

## Research Article



# Stem Epidermal Anatomy of Fourteen Sugarcane Varieties of the Punjab

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**Abstract** | A comparative study of stem epidermis of fourteen sugarcane varieties (*i.e.*, L-118, CP72-2086, CP43-33, HSF-240, CPF-237, SPF-213, COjv-84, CO-1148, COL-54, COL-29, CO-975, BL-4, Triton and BF-162) was carried out using Schultz's maceration method followed by staining with chloroiodide of Zinc. The results indicated substantial differences among the sugarcane varieties for various stem epidermal cell combinations and structures like number of stomata, number and shape of cork, silica, long, and pointed elongated cork cells. It is therefore contended that the structure of the epidermis in sugar cane can be used as a characteristic of variety differentiation.

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**Keywords** | Stem epidermis, Silica cells, Cork cells, Sugarcane, Stomata.

Sugarcane is one of the most important cash crops of Pakistan. For its maximum production in any region it is essential to plant cane varieties which are approved for that region. Planting of sugarcane varieties or cultivars, which have not been approved for unwanted characters can lead to serious economic losses. In order to prevent such losses and for keeping the purity of a variety in the field it is essential for our experts; working in this specific area to have diverse means for differentiating the various varieties. Several attempts have been made to describe the agricultural and morphological features for the description of various sugar cane varieties (Cowgill, 1917; Barber, 1915). These features are number, size, colour of stalks, bud groove, nodal characteristic, ivory patterns, leaf characteristic and root system etc. Cellular studies have shown that sugarcane stem epidermal cells differ (in size, number and distribution of silica cells) between varieties (Frohnmeier, 1914). The plant epidermis plays an important role in defense, pollinator

attraction and water relations that can be attributed to the various specialized cells present in epidermis. The epidermal cells show various morphological specializations that can be added to the varietal descriptions (Prat, 1936; Hubbard, 1948; Chaudhari et al., 2014; Khan et al., 2011; Elahi and Ashraf, 2002). After the work of Artschwager (1930) and (1939), which depend on the varietal modifications of the epidermal structures, little work has been done on these characteristics. His studies, though, presented fairly distinct dissimilarities among varieties. Present study was therefore planned to examine the probability of using some cellular features for the precise documentation of sugarcane varieties.

## Materials and Methods

The study was conducted at the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan. Mature stalks of the fourteen sugarcane field

varieties of Punjab *i.e.*, L-118, CP72-2086, CP43-33, HSF-240, CPF-213, Cojv-84, CO-1148, COL-54, COL-29, CO-975, BL-54, Triton and BF-162 were obtained from the Ayyub Agricultural Research Center, Faisalabad, Pakistan.

### Cotological Study of Stem Epidermis

Twelve months old mature stalks of the sugarcane varieties were collected from experimental sites. Ten individual samples of every variety were tested. Care was taken to select the material at similar phase of growth. Central portion of the mature inter node which had reached their absolute length and were no longer enclosed by the protective leaf cover were used for this study.

### Maceration

Schultz's method of maceration was used (Subramanayam, 1996). A portion of about 5 mm<sup>2</sup> of the stem epidermis with some of the fibrous and cortical tissues was cut from the selected part of the internodes and placed it in a test tube then 4 ml concentrated nitric acid, 2 gm potassium chlorate and 1 ml of distilled water was added. Then mixture was boiled in a test tube and when epidermis detached, the contents were transferred into a Petridish having some water.

### Staining and Slide Preparation

The stem epidermis was mounted on a slide and stained with chloloiodide of Zinc (Van Dillewijn, 1952). Different cytological characters were observed under NIKON Leitzoptiphot research microscope and photographs were taken on Konica Monochrome VX 400.

### Statistical Analysis

The data was statistically analyzed (Steel and Torrie, 1980) by using Analysis of Variance. While for the significance of means, Duncan's multiple range (DMRT) test was used.

## Results and Discussion

Stem epidermis of fourteen sugar cane varieties (*i.e.*, L-118, CP72-2086, CP43-33, HSF-240, CPF-237, SPF-213, COjv-84, CO-1148, COL-54, COL-29, CO-975, BL-4, Triton and BF-162) were studied with the help of light microscope (Figure 1, 2, 3, 4, 5, 6 and Tables 1, 2). The stem epidermis seemed to be made up of several types of cells inclined in a uniform design. The long cells, which occupy the larger portion

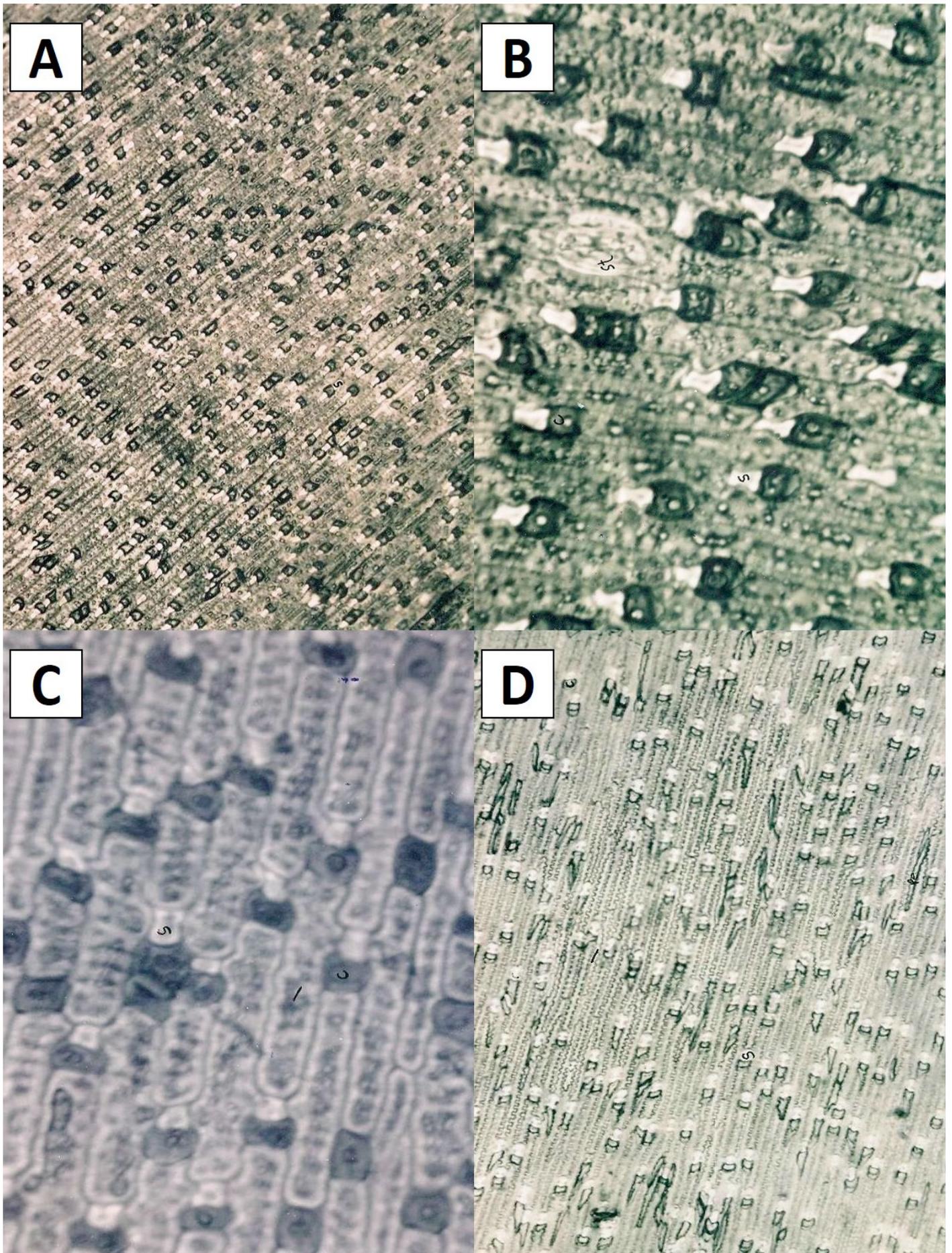
of the epidermal cells, look like a four-sided prism. They differ largely in length while differences in width were less apparent.

**Table 1:** Various stem epidermis characteristics of fourteen sugar cane varieties. Letters indicate statistically significant differences by Duncan's multiple range tests at  $P < 0.05$

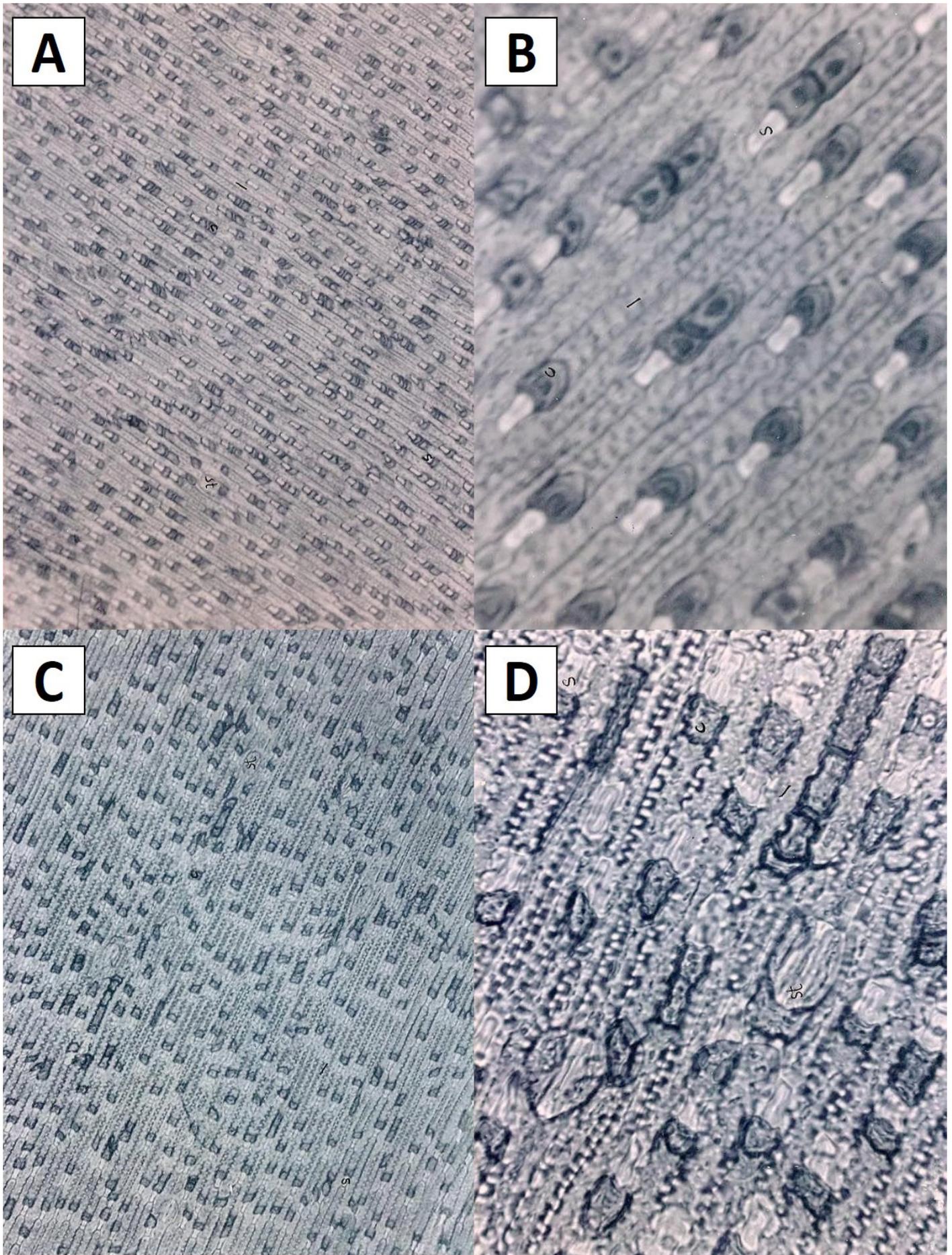
Name of variety	Width of long Cells at its Max point(µm)	Width of long cells at its Min point (µm)	Length of long cells (µm)	Extra cork cells per square (mm)
L-118	17 <sup>de</sup>	6.25 <sup>bc</sup>	90.25 <sup>b</sup>	Scarce
CP72-2086	21 <sup>b</sup>	6.25 <sup>bc</sup>	67 <sup>b</sup>	Abundant
CP43-33	18.45 <sup>c</sup>	7.5 <sup>a</sup>	187.75 <sup>a</sup>	Scarce
HSF-240	13.25 <sup>f</sup>	5.5 <sup>cd</sup>	65.15 <sup>b</sup>	Scarce
CPF-237	18.2 <sup>cd</sup>	5 <sup>d</sup>	103.75 <sup>b</sup>	Absent
SPF-213	14 <sup>f</sup>	6.25 <sup>bc</sup>	77.75 <sup>b</sup>	Absent
COjv-84	18.55 <sup>c</sup>	6 <sup>cd</sup>	81.76 <sup>b</sup>	Abundant
CO-1148	18.75 <sup>c</sup>	6.25 <sup>bc</sup>	131.7 <sup>ab</sup>	Scarce
COL-54	18.8 <sup>c</sup>	6.255 <sup>bc</sup>	112.5 <sup>b</sup>	Absent
COL-29	23.5 <sup>a</sup>	6.5 <sup>abc</sup>	108.75 <sup>b</sup>	Absent
CO-975	18.5 <sup>c</sup>	7.25 <sup>ab</sup>	97.25 <sup>b</sup>	Absent
BL-4	18.2 <sup>cd</sup>	6.25 <sup>bc</sup>	103 <sup>b</sup>	Absent
Triton	14.5 <sup>f</sup>	5.5 <sup>cd</sup>	136.25 <sup>ab</sup>	Absent
BF-162	16.25 <sup>e</sup>	5 <sup>d</sup>	88.75 <sup>b</sup>	Absent

**Table 2:** Various stem epidermis characteristics of fourteen sugar cane varieties

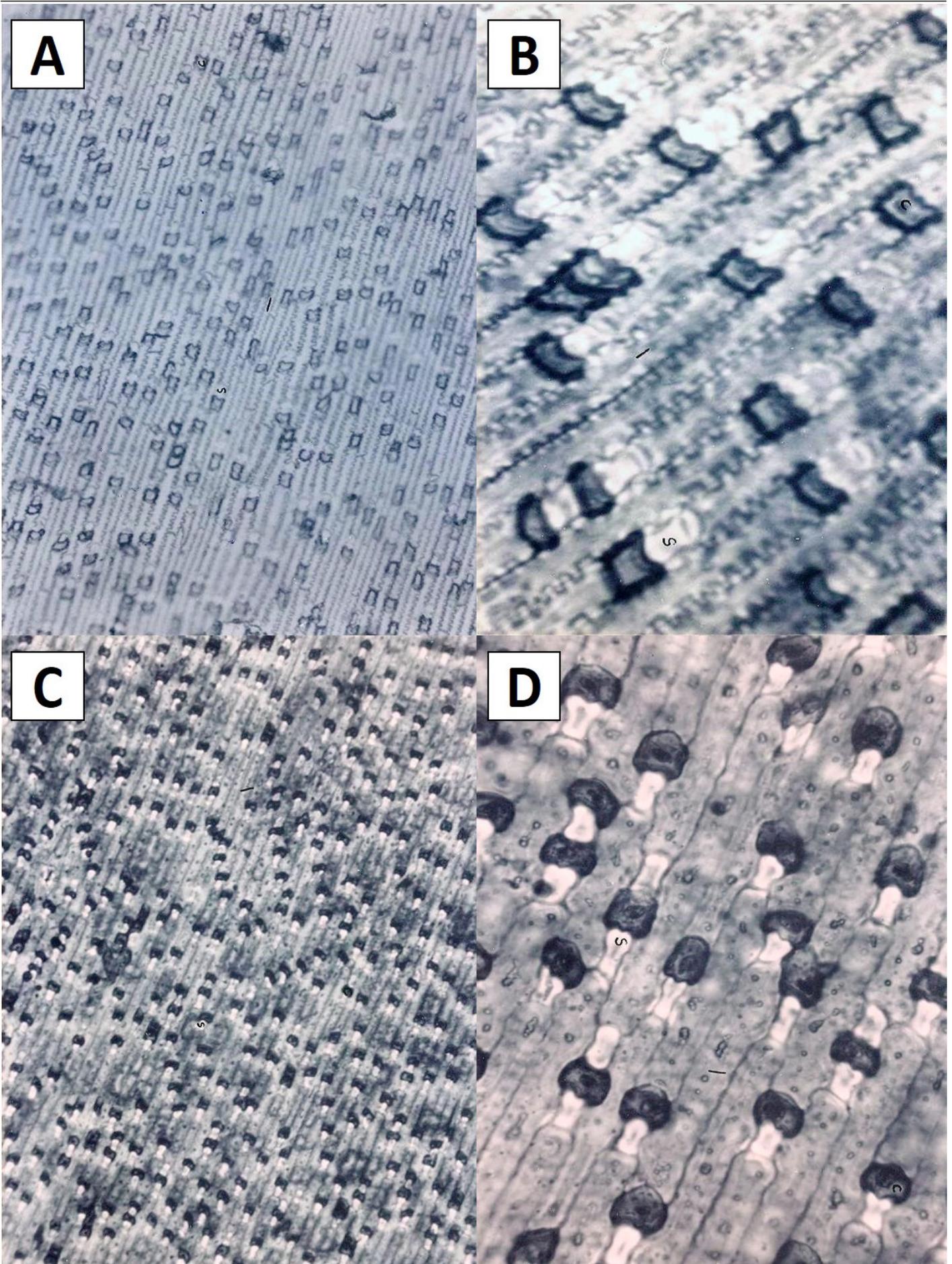
Name of variety	Solitary Cork cells/square (mm)	Pointed elongated cork cells/ square (mm)	Stomata/ square (mm)
L-118	Absent	Absent	Scarce
CP72-2086	Scarce	Absent	Scarce
CP43-33	Scarce	Abundant	Scarce
HSF-240	Absent	Scarce	Scarce
CPF-237	Scarce	Abundant	Scarce
SPF-213	Scarce	Scarce	Scarce
COjv-84	Scarce	Scarce	Absent
CO-1148	Scarce	Absent	Absent
COL-54	Absent	Scarce	Absent
COL-29	Scarce	Scarce	Scarce
CO-975	Scarce	Scarce	Scarce
BL-4	Scarce	Abundant	Scarce
Triton	Absent	Absent	Absent
BF-162	Abundant	Scarce	Scarce



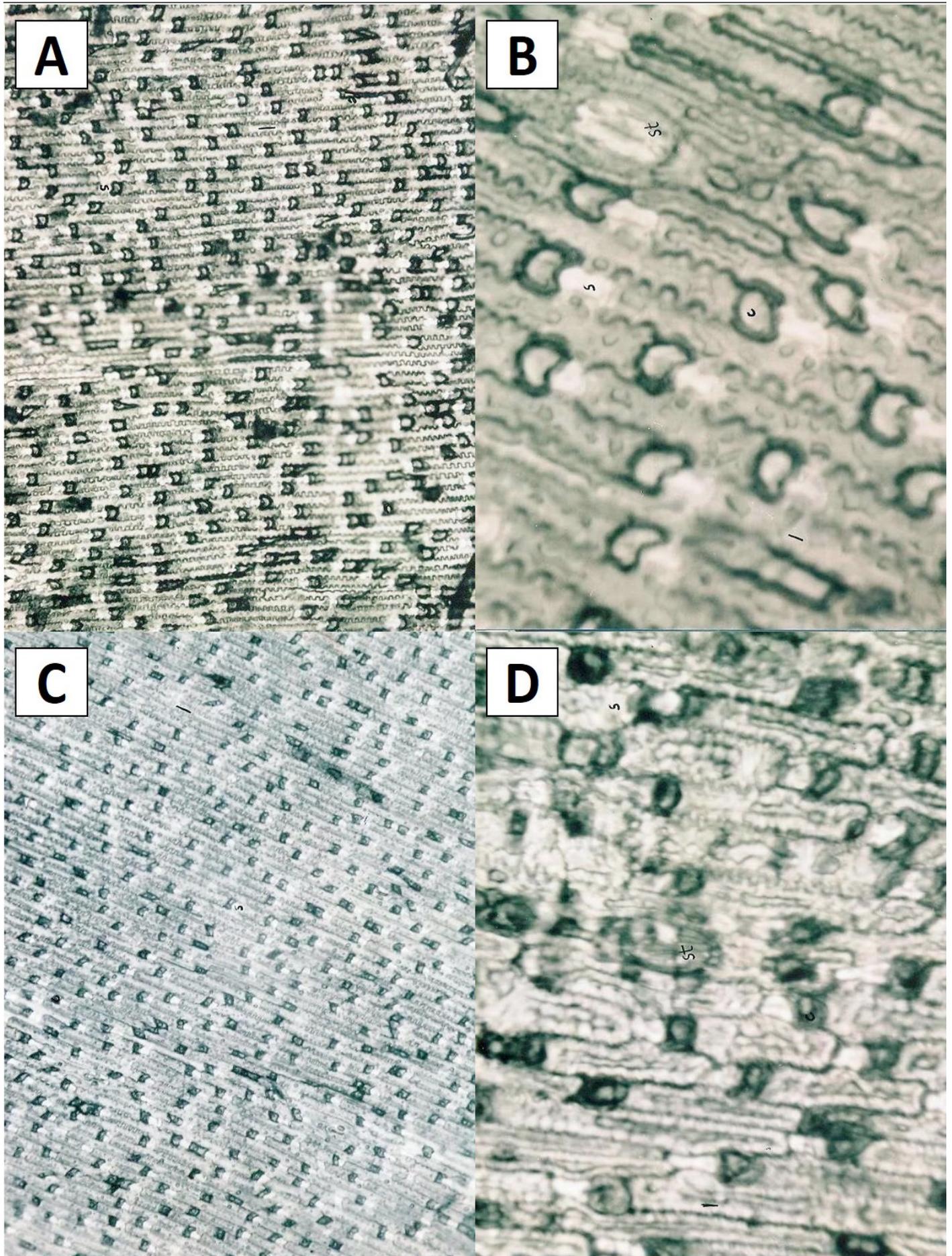
**Figure 1:** Photomicrographs of the stem epidermis of the sugarcane variety: **A:** (X 208); **B:** L-118 (X 416); **C:** CP72-2086 (X 416); **D:** CP43-33 (X 208). *c*, cork cell; *s*, silica cell; *st*, stomata; *l*, long cell; *pe*, pointed elongated



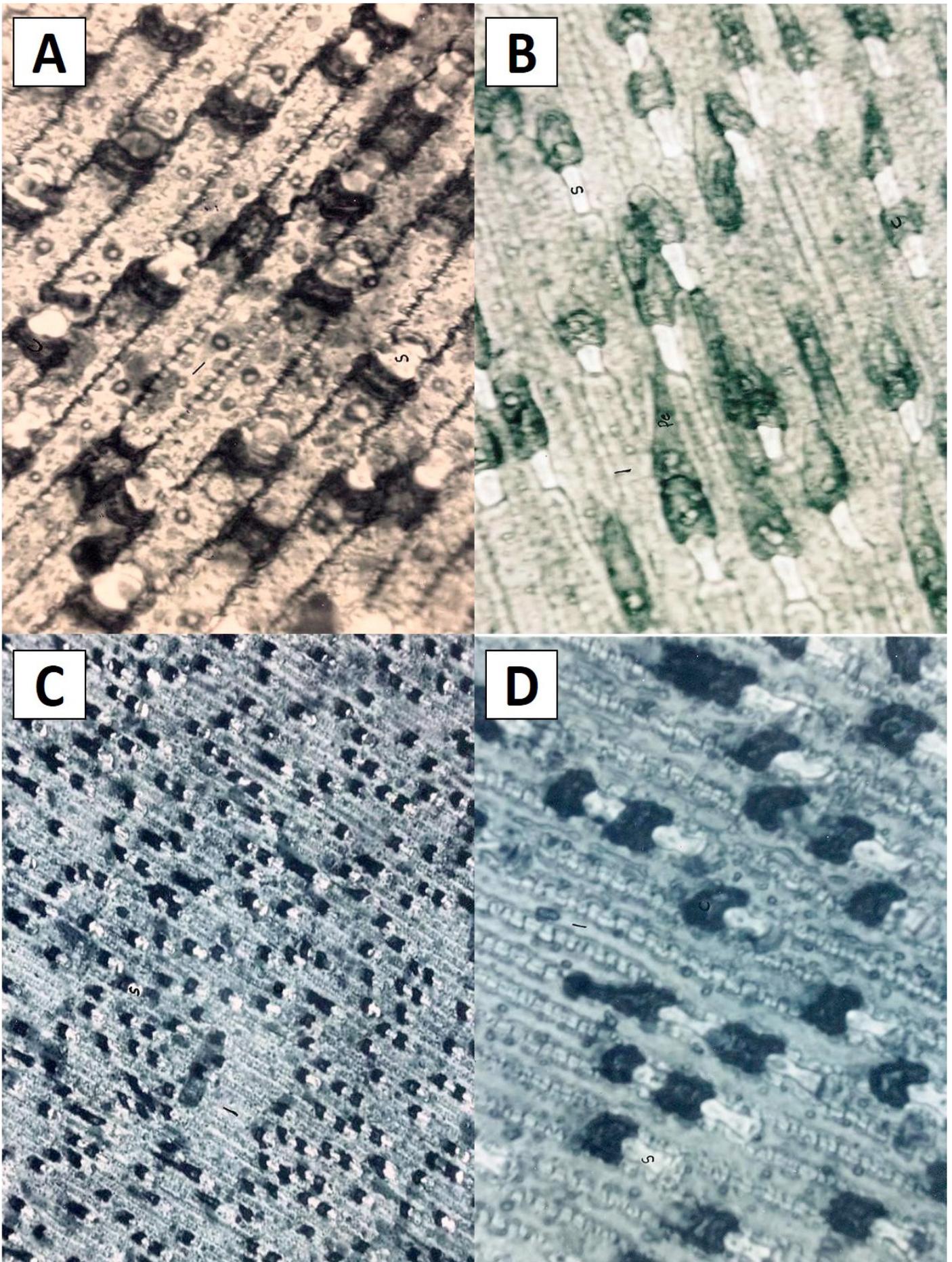
**Figure 2:** Photomicrographs of the stem epidermis of the sugarcane variety: **A:** HSF-240 (X 208); **B:** HSF-240 (X 416); **C:** SPF-213 (X 208); **D:** SPF-213 (X 416). c, cork cell; s, silica cell; l, long cell; st, stomata



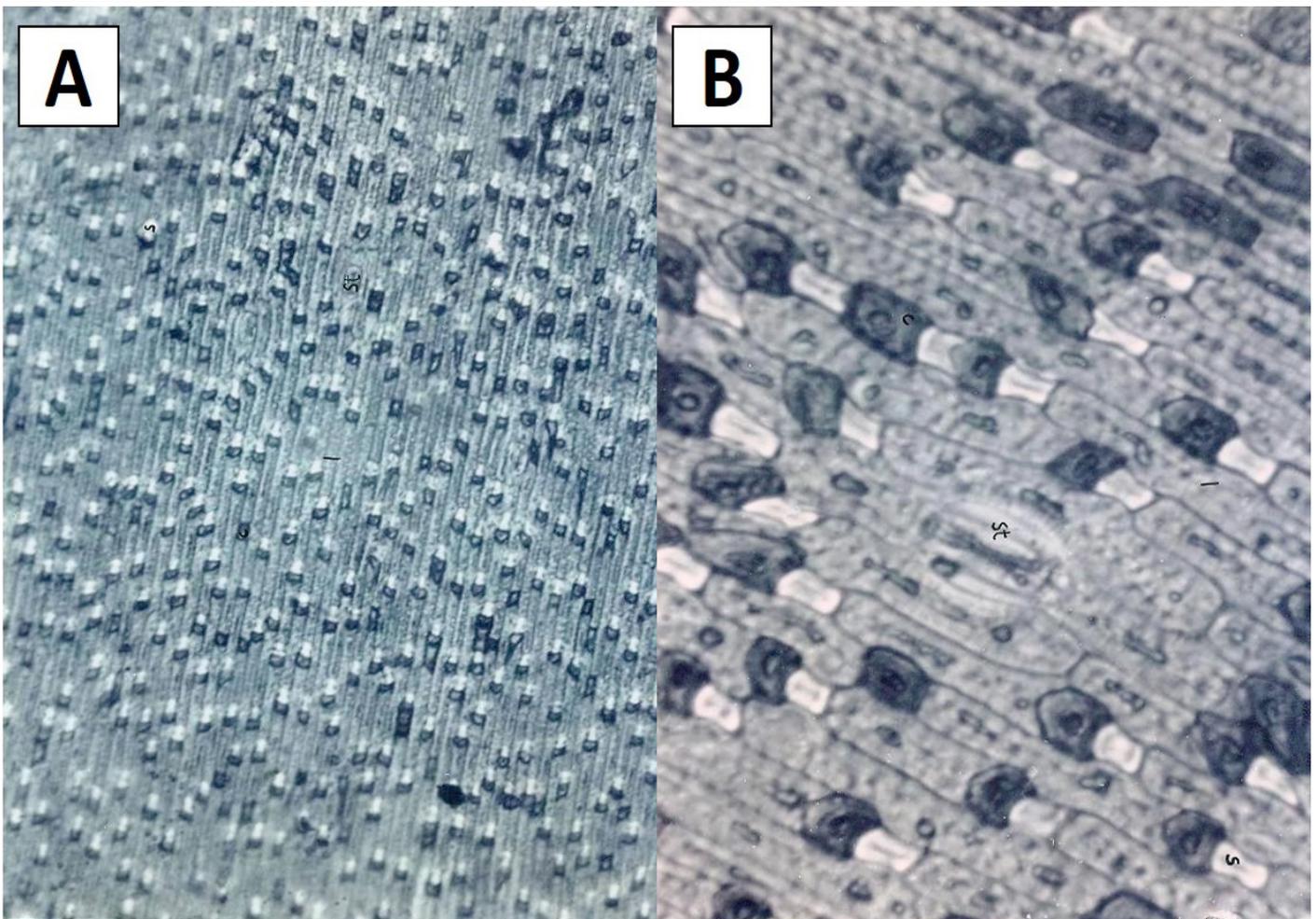
**Figure 3:** Photomicrographs of the stem epidermis of the sugarcane variety: **A:** CO-1148 (X 208); **B:** CO-1148 (X 416); **C:** CoL-54 (X 208); **D:** CoL-54 (X 416). *c*, cork cell; *s*, silica cell; *l*, long cell



**Figure 4:** Photomicrographs of the stem epidermis of the sugarcane variety: **A:** CoL-29 (X 208); **B:** CoL-29 (X 416); **C:** CO-975 (X 208); **D:** CO-975 (X 416). *c*, cork cell; *s*, silica cell; *I*, long cell; *st*, stomata



**Figure 5:** Photomicrograph of the stem epidermis of the sugarcane variety: **A:** Cojv-84 (X 416); **B:** BL-4 (X 416); **C:** Triton (X 208) **D:** Triton (X 416). c, cork cell; s, silica cell; l, long cell; pe, pointed elongated



**Figure 6:** Photomicrographs of the stem epidermis of the sugarcane variety BF-162: **A:** (X 208); **B:** (X 416). *c*, cork cell; *s*, silica cell; *I*, long cell; *st*, stomata

The differences in epidermal cell structure is divided into two groups; *i.e.*, quantitative and qualitative. Qualitative changes consist of the absence of any type of cell as in the varieties L-118, HSF-240, COL-54, Triton, solitary cork cells and in varieties CPF-237, SPF-213, COL-54, COL-29, CO-975, BL-4, Triton, BF-162 extra cork cells were absent (Table 1, 2) in the stem epidermal cell structure which differentiate them from all other varieties. Structural differences of stem epidermal cells among different sugar cane varieties are sometimes so much striking that many varieties can be recognized by only one character. For example, in the epidermal cell structure of sugarcane varieties Triton (Figure 5C, D) and BF-162 (Figure 6) two cork and two silica cells mostly form short cell group, while in HSF-240, the short cell group mostly formed with two cork and one silica cell (Figure 2A, B). Dahlgren and Clifford (1982) stated that the size, shape and distribution scheme of silica bodies on various plant epidermal structures varies from one species to another, and this difference has always been thought of high taxonomic significance.

There are also quantitative differences as there are partial absences or abundance of any type of cells in different varieties as shown in Table 1 and 2. Among the stem epidermal cells of CP43-33, higher number of pointed elongated cork cells and presence of hook like cork cell (Figure 1D) are the distinguishing characters. This increase may be brought about by the omission of long or silica cells, so that two short cell groups join each other directly (Artschwager, 1930). The inconsistencies of the number of stomata among the different varieties play an important role in classification (Ullah et al., 2011). The distribution of stomata, though erratic, offers a valued diagnostic feature. When comparing the number of stomata in the stem epidermal cells of all the sugarcane varieties, few stomata were observed in COL-29 and CO-975, while in varieties COjv-84, CO-1148, COL-54 and Triton stomata were absent (Table 2). Various kinds of epidermal cells and their supplementary design may help in creating a more natural grouping of the genera and tribes of the Gramineae (Metcalf, 1960, 1963; Elahi and Ashraf, 2002). Among quantitative

variations of epidermal cells, the width and length of long cells, the number and distribution of stomata are very important (Elahi and Ashraf, 2002; Ullah et al., 2011). In the stem epidermal cells of all the 14 sugarcane varieties width of long cell at its maximum and minimum point ranged from 23.5 $\mu$ m-13.25 $\mu$ m and 7.25 $\mu$ m -5 $\mu$ m, respectively, while length of long cells ranged from a minimum of 65.15  $\mu$ m in the variety HSF-240, to the maximum of 187.75  $\mu$ m in CP43-33 (Table 1). According to Metcalfe (1960), the long cells exist in different shapes, and can be used for the solution of various taxonomic problems. Van Dillewijn (1952) stated that the sugar cane stem epidermis display a fascinating patterns, which differ significantly in various varieties. Rao and Balasubramanian (1953) suggested that quantitative dissimilarities in size of many organs and cell - types are sometimes linked to chromosome number of the clone. According to Artschwager (1930), it would be necessary to study the epidermis of a large number of canes, in order to trace group relationships and investigates the parental influence in the different crosses.

## Conclusion

The diagnostic importance of sugarcane stem epidermal cell structure has been examined on 14 varieties of commercial value. It is clear from the present study that the design of the epidermis of sugarcane varies greatly among varieties and remains nearly constant within variety. So it is suggested that characters of the sugarcane stem epidermal cells are diagnostic and can provide additional reliable characters for identification of varieties when combined with morphological characters. Further studies should be designed to verify that this epidermal cell configuration remains constant under diverse environmental situations.

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## Authors' Contributions

Nosheen Noor Elahi designed the experiments, helped with interpretation of data and contributed in conducting experiments. Misbah Mughal conducted the experiments and wrote the manuscript.

## Conflict of Interest

Authors have declared no conflict of interests.

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