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EFFECT OF SOME BEAN CULTIVARS PHASEOLUS VULGARIS L. IN THE GREENHOUSE ON ANATOMICAL CHARACTERISTICS

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Abstract
The experiment was taken place at the Horticulture and Landscape Engineering Department, Tikrit University, Greenhouse Unit during the period of 2022–2023 growing season to study the effect of four bean cultivars on anatomical traits. The results revealed significant differences among the cultivars. The results showed Gia Bean cultivar outperformed the others in terms of cuticle thickness on both the upper and lower leaf surfaces, as well as epidermal cell dimensions on the upper surface. Meanwhile, the Seychelles cultivar showed greater values in epidermal cell thickness (upper and lower surfaces), mesophyll tissue thickness, stomatal dimensions on both surfaces, and lower surface epidermal cell dimensions, in addition to having the highest stomatal frequency on the lower surface and the Ferrari cultivar was differentiated by the highest stomatal frequency on the upper surface.

1. Introduction

Phaseolus vulgaris L. (common bean) belongs to the Fabaceae family and the Phaseolus genus, which comprises over 150 species of annual and perennial plants (Allen & Lenne, 1998). It is an herbaceous plant cultivated worldwide, consumed as fresh pods (green beans) or dry seeds. Over approximately 7000 years, beans have evolved from a wild species into one of the main crops, with South America being its center of origin (Kaplan & Lynch, 1999). Beans are nutrient rich vegetables containing 52 – 76 % carbohydrates, 14–33% protein, and amino acids such as lysine (6.4 – 7.6 g/100 g). Recent studies have shown clear variations among common bean varieties (Phaseolus vulgaris) in anatomical characteristics related to growth efficiency and tolerance to different environmental conditions. Studies have shown that water-stress-tolerant varieties exhibited a significant increase in upper and lower epidermal thickness and mesophyll thickness, along with a decrease in stomata density, compared to sensitive varieties (Sadeghi et al., 2023; Almeida Silva et al., 2024). This contributed to reduced water loss and improved water use efficiency. The researchers also indicated that anatomical differences between varieties are important indicators for assessing drought tolerance. Tabatabaiepour et al. (2023) noted that variety variation led to significant differences in the anatomical and physiological characteristics of leaves. Some varieties demonstrated a greater ability to maintain leaf tissue integrity and photosynthetic efficiency when competing with weeds, while others were affected by reduced leaf tissue thickness and disruption of cellular structure. In other side (Bueno & Vendrame, 2024) reported that light intensity and wavelength variations significantly affected the anatomical characteristics of the studied cultivars. They observed an increase in the number of stomata and improved morphological growth characteristics in some cultivars compared to others, indicating a differentiated genetic response to environmental conditions. Kumar et al. (2022) also noted significant differences between drought-tolerant and drought-sensitive genotypes in the number of stomata, cuticle thickness, and vascular bundle size. The drought-tolerant cultivars possessed anatomical characteristics that enabled them to continue growing under stress conditions. Farooq et al. (2023) stated that anatomical changes in the bean plant are among the most important adaptive mechanisms to cope with environmental stresses, and

that the difference in these traits is directly related to the genetic background of the variety (Hazaa & Naser, 2026).

Anatomical studies are crucial in revealing developmental relationships, especially with the advent of modern microscopy. Such studies have been fundamental in plant taxonomy, providing reliable classification decisions , as many anatomical characteristics are diagnostic (Khalid et al., 2009). Anatomical traits are used for taxonomic purposes like for example Al-Mayah (1983) developed diagnostic keys based on epidermal characteristics to study species in the Terminalia genus (Combretaceae family) showing the importance of leaf venation in species differentiation. Similarly, Soltis et al. (2018) employed leaf anatomy in studying complex plant families such as Coniferaceae and Poaceae. The anatomical features of Phaseolus vulgaris L., including leaf anatomy and epidermal layers and stomatal complexes and vascular bundle arrangements were shown to vary among common bean species (Stenglein et al., 2004). The research objective is to study the effect of four bean cultivars on anatomical traits

2. Materials and Methods

2.1 Anatomical Characteristics

2.1.1 Preparation of Transverse Sections:

Transverse leaf sections were studied using fresh field-collected samples. The Samples were immersed in 1 % Sodium Hydroxide (NaOH) for 24 – 48 hours, rinsed with Distilled Water to remove Alkali residues, and fixed in FAA solution for 18 – 24 hours. For preserved dry samples, boiling in water for 5 minutes followed by transfer to 70 % ethanol was employed. The sections were prepared as follows:

Killing and Fixation:

Fresh leaf pieces (2 – 5 mm) were cut and placed in vials containing 20 ml of FAA fixative (Johnson, 1940) for 18 – 24 hours:

50 ml ethyl alcohol
5 ml glacial acetic acid
10 ml formaldehyde (37 – 40 %)
35 ml Distilled Water

Washing and Dehydration:

The Samples were washed twice with 70 % ethanol, preserved in 70 % ethanol, then passed through a graded ethanol series (80 %, 90 %, 95 %) for 2 hours each. Finally, they were placed in absolute ethanol for 2 hours to eliminate residual water.

Clearing and Infiltration:

The Samples were passed through mixtures of absolute ethanol and xylene in volume ratios of 1:3, 1:1, and 3:1, followed by pure xylene (2 hours each) according to (Sass, 1958). Xylene was partially replaced with molten paraffin at 55 – 60°C in an oven for 1 hour, then replaced with pure paraffin and left in the oven with same temperature for 4 – 5 days. This cycle was repeated 5 – 6 times, with a final overnight infiltration as per (Al-Mashhadani, 1992).

Embedding and Mounting:

The Samples were embedded in previously heated paraffin blocks using molds and were left to cool for 24 hours. The blocks were trimmed and mounted on wooden holders and sectioned using a Bright rotary microtome at 10 – 15 µm thickness. Sections were floated on a water bath at 40–45°C, mounted on pre-coated slides (glycerine-albumin) and dried on a hot plate at 40 – 45°C for 4 – 12 hours.

Dewaxing and Staining:

Following (Sass, 1958), slides were processed through:

Xylene (2 – 4 hours at 50°C, twice)
Xylene–absolute the ethanol (1:1) for 5 minutes
Graded ethanol series (30 % , 50 % , 70 % , 80 % , 96 %) for 5 minutes each
0.2% Safranin in 50 % ethanol for 2–24 hours
Ascending ethanol series (30 % to 90 %) for 5 minutes each
1 % Fast Green in absolute ethanol for 3 – 5 seconds

Absolute the ethanol for 5 minutes
Xylene – clove oil (1:1) for 5 minutes
Xylene twice for 3 minutes

Slides were permanently mounted using Canada balsam, covered with cover slips, and left to dry on a hot plate at 40 – 45°C for 24 hours. Sections were examined under an Olympus compound light microscope and photographed

2.1.2 Epidermis Preparation

Epidermal peels were obtained from fully developed leaf sections, including the midrib, lamina, and margin, using the stripping method with fine-tipped forceps. Peels of both adaxial and abaxial surfaces were mounted on glass slides with a drop of glycerin jelly, covered with cover slips, and examined under an Olympus compound microscope using an eyepiece micrometer.

Measurements were taken for 5 – 10 epidermal cells, including radial and tangential wall dimensions, ordinary epidermal cell shape, guard cell dimensions, stomatal complex shape, and stomatal area. Stomatal frequency was calculated using the formula according to (Stace, 1980), terminology followed that of (Dilcher, 1974):

Stomatal frequency = Number of stomata per 2 mm^2 (1)

2.2 Plant Material

The cultivars used in this study are recorded in Table 1.

Table 1. Cultivars used in the study and their origins.

Cultivar Name	Origin	Code
Sonesta	Poland	V1
Ferrari	Poland	V2
Gia Bean	USA	V3
Seychelles	Netherlands	V4

2.3 Studied Traits:

The following anatomical traits were evaluated:

- Upper cuticle thickness (µm).
- Lower cuticle thickness (µm).
- Upper epidermal thickness (µm).
- Lower epidermal thickness (µm).
- Mesophyll tissue thickness (µm).
- Number of stomata.
- Stomatal area.
- Stomatal complex.

3. Result and Discussion

3.1. Thickness of Upper and Lower Cuticle, Upper and Lower Epidermis, and Mesophyll Tissue (µm)

Table 2 presents the quantitative characteristics of the transverse sections of bean leaves, showing clear differences in the thickness of the cuticle layer enveloping the leaves. These differences were evident between the upper and lower surfaces, even within the same cultivar. The Gia Bean cultivar recorded the highest upper cuticle thickness at 7 µm, compared to the lowest thickness recorded by Sonesta at 3 µm. For the lower cuticle, Gia Bean again exhibited the highest thickness at 4 µm, while Sonesta showed the lowest at 2 µm.

Table 2. Quantitative Characteristics of Leaf Cross-Sections in Common Bean Cultivars (µm).

Cultivar	Cuticle Thickness (µm)	Epidermis Thickness (µm)	Mesophyll Thickness (µm)
	Upper	Lower	Upper
Sonesta	3	2	20
Ferrari	4	3	23
Gia Bean	7	4	30
Seychelles	5	3	35

Leaf epidermal cells exhibited both wavy and curved shapes, with more pronounced undulations in the lower surface epidermal walls across all cultivars. Seychelles had the most pronounced nodulations . According to (Esau, 1965), this waviness may be due to abrupt cell expansion during the differentiation of the leaf. The consistent wall shapes across cultivars are likely genetically controlled. The epidermal cells of the studied cultivars were simple and composed of a single cell layer (uniseriate) covering both upper and lower leaf surfaces. Differences was observed in the shape and size of the epidermal cells even within the same layer. Sonesta and Ferrari had barrel shaped cells while Gia Bean and Seychelles showed a mix of spherical and oval shaped cells indicating heterogeneity in epidermal cell morphology across cultivars. Also, cells around the midrib region were more uniformly sized and structured. Differences in epidermal cell thickness were clear with Seychelles recording the highest upper epidermal thickness at 35 µm compared to the lowest in Sonesta at 20 µm. For the lower epidermis Seychelles again had the highest thickness at 23 µm while Sonesta recorded the lowest at 15 µm. Regarding mesophyll tissue, Seychelles had the highest mesophyll thickness among all cultivars measuring 116 µm, while Sonesta had the lowest at 87 µm.

3.2. Stomatal Number and Dimensions

According to Table 3, which outlines stomatal and epidermal cell dimensions on both upper and lower leaf surfaces of the bean cultivars, the Seychelles cultivar had the largest stomata on the lower leaf surface (14 × 8 µm), significantly surpassing the other cultivars, while Sonesta had the smallest (10 × 7 µm). On the upper surface, Seychelles again exhibited significantly larger stomata (12 × 9 µm), whereas Gia Bean had the smallest (9 × 6 µm). Susetyarini et al. (2020) stated that stomatal size is influenced by various internal and external factors. Changes in guard cell thickness in response to sunlight, CO_2, and water conservation affect stomatal size. Larger and longer stomata respond more efficiently to drought and transpiration. The higher stomatal density on the lower epidermis is an adaptive trait as the upper surface is more exposed to light and heat and environmental stress.

In terms of lower epidermal cell size Seychelles recorded the largest average (49 × 21 µm) while Gia Bean had the smallest (43 × 23 µm) with the other cultivars falling in between on the upper surface Gia Bean had the largest cells (39 × 21 µm), while Seychelles had the smallest (34 × 18 µm). These variations in cell dimensions and stomatal size may be attributed to foliar application of nano-fertilizer, which enhances cambial activity and consequently stimulates the production of plant hormones such as cytokinins (responsible for cell proliferation) and auxins (responsible for cell elongation), this agree with finding by (Al-Hujairi, 2020).

Table 3. Dimensions of Stomata and Epidermal Cells on the Upper and Lower Leaf Surfaces of Common Bean Cultivars.

Cultivar	Stomata (Lower Surface)	Stomata (Upper Surface)	Epidermal Cell Size (µm)
	Length (µm)	Width (µm)	Length (µm)
Sonesta	10	7	9
Ferrari	12	6	10
Gia Bean	11	9	9
Seychelles	14	8	12

Note: The average values represent measurements taken from five replicates, divided by the total number of measurements. Values are expressed in micrometers (µm)

3.3. Stomatal Number and Dimensions

Stomata were observed on both leaf surfaces, with a higher density on the lower surface. Their orientation was random. The stomatal complex type on the lower epidermis of all cultivars was diacytic (with two subsidiary cells parallel to the guard cells), whereas the upper surface exhibited a triacytic pattern. Each stomatal complex consists of a pore (stoma) surrounded by a pair of guard cells which regulate gas exchange and stomatal movement.

Stomatal frequency was different among cultivars and between the two leaf surfaces of the same cultivar. The highest stomatal frequency on the lower surface was recorded in Seychelles (104 stomata/mm^2) while Sonesta had the lowest (73 stomata/mm^2). On the upper surface Ferrari had the highest stomatal frequency (24 stomata/mm^2), whereas Gia Bean had the lowest (12 stomata/mm^2), as shown in Table 4.

Table 4. Quantitative Traits of Stomatal Frequency and Stomatal Complex Types in Leaves of Common Bean Cultivars.

Cultivar	Stomatal Frequency (No./mm^2)	Stomatal Complex Type
	Lower Surface	Upper Surface
Sonesta	73	20
Ferrari	80	24
Gia Bean	87	12
Seychelles	104	20

Note: The values represent means of five replicates. Stomatal frequency was calculated per square millimeter (mm^2), not merely by the number of stomata within a microscopic field

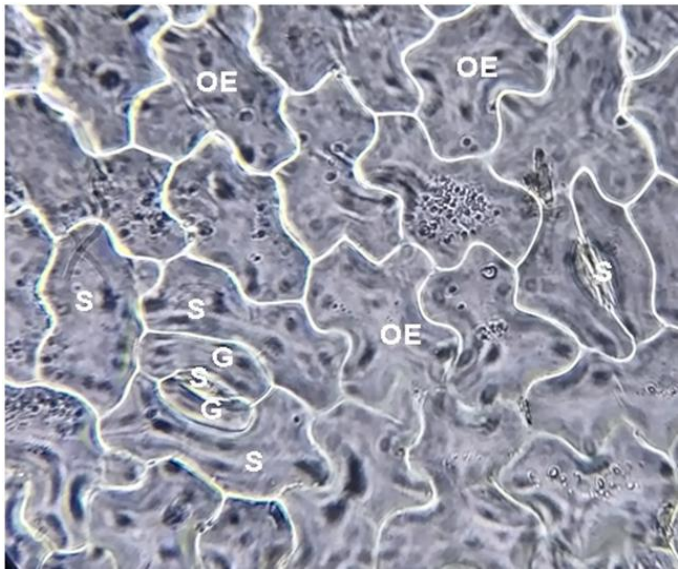

Figure 1. Normal epidermal cells and stomata in the epidermis of a bean plant under power of 40x. G: guard cell , S:subsidiary , OE: ordinary epidermal cells.

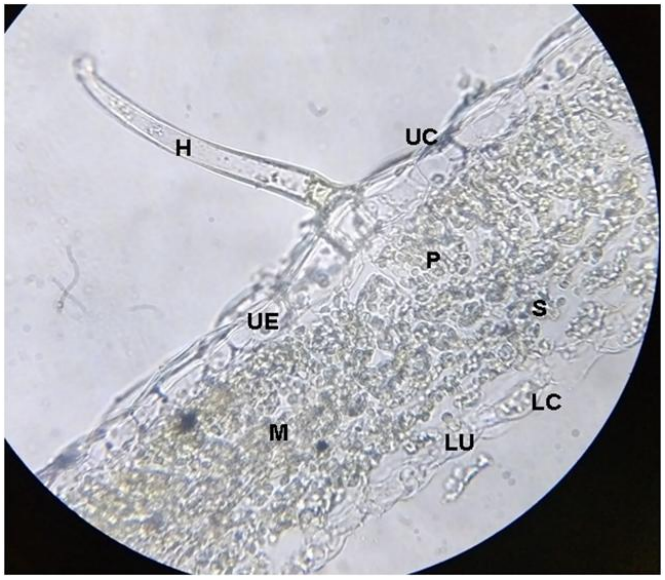

Figure 2. Cross section of a bean leaf showing its tissue components under the strength of 40 x. UC:upper cuticle , LC:lower cuticle , UE:upper epidermis , LE:lower epidermis , m: mesophyll ,P:paliside tissue , S:spongy tissue , H: hair

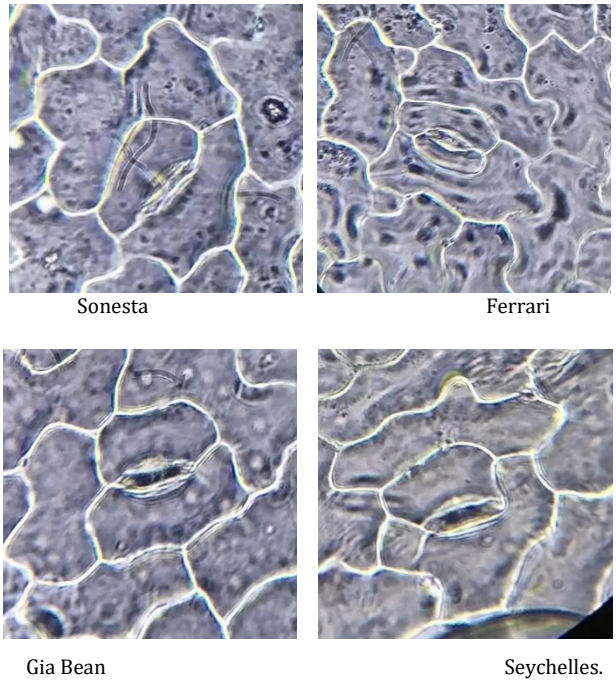

Figure3. The stomata complex in the upper epidermis of studied bean cultivars under strength of 100 x

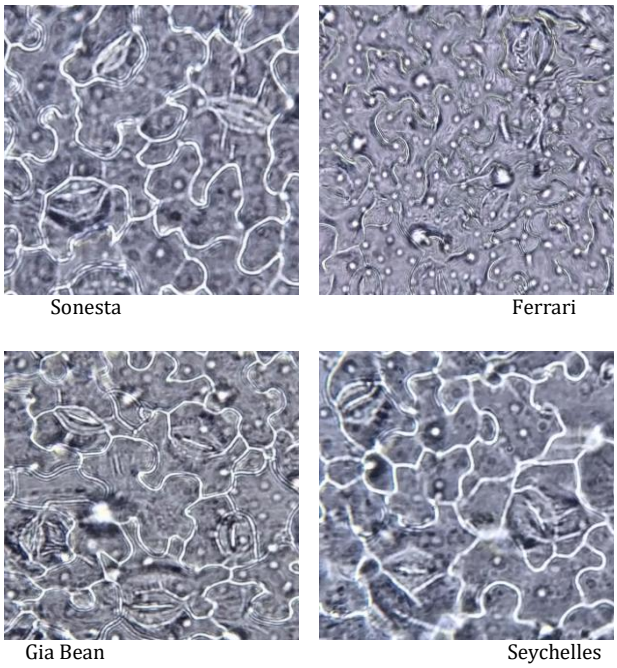

Figure4. The stomata complex in the lower epidermis of studied bean cultivars under strength of 40x

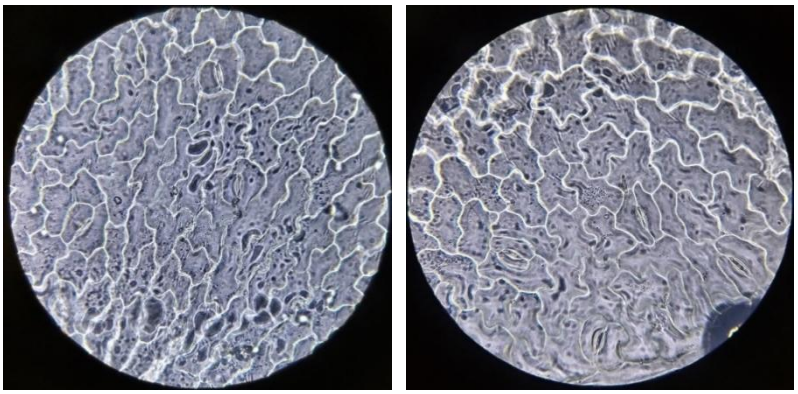

Figure 5. Stomata in the upper epidermis of studied bean cultivars under strength of 40 x.

4. Conclusion

The study demonstrates significant variation in stomatal frequency values among the examined cultivars. Variation in somatal frequency is likely due to differing cultivars responces to environmental factors such as humidity, drought, and light intensity. Esau (1965) also confirmed that stomatal frequency is highly variable under different environmental influences.

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