

## USING LEAF EPIDERMAL CHARACTERS TO IDENTIFY AND DIFFERENTIATE FORMS IN THE *DURANTA ERECTA* COMPLEX (VERBENACEAE)

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### Abstract

*Duranta erecta* L., commonly known as ‘Golden Dewdrop’ or ‘Skyflower’, is a widely cultivated ornamental shrub valued for its aesthetic appeal in various regions, including Nigeria. However, its taxonomy and nomenclature have been subject to considerable ambiguity. This study examines the leaf epidermal morphology and stomatal characteristics of eight distinct forms of *D. erecta* (Thorny Green, Yellow Bush, Plain Yellow, Broad Green 1, Broad Green 2, Green Bush, Variegated Yellow, and Variegated White) to assess their taxonomic relevance. Freshly collected leaves were used to prepare temporary and permanent slides of both abaxial and adaxial surfaces. Standard anatomical techniques were employed for both qualitative and quantitative analyses, and photomicrographs were captured using a light microscope fitted with an Amscope digital camera. All forms were found to be hypostomatic, with stomata restricted to the abaxial surface. The epidermal cells were generally irregular, except in Variegated Yellow, which displayed pentagonal cells on the adaxial side. Anticlinal walls ranged from straight to curved and sinuate. Anisocytic stomatal complexes were common across all forms, with additional tetracytic and paracytic types present in some. Both multicellular peltate glandular and unicellular non-glandular trichomes were observed, predominantly on the abaxial surface. Quantitative data revealed significant inter-form variation in stomatal density, stomatal index, epidermal cell size, and trichome density. Yellow Bush and Plain Yellow had the highest stomatal densities, while Green Bush and Variegated White had the lowest. Broad Green 1 exhibited the largest epidermal cells, whereas Green Bush had the smallest. Trichome density was highest in Yellow Bush and lowest in Variegated Yellow. These anatomical differences highlight the taxonomic utility of leaf epidermal and stomata features in distinguishing forms of *Duranta erecta*.

**Key words:** *Duranta erecta*; Epidermal morphology; Hypostomatic leaves; Taxonomy; Stomatal ontogeny

### Introduction

Plant classification has long been a central focus in the fields of plant taxonomy and systematics. As new morphological, anatomical, molecular, or phytochemical evidence becomes available, plants are frequently reclassified, making taxonomy an evolving and continuous process (Oyeleke, 2004). Traditionally, most taxonomic classifications have relied on external morphological features such as flowers and fruits (Olorode, 1984). However, because these reproductive structures are often seasonal and may not be present year-round, alternative approaches, particularly anatomical studies of vegetative structures like leaves, have gained prominence in modern taxonomy (Davis, 1963).

The leaf epidermis, which forms the outermost layer of cells, serves multiple functions, including protection against water loss through transpiration, regulation of gas exchange, secretion of metabolic substances, and water absorption (Dutta, 2009; Adedeji, 2008). Many leaves exhibit dorsiventral anatomy, where the upper (adaxial) and lower (abaxial) surfaces differ structurally and functionally (Hardie, 2009). These epidermal features such as the shape and arrangement of epidermal cells, the type

and distribution of stomata, subsidiary and guard cells, and the presence and structure of trichomes, are taxonomically significant and have been effectively used to resolve classification challenges within plant genera and families (Adedeji, 2008; Saheed & Illoh, 2010).

The genus *Duranta*, particularly *Duranta erecta* L., presents notable taxonomic challenges. Commonly referred to as ‘Golden Dewdrop’ or ‘Skyflower’, *D. erecta* is a sprawling, evergreen shrub or small tree that may grow up to 7 meters tall with an equal spread (Munir, 1995; Floridata, 2015). It is extensively cultivated as an ornamental plant and commonly used as a hedge. The plant typically forms a bushy clump with arching branches; mature stems are equipped with sharp axillary thorns, which are absent in juvenile specimens (Missouri Botanical Garden, 2018). The leaves are ovate, opposite, and measure between 2.5 and 7.6 cm in length (Floridata, 2015).

Despite its widespread use and recognition, the taxonomy and nomenclature of *D. erecta* remain complex (Bailey, 1913; Munir, 1995; Sanders, 2001). Prior studies have largely focused on gross morphological traits and phytochemical profiles, with limited attention paid to comparative leaf epidermal anatomy across its various cultivated forms. Anatomical traits, particularly stomatal

types and their ontogeny, have been shown to offer valuable taxonomic insights (Zhang *et al.*, 2025; Chua and Lau, 2024). As noted by Ekeke and Agbagwa (2015), the type and pattern of adult stomata can serve as both diagnostic features and indicators of natural taxonomic affinities.

Therefore, this study elucidated the leaf epidermal characteristics and stomatal architecture of eight cultivated forms of *Duranta erecta* to assess their taxonomic significance. The observed intra-specific variations yield diagnostic traits useful for species delimitation and identification within the genus.

## Materials and Methods

**Collection of plant materials:** Eight cultivated forms of *Duranta erecta* were selected from the University of Ilorin main campus (North Central Nigeria). For each form, three mature and healthy plants of comparable age and growth condition were randomly selected. Plants exhibiting signs of disease, mechanical damage, or environmental stress were excluded.

From each plant, five fully expanded, mature leaves were collected from the mid-canopy region to minimize developmental variation. Sampling was conducted during the same season to reduce environmental effects. Geographical coordinates and morphological descriptions of each form are presented in Table 1.

**Preparation of epidermal samples:** Epidermal peels were prepared following the protocol of Abdul Rahaman & Oladele (2009) with slight modifications, consistent with established anatomical procedures (Metcalf & Chalk, 1960). Leaf segments approximately 1 mm<sup>2</sup> were excised from the median region of the lamina. Each segment was immersed in concentrated nitric acid (HNO<sub>3</sub>) in a Petri dish and allowed to macerate for up to 24 hours, depending on leaf thickness. The formation of air bubbles indicated readiness for separation.

Samples were carefully transferred into distilled water to remove residual acid. Using a dissecting needle, the adaxial and abaxial epidermal layers were gently teased apart. Each peel was stained with safranin for three to 5 minutes, rinsed several in distilled water to remove excess stain, mounted in glycerine, and covered with a coverslip following the protocol of Okanume *et al.*, (2021). The

edges were sealed with nail varnish to prevent desiccation. Slides were labeled appropriately for each specimen.

**Microscopic observation and measurement:** Prepared slides were examined under an Olympus microscope at ×40 magnification. An AmScope MU1000 (10MP) digital camera system connected to a computer was used for image capture and measurement. For each species, 10 replicates were prepared per leaf surface (adaxial and abaxial), totaling 20 replicates per morphological form.

Quantitative and qualitative analyses were conducted on epidermal and stomatal features based on the criteria outlined by Abdul Rahaman & Oladele (2009). Parameters measured included epidermal cell length and width, stomatal length and width, stomatal and epidermal cell density, and stomatal complex types. Observations also covered epidermal cell shape, anticlinal wall pattern, and the presence or absence of trichomes, following the classification of Metcalfe & Chalk (1960).

## Statistical Analysis

Quantitative micromorphological data obtained from the adaxial and abaxial epidermal surfaces (stomatal length, stomatal width, stomatal density, stomatal size, epidermal cell density, and epidermal cell size) were expressed as mean ± standard error (SE). Prior to inferential analysis, data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene’s test to verify compliance with parametric assumptions.

Differences among forms were evaluated using one-way Analysis of Variance (ANOVA). Where significant differences were detected ( $p < 0.05$ ), post hoc mean separation was performed using Duncan Multiple range test (DMRT) test to determine pairwise differences among forms.

Pearson’s correlation analysis was conducted separately for adaxial and abaxial epidermal cell size and density. Simple linear regression models were fitted in the scatter plot to quantify the direction and strength of the size and density relationship.

Bar charts were constructed to present comparative mean values of stomata density across forms, while scatter plots were used to depict regression trends and correlation patterns. All statistical analyses were performed at a 95% confidence level ( $\alpha = 0.05$ ).

**Table 1. Morphological descriptions and geographical coordinates of the *Duranta erecta* forms studied.**

S. No.	Form of <i>D. erecta</i>	Source (State)	Geopolitical Zone	GPS Coordinates	Brief Morphological Description
1.	Green bush	Kwara	North Central	8°28'48.30672"N, 4°40'34.9824"E	Erect stem; green leaves with serrate to entire margins; long branches with poorly developed fascicled leaves; often single-noded
2.	Yellow bush	Kwara	North Central	8°28'26.952"N, 4°40'26.9824"E	Well-developed fascicled yellow leaves; serrate to entire margins; branches composed of several nodes and internodes
3.	Variogated yellow	Kwara	North Central	8°28'48.30672"N, 4°40'34.7762"E	Erect stem; variegated yellow leaves with serrate to dentate margins; longer branches; decussate opposite leaves and thorns
4.	Variogated white	Kwara	North Central	8°28'48.40672"N, 4°40'34.7762"E	Erect stem; variegated white leaves with serrate to dentate margins; longer branches; decussate opposite leaves
5.	Thorny green	Kebbi	North Central	8°28'48.30672"N, 4°40'34.9824"E	Erect stem; fully serrated green leaves; upright branches armed with thorns on opposite sides
6.	Broad green 1	Kwara	North Central	8°28'48.30672"N, 4°40'34.9824"E	Widely spreading branches; glabrous leaves with margins ranging from half-serrate to entire
7.	Plain yellow	Kwara	North Central	8°28'48.30672"N, 4°40'34.7762"E	Erect stem; plain yellow leaves with serrate to dentate margins; straight branches with decussate opposite leaves and thorns
8.	Broad green 2	Kwara	North Central	8°28'48.40672"N, 4°40'34.7762"E	Similar to Broad Green 1; widely spreading branches; glabrous leaves with half-serrate to entire margins

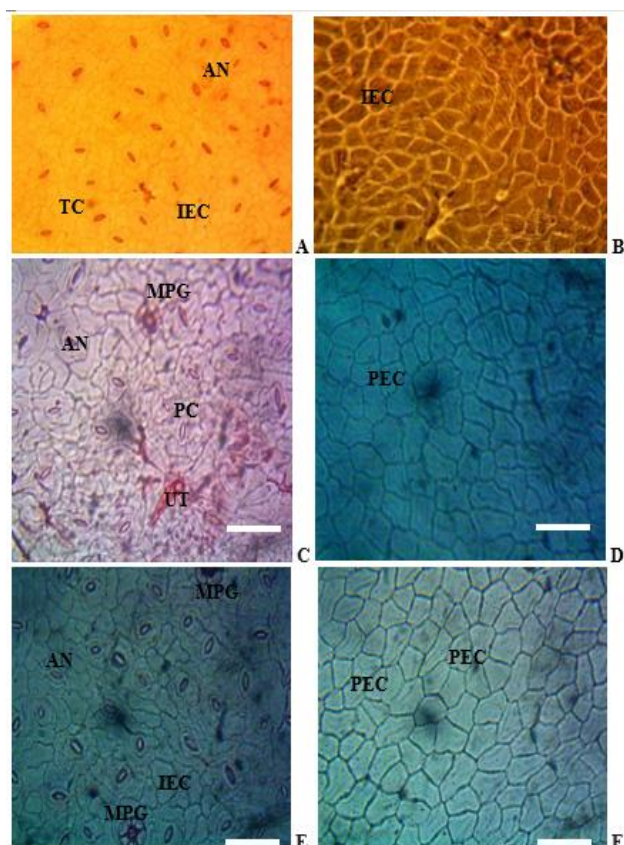


Fig. 1. Photomicrographs of the *Duranta erecta* forms (A: Plain yellow abaxial; B: Plain yellow adaxial; C: Green bush abaxial; D: Green bush adaxial; E: Yellow bush abaxial; F: Yellow bush adaxial. AN: anisocytic stomata; PC: paracytic stomata; TC: tetracytic stomata; IEC: Irregular epidermal cell; PEC: Polygonal epidermal cell; UT: Unicellular trichomes; MPG: Multicellular peltate gland).

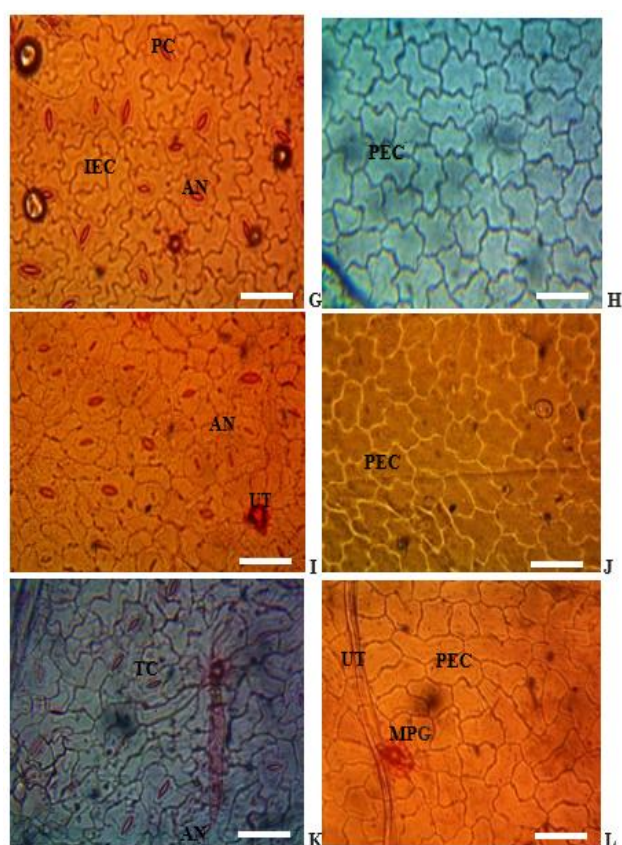


Fig. 2. Photomicrographs of the *Duranta erecta* forms (G: Broad green 1 abaxial; H: Broad green 1 adaxial; I: Broad green 2 abaxial; J: Broad green 2 adaxial; K: Thorny green abaxial; L: Thorny green adaxial. AN: anisocytic stomata; PC: paracytic stomata; TC: tetracytic stomata; IEC: Irregular epidermal cell; PEC: Polygonal epidermal cell; UT: Unicellular trichomes; MPG: Multicellular peltate gland).

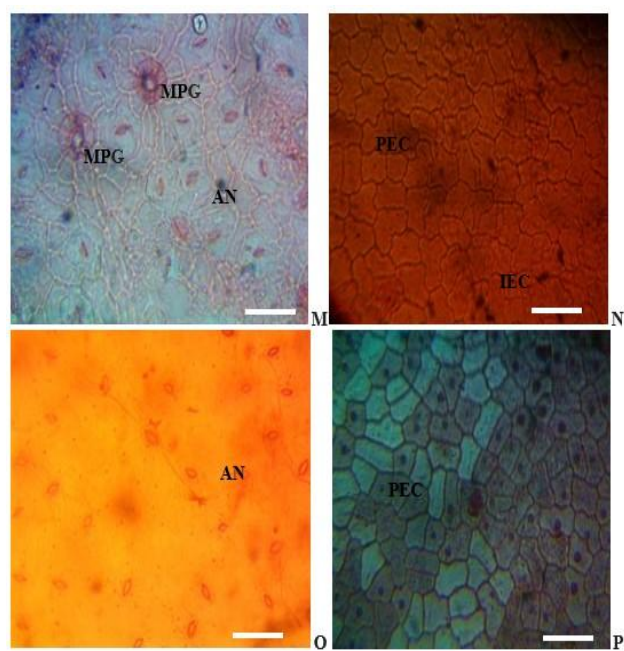


Fig. 3. Photomicrographs of the *Duranta erecta* forms (M: Variegated white abaxial; N: Variegated white adaxial; O: Variegated yellow abaxial; P: Variegated yellow adaxial; AN: Anisocytic stomata; IEC: Irregular epidermal cell; PEC: Polygonal epidermal cell; MPG: Multicellular peltate gland).

**Results**

**Epidermal and stomatal features:** Analysis revealed notable variation in the epidermal and stomatal characteristics across the eight forms of *Duranta erecta* (Figs. 1-3; Tables 2 and 3). All forms exhibited hypostomatic leaves, with stomata confined to the abaxial surface. Thorny Green displayed prominent striations around stomata.

Epidermal cells were predominantly irregular in shape, except in Variegated Yellow, which exhibited pentagonal cells on the adaxial surface. Anticlinal walls on the abaxial surface were generally sinuate or wavy, whereas the adaxial walls ranged from straight to curved in Plain Yellow, Green Bush, and Yellow Bush, and were sinuate in Broad Green 1, Thorny Green, Variegated Yellow, and Variegated White.

All forms possessed anisocytic stomata, a diagnostic feature of *Duranta erecta*. Additional stomatal types were also recorded: tetracytic stomata in Thorny Green, Yellow Bush, and Plain Yellow; and paracytic stomata in Broad Green 1 and Green Bush.

Both multicellular peltate glands and unicellular non-glandular trichomes were observed, with greater trichome density typically on the abaxial surface.

### Quantitative Characteristics

**Stomatal features:** Stomatal length varied from  $18.64 \pm 1.14 \mu\text{m}$  in Variegated White to  $24.69 \pm 0.18 \mu\text{m}$  in Thorny Green. Stomatal width ranged from  $12.27 \pm 0.84 \mu\text{m}$  (Green Bush) to  $16.03 \pm 0.58 \mu\text{m}$  (Variegated White). Although the size differences were not statistically significant, variation in stomatal index and frequency was notable, Plain Yellow recorded the highest stomatal index ( $37.31 \pm 2.27 \mu\text{m}$ ), while Yellow Bush had the highest frequency ( $7.60 \pm 0.90 \mu\text{m}$ ). Also Stomatal density varied among the studied forms (Fig. 4). The highest stomatal density was recorded in Plain Yellow ( $155.92 \pm 14.91 \text{ mm}^{-2}$ ), followed by Yellow Bush ( $152.63 \pm 10.96 \text{ mm}^{-2}$ ). Broad Green 2 exhibited moderately values ( $133.55 \pm 6.36 \text{ mm}^{-2}$ ), while the Thorny Green ( $48.03 \text{ mm}^{-2}$ ) showed the lowest stomata density ( $48.03 \pm 3.11 \text{ mm}^{-2}$ ) (Fig. 4; Table 2).

**Epidermal cell features (Abaxial Surface):** Thorny Green had the largest epidermal cells ( $41.13 \times 21.68 \mu\text{m}$ ) and one of the lowest cell densities ( $280.26 \pm 15.97 \mu\text{m}$ ), while Variegated White exhibited the smallest cells ( $24.89 \pm 1.33 \mu\text{m} \times 19.62 \pm 2.14 \mu\text{m}$ ) and the highest density ( $348.03 \pm 4.54 \mu\text{m}$ ). Broad Green 1 recorded the lowest density overall ( $201.98 \pm 8.88 \mu\text{m}$ ), though not significantly different from Variegated Yellow ( $203.95 \pm 8.49 \mu\text{m}$ ). A moderate negative correlation was observed between epidermal density and cell size ( $r = -0.537$ ,  $p = 0.170$ ) (Fig. 5; Table 2). Although the trend suggests an inverse size to density relationship, the association was not statistically significant at  $p < 0.05$ . The distribution pattern indicates partial structural trade-off, with Broad Green 1 exhibiting markedly larger cell size at relatively low density, contributing to variability within the dataset.

**Epidermal cell features (Adaxial Surface):** Yellow Bush displayed the highest epidermal density ( $739.47 \pm 31.47 \mu\text{m}$ ), while Thorny Green had the lowest ( $416.44 \pm 12.98 \mu\text{m}$ ), comparable to Broad Green 1 ( $549.67 \pm 19.03 \mu\text{m}$ ). Broad Green 1 also had the longest epidermal cells ( $39.49 \pm 2.26 \mu\text{m}$ ), whereas Yellow Bush had the shortest ( $28.30 \pm 1.37 \mu\text{m}$ ). The widest cells were recorded in Thorny Green ( $32.75 \pm 1.80 \mu\text{m}$ ), while Plain Yellow had the narrowest ( $21.10 \pm 1.43 \mu\text{m}$ ). Variegated White had the largest overall epidermal cell size ( $1249.67 \pm 77.90 \mu\text{m}$ ), while Green Bush had the smallest ( $625.02 \pm 44.97 \mu\text{m}$ ), not significantly different from Yellow Bush ( $649.51 \pm 36.76 \mu\text{m}$ ).

In contrast to the abaxial, the adaxial surface exhibited a strong and statistically significant negative correlation between epidermal density and cell size ( $r = -0.859$ ,  $p = 0.006$ ) (Fig. 6; Table 2). Linear regression analysis confirmed a pronounced inverse relationship, indicating that increases in epidermal density are associated with substantial reductions in cell size. Accessions such as Yellow Bush and Plain Yellow clustered in the high-density, small-cell region, whereas Thorny green and Broad Green 2 occupied the low-density, large-cell region.

**Trichome density:** Yellow Bush exhibited the highest trichome density ( $1.30 \pm 0.21 \mu\text{m}$ ), though not statistically different from Broad Green 2 ( $0.70 \pm 0.21 \mu\text{m}$ ) and Green Bush ( $0.80 \pm 0.36 \mu\text{m}$ ). Variegated Yellow recorded the lowest trichome density, which was not significantly different from those observed in Variegated White, Broad Green 1, Plain Yellow, and Thorny Green (Table 2).

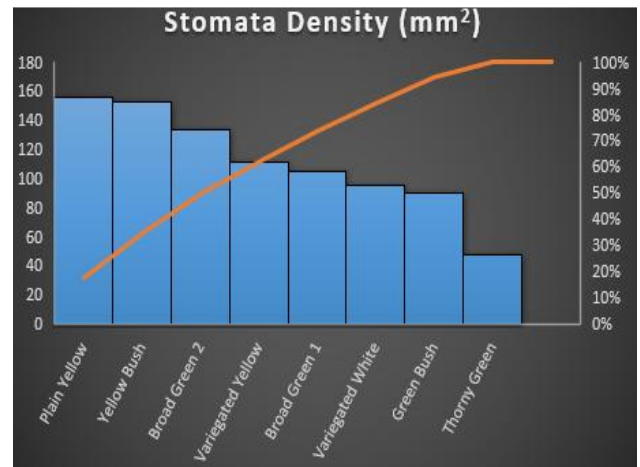


Fig. 4. Variation in the stomata density among the morphological forms of *Duranta erecta*.

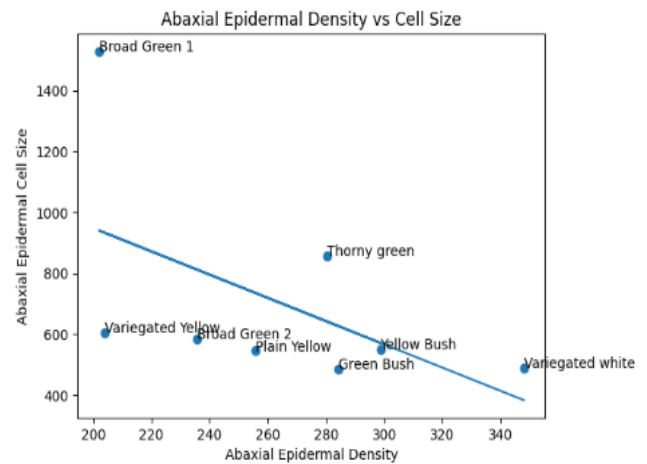


Fig. 5. Scatter plot analysis of epidermal cell size and density on the abaxial leaf surfaces.

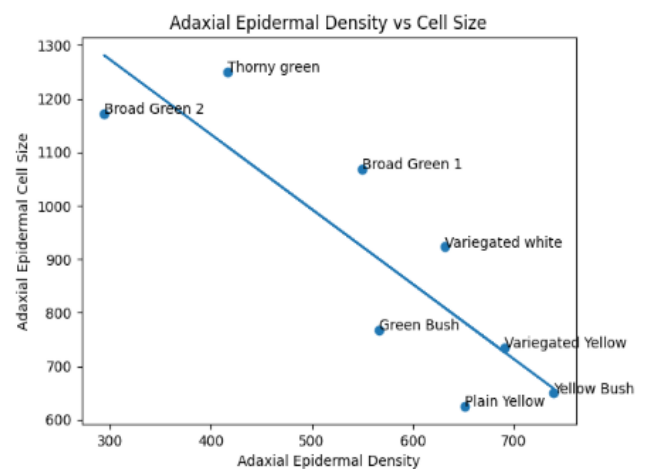


Fig. 6. Scatter plot analysis of epidermal cell size and density on the adaxial leaf surfaces.

Table 2. Quantitative leaf epidermal characteristics of *Duranta erecta* forms studied.

Species	Leaf surfaces	Stomata density (mm <sup>-2</sup> )	Stomata length (µm)	Stomata width (µm)	Stomata size (µm)	Stomata index (%)	Stomata frequency (mm <sup>-2</sup> )	Trichomes	Epidermal length (µm)	Epidermal width (µm)	Epidermal density (mm <sup>-2</sup> )	Epidermal size (µm)
Thorny green	Abaxial	48.03±3.11 <sup>d</sup>	24.69±0.18 <sup>a</sup>	12.70±0.65 <sup>b</sup>	245.93±12.37 <sup>a</sup>	14.77±1.0 <sup>c</sup>	19.33±1.88 <sup>a</sup>	0.30±0.15 <sup>b</sup>	41.13±3.02 <sup>a</sup>	21.68±1.87 <sup>b</sup>	280.26±15.97 <sup>bc</sup>	857.94±47.77 <sup>b</sup>
	Adaxial	-	-	-	-	-	-	-	38.74±2.50 <sup>ab</sup>	32.75±1.80 <sup>a</sup>	416.44±12.98 <sup>d</sup>	1249.67±77.90 <sup>a</sup>
Yellow bush	Abaxial	152.63±10.96 <sup>a</sup>	21.97±0.64 <sup>b</sup>	13.24±0.41 <sup>b</sup>	227.89±8.51 <sup>a</sup>	33.51±1.40 <sup>a</sup>	7.60±0.90 <sup>c</sup>	1.30±0.21 <sup>a</sup>	26.37±1.39 <sup>b</sup>	20.75±1.85 <sup>b</sup>	298.69±10.11 <sup>b</sup>	549.27±57.94 <sup>b</sup>
	Adaxial	-	-	-	-	-	-	-	28.30±1.37 <sup>d</sup>	23.28±1.36 <sup>cd</sup>	739.47±31.74 <sup>a</sup>	649.51±36.76 <sup>d</sup>
Plain yellow	Abaxial	155.92±14.91 <sup>a</sup>	19.18±0.52 <sup>cd</sup>	13.89±0.90 <sup>ab</sup>	211.05±17.87 <sup>a</sup>	37.31±2.27 <sup>a</sup>	6.37±0.43 <sup>c</sup>	0.50±0.17 <sup>b</sup>	24.18±0.89 <sup>b</sup>	22.26±2.50 <sup>b</sup>	255.92±11.79 <sup>cd</sup>	545.07±76.59 <sup>b</sup>
	Adaxial	-	-	-	-	-	-	-	29.91±1.58 <sup>cd</sup>	21.10±1.43 <sup>d</sup>	651.97±45.49 <sup>b</sup>	625.02±44.97 <sup>d</sup>
Broad green 1	Abaxial	105.27±9.36 <sup>c</sup>	21.33±1.06 <sup>bc</sup>	14.51±0.89 <sup>ab</sup>	247.72±26.32 <sup>a</sup>	33.90±2.26 <sup>a</sup>	8.84±0.73 <sup>bc</sup>	0.40±0.16 <sup>b</sup>	38.45±5.57 <sup>a</sup>	34.84±4.70 <sup>a</sup>	201.98±8.88 <sup>e</sup>	1528.10±440.92 <sup>a</sup>
	Adaxial	-	-	-	-	-	-	-	39.49±2.26 <sup>a</sup>	26.69±1.55 <sup>abcd</sup>	549.67±19.03 <sup>d</sup>	1068.33±107.45 <sup>ab</sup>
Broad green 2	Abaxial	133.55±6.36 <sup>ab</sup>	20.58±0.92 <sup>bcd</sup>	14.09±0.85 <sup>ab</sup>	231.21±22.15 <sup>ab</sup>	36.30±1.04 <sup>a</sup>	9.68±0.96 <sup>bc</sup>	0.70±0.21 <sup>a</sup>	27.63±1.22 <sup>b</sup>	21.00±1.37 <sup>b</sup>	235.53±12.51 <sup>de</sup>	584.69±50.04 <sup>b</sup>
	Adaxial	-	-	-	-	-	-	-	37.90±5.27 <sup>abc</sup>	28.06±3.56 <sup>abc</sup>	294.08±33.80 <sup>e</sup>	1172.14±173.01 <sup>ab</sup>
Green bush	Abaxial	90.13±4.60 <sup>c</sup>	19.64±0.70 <sup>bcd</sup>	12.27±0.84 <sup>b</sup>	188.12±13.37 <sup>a</sup>	24.35±1.48 <sup>b</sup>	11.77±1.22 <sup>b</sup>	0.80±0.36 <sup>c</sup>	27.46±2.29 <sup>b</sup>	17.95±1.08 <sup>b</sup>	284.21±14.93 <sup>bc</sup>	485.59±43.15 <sup>b</sup>
	Adaxial	-	-	-	-	-	-	-	33.69±2.34 <sup>abc</sup>	23.75±1.89 <sup>bcd</sup>	566.44±22.65 <sup>c</sup>	768.43±32.92 <sup>cd</sup>
Variegated yellow	Abaxial	111.18±5.50 <sup>bc</sup>	20.62±0.76 <sup>bcd</sup>	14.21±0.89 <sup>ab</sup>	233.30±19.94 <sup>a</sup>	35.38±1.52 <sup>a</sup>	9.49±0.94 <sup>bc</sup>	0.20±0.13 <sup>b</sup>	30.84±1.90 <sup>b</sup>	19.28±1.93 <sup>b</sup>	203.95±8.49 <sup>e</sup>	603.36±70.64 <sup>b</sup>
	Adaxial	-	-	-	-	-	-	-	30.93±1.05 <sup>bcd</sup>	23.95±0.88 <sup>bcd</sup>	690.79±15.44 <sup>ab</sup>	733.75±14.16 <sup>cd</sup>
Variegated white	Abaxial	96.05±3.42 <sup>c</sup>	18.64±1.14 <sup>d</sup>	16.03±0.58 <sup>a</sup>	236.54±20.60 <sup>a</sup>	21.59±0.50 <sup>b</sup>	11.70±1.09 <sup>b</sup>	0.50±0.17 <sup>b</sup>	24.89±1.33 <sup>b</sup>	19.62±2.14 <sup>b</sup>	348.03±4.54 <sup>a</sup>	487.62±59.63 <sup>b</sup>
	Adaxial	-	-	-	-	-	-	-	31.50±2.14 <sup>abc</sup>	30.39±3.11 <sup>ab</sup>	632.24±23.93 <sup>bc</sup>	923.10±78.84 <sup>bc</sup>

Each values with the same alphabet are not significantly different ( $p \leq 0.05$ ) from one another across the column

**Table 3. Qualitative leaf epidermal characteristics of *Duranta erecta* forms studied.**

Species	Leaf surfaces	Stomata type	Anticlinal wall pattern	Epidermal cell shape
Thorny green	Abaxial	Tetracytic, Anisocytic	Sinuate	Irregular
	Adaxial	-	Sinuate	Irregular
Yellow bush	Abaxial	Tetracytic, Anisocytic	Sinuate	Irregular
	Adaxial	-	Straight to curve	Irregular
Plain yellow	Abaxial	Anisocytic, Tetracytic	Sinuate	Irregular
	Adaxial	-	Straight to curve	Irregular
Broad green 1	Abaxial	Paracytic, Anisocytic	Sinuate	Irregular
	Adaxial	-	Sinuate	Irregular
Broad green 2	Abaxial	Anisocytic	Sinuate	Irregular
	Adaxial	-	Sinuate	Irregular
Green bush	Abaxial	Paracytic, Anisocytic	Sinuate	Irregular
	Adaxial	-	Straight to curve	Irregular
Variegated yellow	Abaxial	Anisocytic	Sinuate	Irregular
	Adaxial	-	Straight to curve	Pentagonal
Variegated white	Abaxial	Anisocytic	Sinuate	Irregular
	Adaxial	-	Sinuate	Irregular

## Discussion

The micromorphological analysis of leaf epidermal surfaces in eight forms of *Duranta erecta* revealed several diagnostic features and intraspecific variations of taxonomic significance. While these features may not serve as definitive delimiters at the species level, they hold substantial value for classification and identification within the genus.

All forms displayed a hypostomatic leaf condition, with stomata confined to the abaxial surface. This observation is consistent with earlier reports by Ajuziogu *et al.*, (2018), who described a similar condition in various members of the Verbenaceae family. The abaxial restriction of stomata, as also noted by Mbagwu *et al.*, (2008) and Pasta *et al.*, (2025), likely represents an adaptive trait for water conservation, an advantageous feature for survival in diverse or drought-prone environments.

The comparative morphology of the eight forms revealed a suite of shared features, particularly on the lower epidermis, including the presence of stomata, trichomes, subsidiary cells, and comparable epidermal cell densities. These shared traits underscore the close relationships among the forms and support their classification within a single species complex. This finding resonates with the work of Moroni *et al.*, (2019), who reported remarkable morphological uniformity across various *Duranta* accessions, especially in habit, floral, and fruit structures.

Despite these similarities, significant micromorphological differences were observed. Most forms exhibited sinuate anticlinal walls on the abaxial surface; however, Broad Green 2 showed a distinct pattern, with curved to straight walls abaxially and sinuate walls adaxially. These variations align with Stace (1966) observations that anticlinal wall morphology is often influenced by environmental factors, such as humidity. On the adaxial surface, a broader range of anticlinal wall patterns was recorded, from straight to curved (Plain Yellow, Green Bush, Yellow Bush, Variegated Yellow) to predominantly sinuate in others.

The consistent presence of anisocytic stomata across all forms contrasts with the anomocytic stomata reported by Ajuziogu *et al.*, (2018), but aligns with findings by Gole *et al.*, (2013) and Sack & Buckley (2016), suggesting that anisocytic

stomata may represent a conserved ancestral trait within the genus. The additional occurrence of tetracytic stomata in Thorny Green, Yellow Bush, and Plain Yellow, as well as paracytic stomata in Broad Green 1 and Green Bush, (not previously emphasized for *Duranta erecta*) provides further micromorphological evidence useful for distinguishing among these forms. This is further corroborated by Choi *et al.*, (2022) who reported that, in horticultural systems, where vegetative similarity complicates identification, stomatal configuration combined with density metrics offers reliable micromorphological diagnostic criteria.

Trichome morphology also contributed valuable taxonomic information. All forms possessed multicellular peltate glandular trichomes, in agreement with the observations of Manokari & Shekhawat (2016), who documented similar structures in both *In vitro* and field-grown *Duranta erecta*. However, the quantitative variation in trichome density observed in the present study expands on earlier qualitative reports, although delicate, further highlights the potential of epidermal traits in delimiting morphological forms. Trichome density has been linked to herbivore defense, microclimatic buffering, and boundary-layer resistance (Glas *et al.*, 2012; de Campos *et al.*, 2021), implying that such variation may have both ecological and horticultural implication.

Quantitative analysis revealed a pronounced inverse relationship between epidermal cell size and density, particularly on the adaxial surface ( $r = -0.859, p < 0.01$ ). This strong negative correlation reflects a developmental trade-off between cell expansion and cell proliferation, consistent with broader models of epidermal optimization (Dow *et al.*, 2014; Pillitteri & Torii, 2012). The weaker, non-significant relationship observed on the abaxial surface suggests differential developmental regulation between epidermal layers. Such surface-specific coordination has been associated with functional specialization in response to irradiance gradients and thermal exposure (Sack & Buckley, 2016; Xiong & Flexas, 2020).

From an ecological perspective, forms such as Yellow Bush and Plain Yellow, which exhibited higher stomatal densities, may possess enhanced gas exchange capacity under high-light conditions. Increased stomatal density is often associated with improved photosynthetic

responsiveness but may incur higher transpirational cost (Franks & Beerling, 2009; Xiong & Flexas, 2020). Conversely, Thorny Green and Variegated White forms characterized by larger epidermal cells and comparatively lower densities may reflect structural strategies associated with moderated transpiration or ornamental selection. These patterns suggest that micromorphological variation in *Duranta erecta* may represent functional adjustments to light exposure and water-use optimization.

Epidermal density and stomatal characteristics are known to exhibit phenotypic plasticity under varying environmental regimes (Franks & Beerling, 2009). However, the clustering of specific horticultural forms with consistent anatomical profiles suggests that at least part of the variation may be genetically stabilized. Manokari & Shekhawat (2016) demonstrated relative structural stability of trichomes across *In vitro* and field conditions, supporting a genetic basis for certain epidermal traits. Resolving plastic versus heritable components would require controlled environmental trials or molecular analysis.

The combination of shared and distinctive epidermal traits observed across the eight forms underscores their close evolutionary relationship while also providing diagnostic criteria for their separation. These findings are consistent with classical descriptions of dicotyledonous epidermal structures by Metcalfe & Chalk (1960), particularly regarding the role of subsidiary cell arrangement in stomatal classification.

### Conclusion

This study provides a comprehensive assessment of epidermal micromorphology in eight morphological forms of *Duranta erecta*, highlighting both their shared features and distinguishing characters. The consistent hypostomatic condition, predominance of anisocytic stomata, and widespread occurrence of multicellular peltate and unicellular trichomes underscore a close phylogenetic relationship among the forms.

However, variations in epidermal cell shape, anticlinal wall architecture, stomatal complex types, and quantitative parameters such as stomatal density and cell dimensions offer reliable diagnostic features for form-level delimitation. These findings demonstrate that epidermal micromorphology traits are not merely supportive but diagnostically decisive, and can be a valuable tool for the taxonomy, classification, and identification of *Duranta erecta* forms.

Beyond morphology-based identification, these epidermal markers provide a structured framework for integrative taxonomy. When combined with molecular markers like plastid barcodes (*rbcL* and *matK*) or nuclear regions (ITS), micromorphological characters can help resolve fine-scale relationships, test the taxonomic status of morphotypes, and clarify intra- versus interspecific boundaries. Such integrative approaches will enhance the resolution and contribute to a more stable and predictive classification system within *Duranta*.

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