ANATOMICAL DIFFERENCES IN THE STEM EPIDERMAL STRUCTURE OF SUGARCANE VARIETIES GROWN IN SOUTH AFRICA

By G. ROTH

South African Sugar Association Experiment Station

Introduction

For optimum production it is important to plant approved cane varieties in suitable environments. The indiscriminate planting of varieties which have not been screened for undesirable features can lead to heavy financial losses being incurred, not only by the individual, but by an entire sugar industry. In order to prevent such disastrous consequences phytosanitary regulations are laid down, and for their effective enforcement it is essential to have reliable means for identifying the different varieties. The search for methods of separation and identification has now been carried out for over half a century.

Sugarcane varieties differ in many characteristic features. These include the size and form of the plant, pattern of root development, size and shape of the leaf dewlap, bud, auricle and ligule, and the microscopic morphology of the stem epidermis. Some varieties are known to be resistant, while others are susceptible, to particular diseases. In addition, the growth and arrangement of the stomata, the number and disposition of the root primordia in the root ring and the pattern of leaf and bud emergence, may be used for variety identification.

Examination of the structure of sugarcane stems has shown variations in the number of silica cells between varieties[12] and in the development and structure of these cells[7]. Anatomical studies have indicated that epidermal cells vary in size and cell wall thickness, and in the distribution of the stomata[9, 10, 11]. The ligule itself has been used as a basis for taxonomy, since environmental conditions have little influence on this feature[2, 8]. Further work on the classification of wild and cultivated sugarcanes has given new impetus to the use of the ligule and dewlap as organs for variety identification[3, 4, 5, 6]. Since the work of Artschwager[1], which relied on the varietal differences of the epidermis, little attention has been paid to this aspect. His studies, however, showed quite pronounced differences between varieties.

The research work reported here is based on that of Wieler[12] and Artschwager[1] and relies on the anatomical structure of the stem epidermis. This study was designed to investigate the possibility of using some anatomical characteristics for the accurate identification of cane varieties.

Laboratory Technique

Sufficient material for the study of the stem epidermis of 22 different varieties, including most of the released varieties in South Africa, was obtained from the collection plots at the South African Sugar Association Experiment Station. The selected cane was grown on the same fields, under similar conditions, and was all of the same age and stage of development. The following varieties were used:

Uba, P.O.J.2714, P.O.J.2725, P.O.J.2878, Co.301, Co.331, N:Co.292, N:Co.293, N:Co.310, N:Co.334, N:Co.339, N:Co.376, N:Co.382, N.50/211, N.51/168, N.51/539, N.52/219, N.53/216, N.55/805, N.10, C.B.36/14, C.B.38/22.

Cane samples were taken by severing the stick about 2 cm above the eighth clearly developed node, counting from the top. The stick was topped 20 cm above this point, and the rind of the sample sliced off to form four strips about 1.5 cm wide. The lower portion of each strip, representing the centre of the eighth internode, was then scored twice longitudinally and three times horizontally to yield nine rectangular sections, each about 1 cm long and $\frac{1}{2}$ cm wide. The strips with the scored section at the base were then dipped into a boiling solution of 20 g KCl or $KClO_3$ per litre of 65% nitric acid. In due course the epidermis came away, yielding nine separate pieces from each strip.

Another method, described by Artschwager[1], was used with a slight modification. Pieces of epidermis, with the adhering cortical and fibrous tissues, were cut from the central part of the eighth internode and placed in a test-tube containing the above solution and brought to the boil. After the epidermis had been obtained in the form of thin pellicle, the contents of the test-tube were emptied into a 100 ml beaker containing 80 ml water.

With both techniques the pieces of epidermis, separated from cortical and fibrous tissue, were retrieved using a fine, drawn-glass rod and transferred to 25% alcohol. After rinsing repeatedly in fresh diluted alcohol to remove all traces of nitric acid, the washed samples were mounted on microscope slides, stained with either chlor-zinc-iodide, Sudan III, Nile Blue and other stains, fitted with a cover slip and examined and photographed. On an average, about 15 to 20 samples of each variety were tested.

Initially, an attempt was made to find those characteristics which were sufficiently uniform that they could be used for identification. Exhaustive

studies showed there were variations in the composition of the epidermis of the different internodes, when long and short internodes were compared. Though complete uniformity at different localities in the stem was not obtained, those samples taken next to the bud region, or from the middle portion of the internode, indicated that reasonable uniformity could be obtained in the epidermis of long internodes, located 6 to 9 nodes below the top of the stick. To eliminate the errors which may result through slight variations in the composition of the epidermis of the same variety, the eighth internode was used almost entirely throughout this investigation.

This technique was used successfully for most of the varieties tested, staining being most effective when using chlor-zinc-iodide. The critical factor was found to be the duration of treatment in boiling nitric acid (HNO_3) containing potassium chlorate ($KClO_3$) or potassium chloride (KCl). If this is continued for too long, the cell layers which stain dark blue with chlor-zinc-iodide (a mixture of zinc chloride, potassium iodide and iodine) separate completely in the nitric acid solution. Removal of the samples at the right time and their subsequent washing and staining, are techniques which require experience. Specimens of any one variety obtained using this technique are similar.

Variations are, however, greater in some varieties than in others, and differences can sometimes be seen in the length and width of the cells in any one variety, and in the number and pattern of the cork and silica cells. The long cells, which constitute the greater part of the epidermis, form a prism and vary greatly in size, length and thickness of the cuticle wall. These are important factors in the comparison and identification of varieties, as has been shown by Artschwager[1]. It is evident that the variations between the cells of different varieties are much more marked than the variation within those of the same variety. These can be seen in the shape, length and width of the cells of the epidermis; the pattern of the cell walls; the number, pattern and structure of the walls of the cork cells; and the number and shape of the silica cells. In some varieties there are many stomata, in others they are rare or absent.

The use of Sudan III as a stain revealed certain differences which were not apparent when chlor-zinc-iodide was used. The cork cells became clearer, and in some varieties the middle lamella forms a characteristic wavy line, as is demonstrated in Plate I, 5–7. In other varieties this wavy line is very difficult to find.

Care must be taken when the stained samples are mounted on slides because of the morphological differences of the outer and inner wall of the epidermis. The outer wall of the long cells is thicker and more strongly developed than the inner wall. It is evident from the microscopic observations that the difference in the epidermis is due to the existence of different layers. The inner wall gives a cellulose reaction while the outer wall or epicuticula has excellent properties for absorbing certain dyes, particularly chlor-zinc-iodide. If the maceration process is not stopped at the right point, the stain will not be absorbed because the epicuticula becomes separated from the epidermis and dissolves in the nitric acid. Samples containing the epicuticula, which are stained with chlor-zinc-iodide, show the long cells clearly contrasted from the cork and silica cells. However, only the details on the external surface of the wall are clearly visible, while microscopic observations of the inner wall of the epidermis give more details of the internal structure of the cuticle, cork and silica cells. More detailed results of the structure and component parts can be obtained if the epicuticula is removed and the samples stained with Alcian Blue, or Safranin, or a double stain with Sudan III plus Brilliant Cresyl Blue.

The last-mentioned stains gave excellent results with the cork cells of the epidermis, which are the second group of cells to be used as a diagnostic criterion (Plate I, 1–8). The cork cells of sugarcane are short and vary in size and form. They occur singly, in pairs, or in groups of four or more, depending on the variety. With Sudan III, the cork cells stain dark brown, while the long cells remain transparent.

A third type of cell frequently found in association with the cork cells, are the silica cells. Depending on the variety or age of sugarcane and other as yet unknown factors, their numbers vary. Samples stained with chlor-zinc-iodide gave a better separation of the silica cells from the cork cells than the other stains (Plate II, 1–8). From this illustration and from the colour-prints in Plate III, it is evident that there is a difference in the number, shape and size of silica cells in different varieties, but a very uniform pattern within one variety.

Stomata are rare in the epidermis of the stem, but it was found that they were relatively high in number in some varieties, while in others they were almost absent. From the observations made it is difficult to say whether this feature may be used as a factor for diagnostic work or not, hence further studies will have to be carried out.

Varietal Characteristics

Eight of the 22 varieties examined are described here, and some of their characteristics are demonstrated in the colour illustration, Plate III, 1–8.

Co.331: The structure of the epidermis in variety Co.331 is reasonably uniform (Plate III, 1). The long cells are relatively short, occasionally slightly elongated, but regular in pattern. Extreme cell measurements of 56μ to 130μ × 8μ to 15.5μ, with an average of 108μ × 12μ were found in this variety. Each long cell is accompanied by a short cell, although occasionally the short cells appear in pairs. The short cells may be trapezoidal, rectangular, or square in shape, measuring 5μ to 8μ × 8μ to 15μ, with an average of 6μ × 14μ. Silica cells are similar in number to the long or the cork cells, but shaped rather like a dumb-bell. Stomata are found only in small numbers, and they occur irregularly. A comparison with samples of Co.331, using different magnifications and stains, is illustrated in

Plates I and II (1 to 6). It is evident from these illustrations that the pattern within either variety is uniform.

N:Co.293: The pattern of the epidermis in N:Co.293 is illustrated in Plate III, 2. The long cells are usually very irregular and vary in length and width. Extreme cell measurements of 49μ to 327μ × 4μ to 11.5μ were found in this variety. The short cork cells differ quite considerably from those of the variety Co.331 described above. Many of them are elongated or hair-like, others are pointed, some without, but most with, silica cells. The majority occur singly, and appear in many cases as double short cork-cells. Extreme measurements of 15μ to 45μ × 4μ to 14μ, with an average of 21.5μ × 10.5μ were obtained using 100 of the cork cells. The silica cells in this variety are uniform and elongated. Only a few stomata were observed during the investigation.

N:Co.310: The epidermal structure in this variety is reasonably uniform as can be seen in Plate II, 3 and 8, and Plate III, 3. In both these illustrations it is evident that the long cells have a certain variation in length, but are regular in width. In general, the long cells are shorter than those of variety Co.331, with measurements in extreme cases of 52μ to 105μ × 7.5μ to 12.5μ, with an average of 86μ × 11.5μ. Occasionally, it was found that the long cells are wider in the centre than at the ends. The cork or short cells generally occur singly or in pairs, and are variously shaped. Many of them are rectangular, elongated, lunate to reniform, trapezoidal or irregular. Extreme measurements of 6μ to 38μ × 7μ to 14μ, with an average of 16.5μ × 10.5μ were taken from more than 100 cork cells. About 60 to 70 per cent of the cork cells are accompanied by silica cells. Frequently, two rows of long cells end in one. The silica cells are usually rectangular and elongated but may also appear constricted in the middle with swollen ends. Stomata were occasionally found, the counts being higher than in the other varieties described here.

N:Co.339: The epidermis of the variety N:Co.339 appears very regular and its pattern is characteristic (Plate III, 4). The long cells are uniform and appear comparatively long, being double or treble the size of those in Co.331 and N:Co.310. The large long cells show thick, often silicified walls and very often the cells fuse. Extreme measurements of 89μ to 285μ × 8μ to 15μ, with an average of 246μ × 11.5μ, were found in this variety. Many rows of cells terminate abruptly. The short or cork cells appear in various forms: spherical, trapezoidal, rectangular, triangular or hair-like. They are distinct in paired groups of up to four or more in number and are surrounded by a thick wall (Plate II, 4). They measure 7μ to 13μ × 10.5μ to 25μ, with an average of 13μ × 26μ. Stomata are rare, their numbers varying from sample to sample. The only variety similar to N:Co.339 is N.Co.334 (Plate I, 3 and 8, and Plate II, 5). There are, however, some differences in the length of the long cells as well as in the pattern of the cork cells and in the number of silica cells.

N:Co.376: The structure of the epidermis of the variety N:Co.376 is uniform and the long cells are of regular shape (Plates I (2 and 7) and II (2 and 7), and Plate III (5)). The long cells are elongated, rectangular in shape, or occasionally pointed at the end. Cell sizes vary from 62μ to 240μ × 8μ to 14μ, with an average of 217μ × 11μ. Variation in cell length is a common feature of this variety. The cork cells may appear in various shapes: square, rectangular, trapezoidal, elongated or irregular. They measure from 8μ to 15μ × 12μ to 31μ with an average of 10.5μ to 19μ. The short or cork cells are usually uniform in pattern but they are not always accompanied by a silica cell. They usually appear singly or in pairs and one or two fat globules may be observed in the centre of each cell. The number of stomata vary from none to a few.

N.50/211: The structure of the epidermis of N.50/211 is characterised by the relatively irregular size of the long cells, which are rectangular in shape with square ends. The cell form is to some extent irregular and the width of one cell may vary (Plate III, 6). Measurements of 41μ to 97μ × 8μ to 14.5μ, with an average of 82μ × 9.5μ, were found to be usual for this variety. Cork cells are found singly and in pairs, with extreme dimensions of 6μ to 12.5μ × 8μ to 15μ and with an average of 7.5μ × 13μ. Silica cells are quite abundant and nearly every cork cell is accompanied by a silica cell. The form of the silica cells is similar to those of N:Co.310, but tends to be more elongated than the cork cells. The cell wall of the long cells is very often silicified and thick. Stomata are extremely rare in this variety.

N.55/805: The pattern of the epidermis of this variety is very similar to that of N.50/211 (Plate III, 7). The long cells are characterised by their irregular length and width, while the cell wall is thick and heavily silicified. The zig-zag line of the epidermis wall is clear and characteristic. The cork cells are occasionally absent from between two long cells, the latter having maximum dimensions of 39μ to 86.5μ × 8.5μ to 14μ, and an average of 70μ × 10μ. The cork cells are very regular in shape and size, and are either rectangular or square, their diameter being 8.5μ to 14μ × 8.5μ to 15.5μ, with an average of 9μ × 10.5μ. The silica cells vary from rectangular to oval in shape and do not accompany every cork cell. They are smaller in diameter than the cork cells, and are smaller than the silica cells of other varieties. Stomata were found in relatively higher numbers than in other varieties.

C.B.36/14: The epidermis of this variety has a very regular pattern, characterised by relatively long cells which are straight and of constant width (Plate III, 8). Maximum cell measurements are 160μ to 195μ × 8μ to 12μ, the average being 187μ × 10.5μ. The cork cells are irregular, square, trapezoidal, rectangular or pointed in shape, and appear singly or in pairs. The size of the cork cells is 8μ to 18μ × 8μ to 10μ, the average being 12.5μ × 9.5μ. The silica cells, which are usually associated with the cork cells, are elongated and of

dumb-bell shape. Some were observed without an associated cork cell. Stomata were found to be extremely rare in this variety.

Conclusion

The diagnostic value of epidermal structures, has been investigated on 19 released and three restricted varieties of potential commercial interest. From the descriptions provided it can be seen that the pattern of the epidermis of sugarcane varies a great deal between varieties but can be reasonably uniform within any one variety. To establish such uniformity the determinative sample was taken from the centre of the eighth internode, provided that this internode was longer than three inches and that all the varieties had been grown under similar environmental conditions. Further studies will be necessary to prove whether this epidermal pattern remains stable under different environmental conditions, but there is every reason to think that some microscopic characteristics of the outerwall of the epidermis can be used to identify sugarcane varieties.

It will be obvious to the reader who has studied the description of the eight varieties dealt with in this paper that, apart from differences in epidermal patterns, there are noticeable variations in the components making up the pattern. These have been described in detail and are themselves of diagnostic value. They include the length and width of the long cells, the occurrences, size and shape of the short or cork cells, and the number and size of the silica cells. Further research will be necessary to determine whether the number of stomata may be used as a criterion for variety identification.

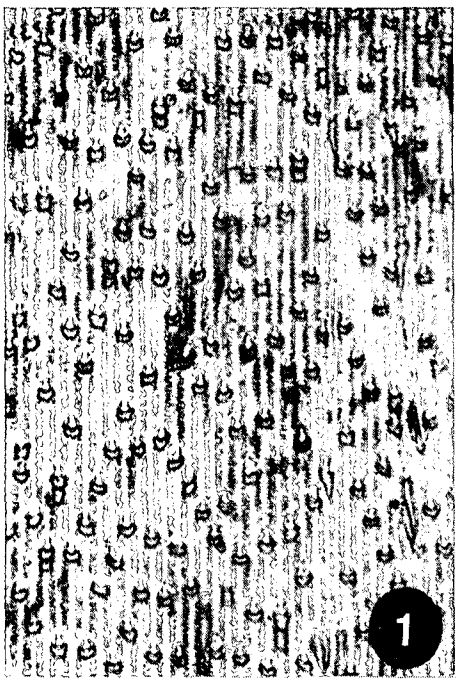
Comparison of the illustrations in Plates II and III, show that varieties N:Co.293, N:Co.339 and N:Co.334 are similar in character, as are the varieties N.50/211 with N.55/805. All the other varieties examined are obviously different. Further studies will have to be carried out under different environmental conditions to determine the value of comparative microscopic morphology of the epidermis of sugarcane as an aid to variety identification.

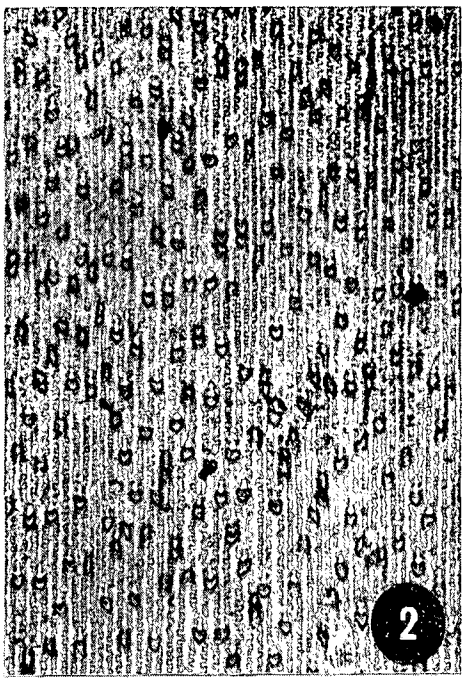
Summary

The comparative microscopic morphology of the epidermal tissue of eight varieties of sugarcane used in South Africa is described. The stem epidermis of sugarcane differs in its structural pattern, such differences being correlated with the varieties themselves. Variations were found in the length and width of the long cells, cork cells, and silica cells. Preparation of samples and the staining methods employed are described in detail.

References

1. Artschwager, E. (1930). A comparative study of the stem epidermis of certain sugarcane varieties. J. agric. Res. 41: 853-865.
2. ——— (1940). Morphology of the vegetative organs of sugarcane. J. agric. Res. 60: 503-549.
3. ——— (1942). A comparative analysis of the vegetative characteristics of some variants of *Saccharum spontaneum*. U.S. Dep. agric. tech. Bull. 811: 55p.
4. ——— (1948). Vegetative characteristics of some wild forms of *Saccharum* and related grasses. U.S. Dep. agric. tech. Bull 951: 69p.
5. ——— (1951). The role of the ligule in sugarcane taxonomy. Am. J. Bot. 38: 144-146.
6. ——— (1951). Structure and taxonomic value of the dewlap in sugarcane. U.S. Dep. agric. tech. Bull. 1038: 12p.
7. Frohnmeyer, M. (1914). Die Entstehung und Ausbildung der Kieselzellen bei den Gramineen. Biblthca. bot. 86: 39p.
8. Jeswiet, J. (1916). Beschrijvingdersoorten van het Suikerriet. Eerste Bijdrage. Morphologie van het suiderriet. Arch. v. Suikerindus. Nederland. Indie Meded. 24: 67-137.
9. Mamili-Calvino, Eva (1921). Studi anatomici e fisiologici sulla canna da zucchero. (Le Staz. Sperim. Agr. Italiane.) 54: 345-395.
10. ——— (1922). Estudios anatomicos y fisiologicos sobre la canna da azucar en Cuba. Mem. Soc. Cubana Hist. Nat. "Felipe Poey" 4: 156-211.
11. ——— (1925). Physiological anatomy of leaves of the sugarcane. (*Saccharum officinarum* L.). The Ref. Book Sug. Ind. of the World, 60-65.
12. Wieler, A. (1898). Beiträge zur Anatomie des Stockes von *Saccharum*. Beitr. wiss. Bot. B. II: 141-164.





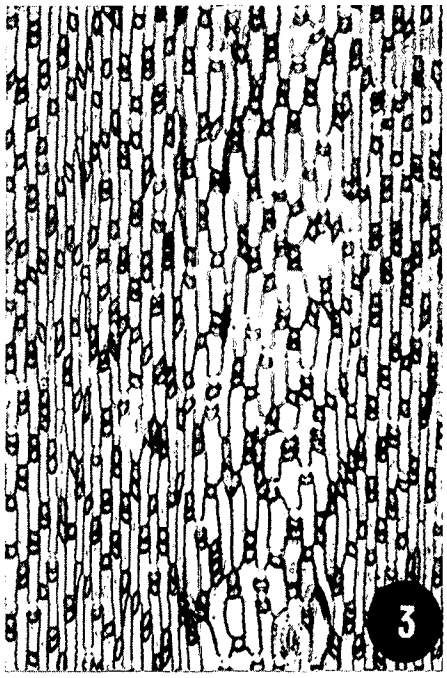


PLATE 1: Epidermal issues of cane varieties stained with Sudan III.

(1) Co 331. Note the clear zig-zag lines of the cells and the form of the cork cells.

(2) N:Co. 376.

(3) N:Co. 334.

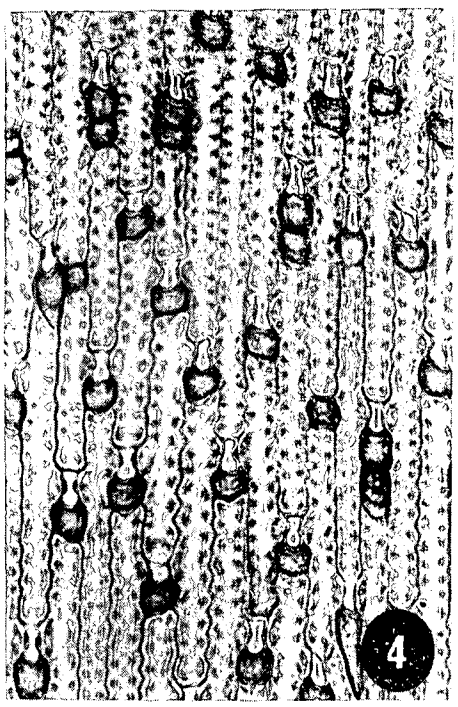
(4) N.10. Inner wall of the epidermis—highly magnified.

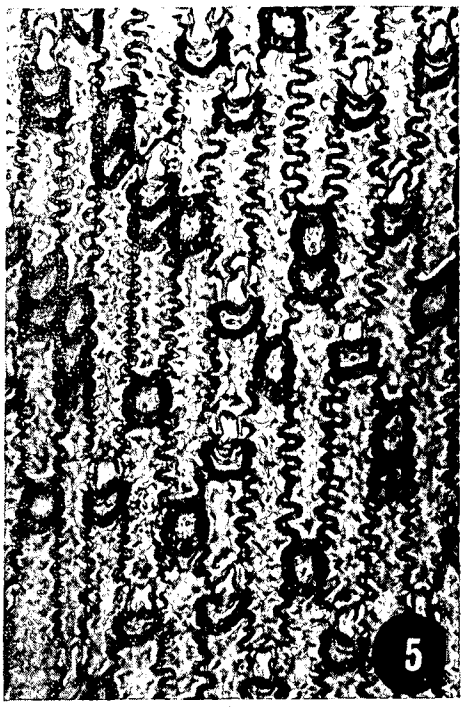
(5) N:Co. 382. Outer wall of epidermis—highly magnified.

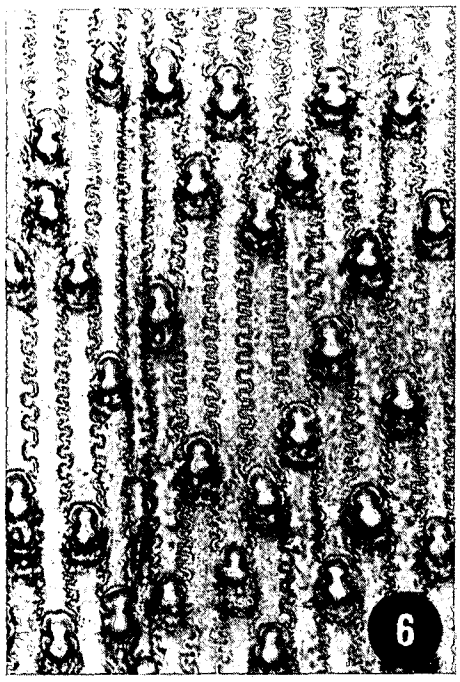
(6) Co. 331. As for Fig. 1, but highly magnified.

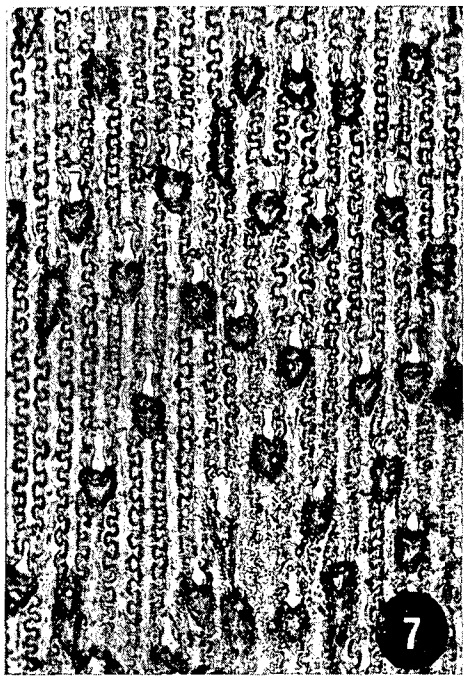
(7) N:Co. 376. Highly magnified.

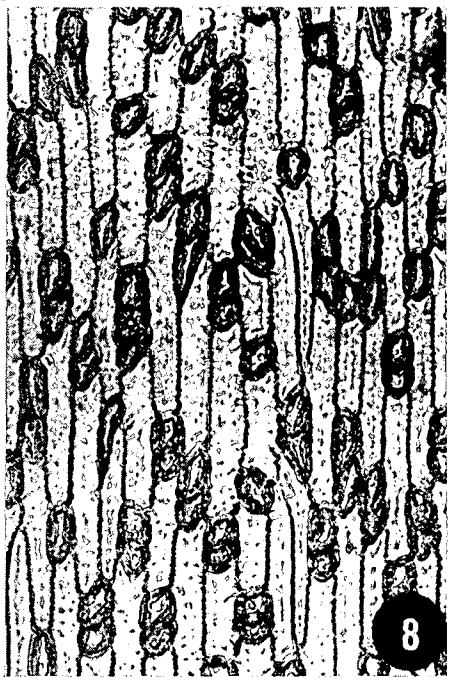
(8) N:Co. 334. Inner wall of the epidermis —highly magnified.

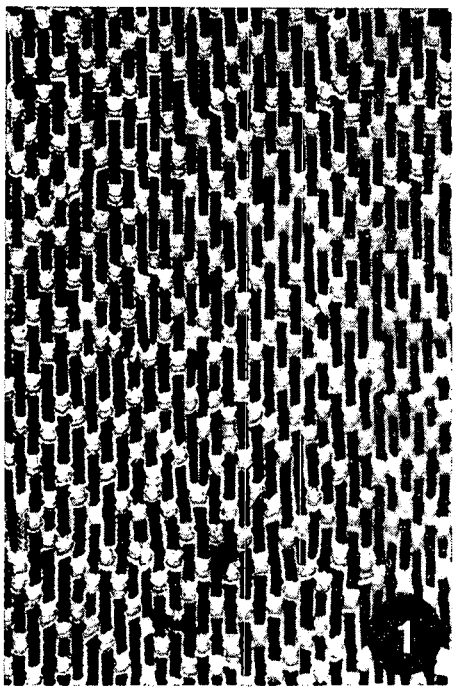


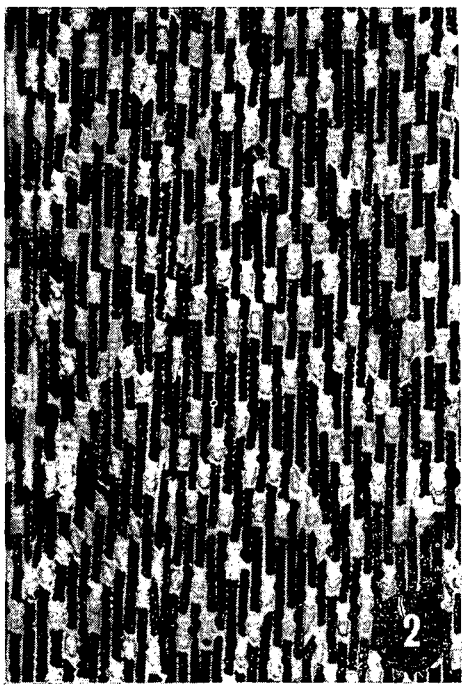


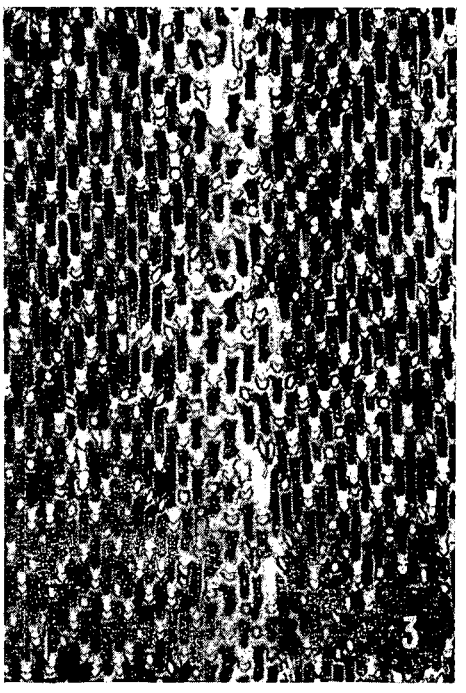


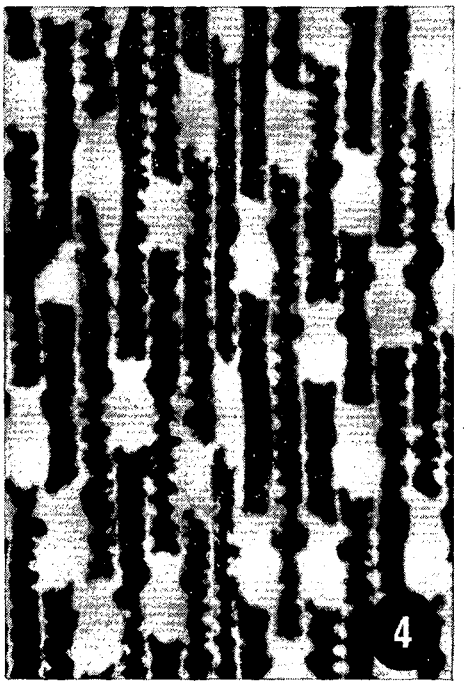












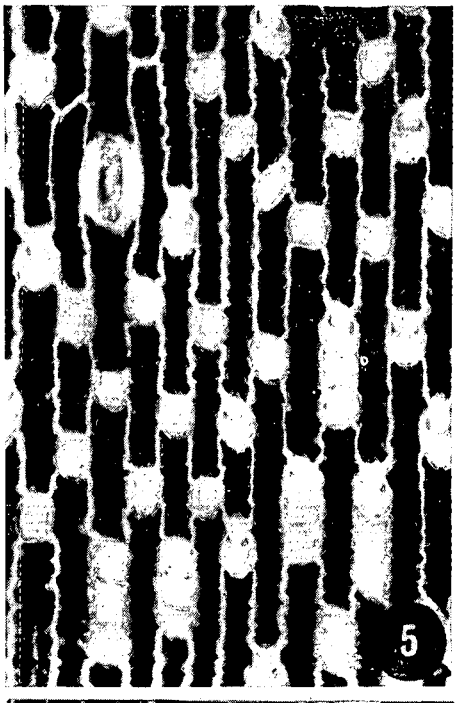


PLATE 2: Epidermal tissues of cane varieties stained with chlor-zinc iodide.

(1) Co. 331—low power magnification.

(2) N:Co. 376—low power magnification.

(3) N:Co. 310—low power magnification.

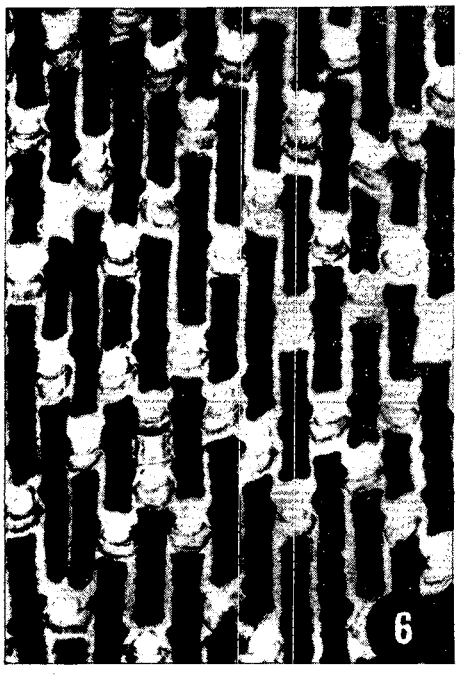
(4) N:Co. 339—high power magnification.

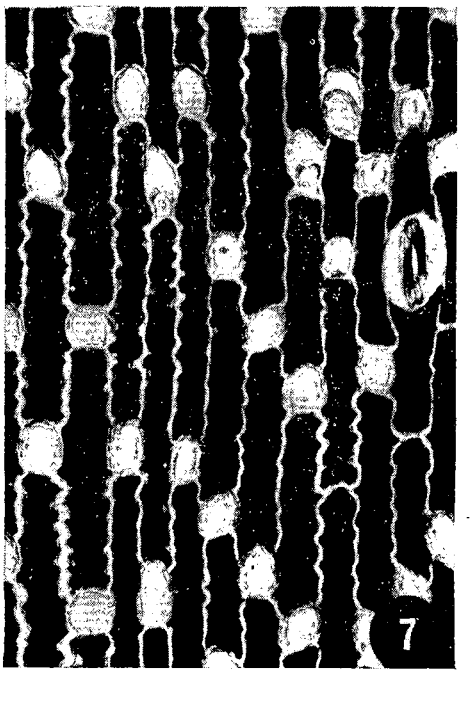
(5) N:Co. 334—high power magnification.

(6) Co. 331—high power magnification.

(7) N:Co. 376—high power magnification.

(8) N:Co. 310—high power magnification.





Discussion

Mr. Wilson (in the chair): **The zig-zag structure** of the long cell indicates a contraction of cell wall and unless an estimate is made of the degree of contraction an entirely wrong idea of the cell length might be obtained.

Dr. Roth: The zig-zag structure is the actual cell wall pattern on the outer layer of the epidermis. The more silica there is in the cell, the greater will be the contrast.

Mr. Rault: It is important from a milling point of view that the different fibre structures in cane should be studied.

Mr. Turck: Is it possible for the ripeness of cane to be determined by the amount of starch in the cells?

Dr. Roth: A stick of cane has approximately the same amount of starch in the top node as in the bottom node. There is a big difference however in starch in samples taken from corresponding nodes of different varieties grown under the same conditions.

Mr. du Toit: Starch content certainly differs in different varieties. It would be too cumbersome to use starch content as an indication of drying off.

When cane is cut starch disappears rapidly due to cane deterioration.

I think this method of Dr. Roth's of cane identification will be very useful, particularly when used in conjunction with other methods.

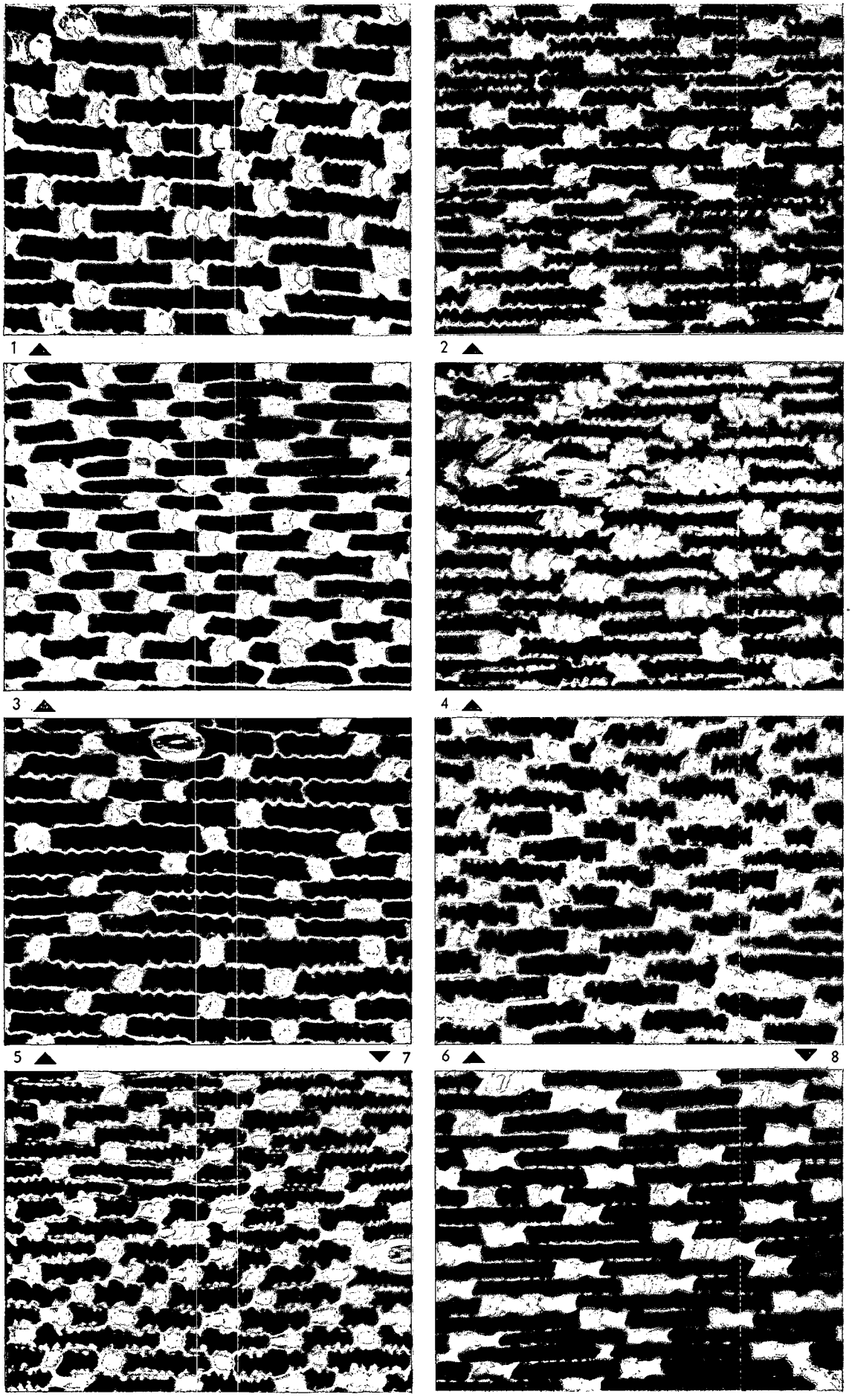


Plate III
Epidermal tissue of 8 varieties of sugarcane stained with chlor-zinc-iodide. (x750).
1. Co.331
2. N:Co.293
3. N:Co.310
4. N:Co.339
5. N:Co.376
6. N.50/211
7. N.55/805
8. C.B.36/14