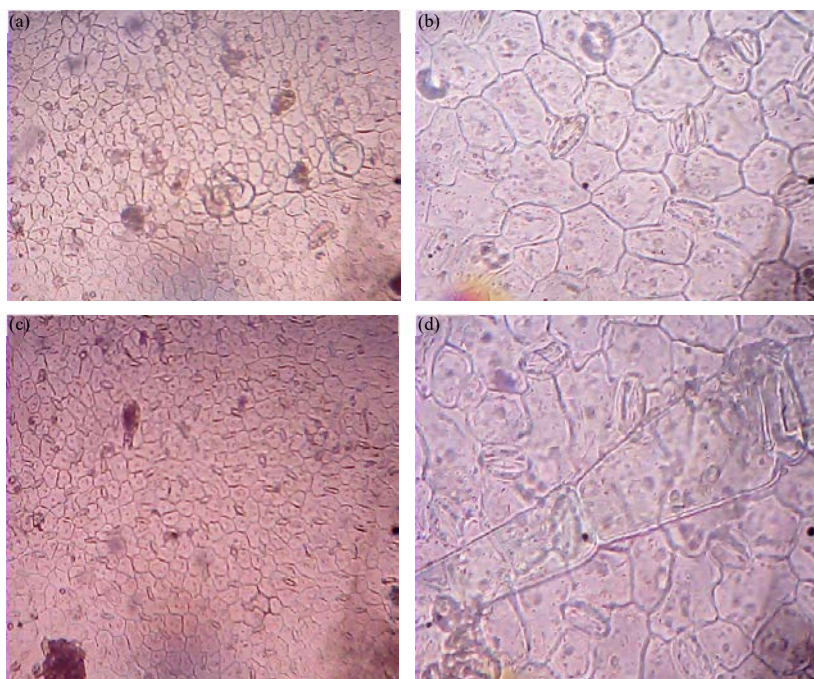


Plate 2(a-d): Photomicrographs of adaxial and abaxial leaf epidermal, (a) Adaxial (X100), (b) Adaxial (X400), (c) Abaxial (X100) and (d) Abaxial (X400) of *Citrullus lanatus* (Kaolack)

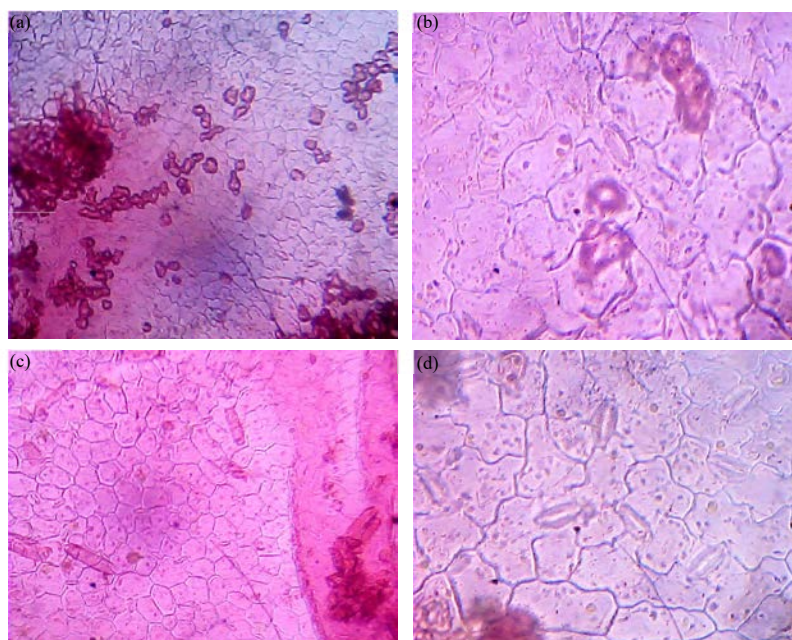


Plate 3(a-d): Photomicrographs of adaxial and abaxial leaf epidermal, (a) Adaxial (X100), (b) Adaxial (X400), (c) Abaxial (X100) and (d) Abaxial (X400) of *Citrullus lanatus* (Grey belle)

watermelon examined on both adaxial and abaxial surfaces (Table 2). Generally, the epidermal cells of the adaxial are wider than the abaxial except in Grey belle where the widest

epidermal cells were noticed on the abaxial surface (Table 2). The epidermal cell width of the adaxial surface varies from 49.15 μm in sugar baby to 54.10 μm in Collos F1. The abaxial

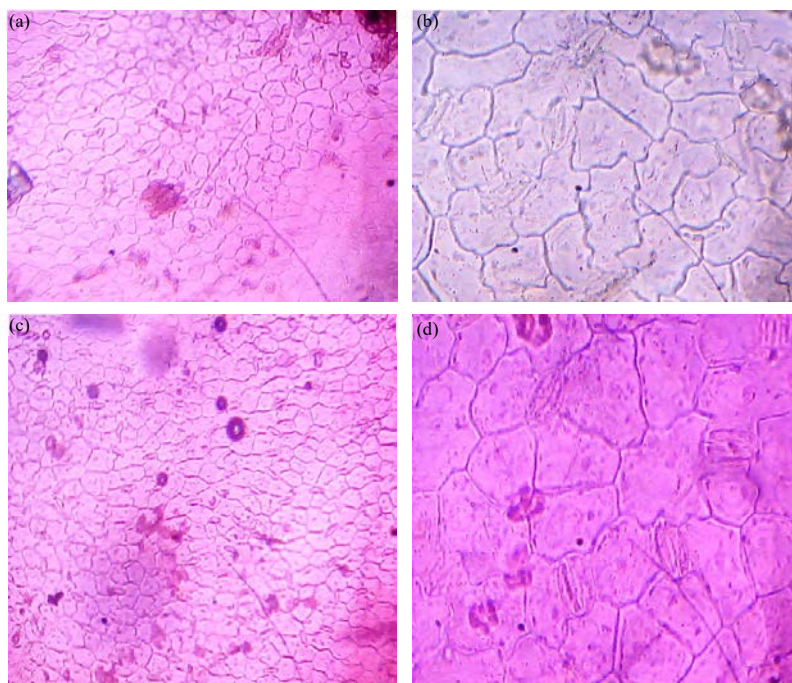


Plate 4(a-d): Photomicrographs of adaxial and abaxial leaf epidermal, (a) Adaxial (X100), (b) Adaxial (X400), (c) Abaxial (X100) and (d) Abaxial (X400) of *Citrullus lanatus* (Collos F1)

cells varied from 44.54 μm in sugar baby to 67.67 μm in Grey belle. The widest (54.10 μm) epidermal cell on the adaxial surface was recorded with Collos F1 while the widest (67.67 μm) on the abaxial was recorded in Grey belle. In addition, sugar baby recorded the narrowest (49.15 and 44.54 μm) on both adaxial and abaxial epidermal cells. The epidermal highest densities of both adaxial and abaxial surfaces were found on Kaolack (203.49 and 169.91 mm^{-2}) while the lower density was recorded with Grey belle (105.94 and 110.65 mm^{-2}) on both adaxial and abaxial, respectively. The value for epidermal cell density in the abaxial of Kaolack was similar to Collos F1 (165.84 mm^{-2}). The relative abundance of epidermal cells was highest (92%) on the adaxial of Kaolack while Collos F1 was recorded with a less (80%) relative abundance of epidermal cells. With respect to the relative abundant of epidermal cells of the abaxial, the highest (89%) was found in sugar baby while lesser (80%) on both Kaolack and Collos F1 has the same relative abundant of epidermal cells in abaxial (Table 2).

There was a significant difference in the stomata dimensions of quantitative attributes of the four varieties of watermelon examined (Table 2). The length, breadth, guard cell, density, relative abundance and stomata index vary among all four varieties (Table 2). Generally, the values for all these parameters were found more on the adaxial than the abaxial. The longest stomata on the adaxial was recorded with

Collos F1 (34.48 μm) followed by Kaolack (30.46 μm) while Grey belle had the shortest stomata (27.90 μm). This result was similar to sugar baby stomata length (28 μm). For abaxial, the longest stomata were found in Collos F1 (32.51 μm) while the shortest was on sugar baby (24.41 μm).

The adaxial stomata breadth was highest in Collos F1 (18.77 μm) while the least was recorded with Grey belle (14.85 μm). The stomata breadth on the abaxial surface was widest on Collos F1 (18.35 μm) followed by Kaolack (17.15 μm) while the lowest value was recorded in Grey belle (11.44 μm). The guard cell area, stomata density, relative abundance and stomata index showed significant differences on both surfaces. In general, adaxial possessed the highest value for all these four parameters (Table 2). Kaolack possessed the highest guard cell, stomata density, relatively abundant and stomata index (505.55 μm , 32.30 mm^2 , 20.00% and 19.86) on adaxial while the least 325.58 μm , 16.92 mm^2 , 8.00% and 12.36 μm were obtained from Grey belle and sugar baby, respectively.

Furthermore, the highest value for abaxial guard cell area (444.63 μm) was Collos F1, this result was similar to Grey belle (439.31 μm) while the lowest value was recorded on sugar baby (220.08 μm). Kaolack variety highest stomata density, relatively abundant and stomata index (42.86 mm^2 , 20.00% and 20.23) similar to Collos F1 (40.95 mm^2) while Grey belle gave the lowest (19.20 mm^2) stomata density.

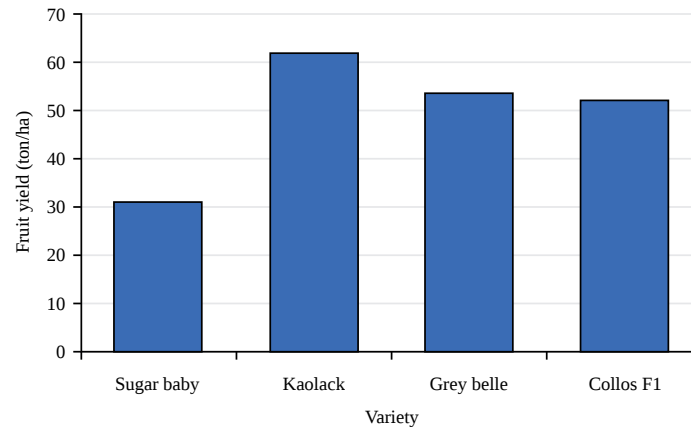


Fig. 1: Fruit yield of the four varieties

Figure 1 shows the result of the fruit yield of the four watermelons used for the study. It was revealed that the highest fruit yield was obtained following the order of Kaolack>Grey belle>Collos F1>Sugar baby.

DISCUSSION

The fruit yield of the four varieties tested was significantly influenced by epidermal cell and stomata density, guard cell area and stomata index. Stomata and epidermal cells play an important role in the control of water evaporation and gas exchange in the leaf. The two guard cells of stomata regulate CO₂ uptake and transpiration by changing the size of stomata pores¹⁴. Statistical analysis shows significant variation among the fruit yield and stomata traits like guard cell area, stomata density, percentage relative abundance and stomata index measured on the adaxial and abaxial surface of watermelon fruit varieties. Kaolack variety shows significantly the highest value of stomata density, guard cell area and index with the highest fruit yield followed by Collos F1 than the other varieties. The highest yield of the Kaolack variety could be due to higher stomata size causing higher photosynthetic efficacy thereby increasing the fruit yield. Significant correlations between stomata features and crop yield were also observed in different crops^{15,16}. This result was in agreement with the findings of Sarwar *et al.*¹⁷ on rice. Similar correlations were observed by Shahinnaia *et al.*⁶ on wheat whereby increases in stomata density, stomata index and guard cell area were all increased with the yield of wheat. This aligned with the findings of Ruggiero *et al.*¹⁸, who reported that the opening and closing of stomata are regulated by changes in the turgor pressure of guard cells. When guard cells absorb solutes, the water potential inside the cells decreases, driving the osmotic flow of water into the guard cells. This results in an increase in

turgor pressure, causing the guard cells to swell and the stomatal pores to open. The longest and widest stomata (length and breadth) were taken with Collos F1 followed by Kaolack variety although the higher value of length and breadth of stomata of Collos F1 does not correlate with yield while Kaolack variety having similar length and breadth of stomata produced the highest yield. This may be attributed to a combination of factors such as density, guard cell area and stomatal index. Similar correlations between stomatal characteristics and grain yield have been observed by Sarwar *et al.*¹⁷, Aminian *et al.*¹⁹ and White *et al.*²⁰ reported that stomatal numbers are linked to photosynthetic activity, while Avci and Ayyün²¹ found a significant impact of stomatal features on Turkish hazelnut. Specifically, leaves with larger stomata exhibited higher photosynthesis rates compared to those with smaller stomata. Leaves are considered the primary photosynthetic organs in higher plants and, therefore, the most important carbon sources^{20,22,23}. This finding contributes to the understanding of the physiological factors that influenced watermelon productivity.

This information can be utilized to select or develop watermelon varieties with desirable leaf epidermal traits that enhance productivity and contribute to sustainable watermelon production. Investigating the relationship between leaf epidermal traits and fruit yield in watermelons is essential for understanding the physiological processes influencing crop productivity. By identifying specific leaf epidermal traits that correlate with higher fruit yield, farmers can select varieties and implement management practices that maximize watermelon productivity. By focusing on the genetic traits associated with high fruit yield, it is possible to develop improved watermelon varieties that are more resilient to environmental stresses and can deliver higher yields, contributing to the sustainability and profitability of watermelon production.

CONCLUSION

The leaf epidermal studies conducted on the four varieties of watermelon provided valuable insights into the relationship between leaf characteristics and fruit yield. These results highlighted the importance of leaf epidermal traits in watermelon productivity and suggested that breeders and growers should consider these characteristics when selecting or developing new varieties. Manipulating stomatal density and size through breeding or cultivation practices may be a promising approach to enhancing fruit yield in watermelon crops.

SIGNIFICANCE STATEMENT

Understanding the relationship between leaf epidermal traits and fruit yield in watermelons is crucial, providing insights into how these microscopic leaf characteristics influence overall plant productivity. By identifying key epidermal features linked to improved fruit yield, the research could inform breeding strategies to enhance watermelon crop performance, potentially leading to more resilient and higher-yielding varieties. This work advances understanding of plant morphology's role in agricultural success and offers valuable applications for optimizing fruit production.

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