LOWER EPIDERMIS OF LEAF MIDRIB AS AN INDICATOR OF ITS HARDNESS IN SUGARCANE

By K. L. Khanna and S. L. Sharma
(Central Sugarcane Research Station, Pusa)

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I. INTRODUCTION

Issac (1939) while working on the insect-pests of sugarcane, reported that the hardness of leaf midrib was a major factor in imparting resistance to the top-borer, *Scirpophaga nivella* Fabr., and that this character could be used in breeding and selection of new varieties. Thuljaram Rao and Venkataraman (1941) confirmed these observations and reported its inheritance in sugarcane hybrids.

In view of the importance thus given to the suggestion, and the likely bearing and far-reaching effect of these findings on the future breeding programme and servicing of sugar industry in India, work was simultaneously undertaken at this station as well to critically examine the hypothesis in detail, first by evaluating the hardness of leaf midrib in terms of both its physical manifestation and anatomical structure (Khanna, 1940, 1941) and then by correlating the index of hardness with the infestation data.

Thuljaram Rao (1947) gives a method for the estimation of midrib hardness which is slow and laborious as all methods involving camera lucida drawings are, and which as such cannot be utilised to collect a large number of observations, so essential in the first place, for determining the hardness of midrib of a particular variety by subjecting the data to statistical examination, and in the second place, for the purpose in view, namely, the quick selection of seedlings, got out of a certain cross and the speedy evaluation of the role played by parents in imparting the hardness of midrib to their progeny. Because of the highly heterozygous nature of genetical constitution of genus Saccharum as a whole, the study of a large number of seedlings resulting from a cross with a view to find out the probability of their inheriting a desired character, cannot be overemphasised.

The present contribution deals with epidermis in relation to the hardness of leaf midrib as determined by a physical method.
II. Material and Methods

Thirteen varieties, namely, Co 210, Co 213, Co 285, Co 299, Co 313, Co 331, Co 421, Co 513, P.O.J. 2725 Saraitha and Chin (Saraitha group), Sewari (Nargori group) and Hemja (Mungo group) representing a very wide range of hardness of leaf midrib were selected for this study. Three one-inch pieces cut from the midrib of standard leaves of stalks of the same age, one from each at a distance of 3" from the transverse mark, formed a sample for a variety. Half of each piece after removing the upper epidermis was macerated in 10% nitric acid. The lower epidermis was mounted in Canada Balsam after the usual process of washing, staining with Safranin and dehydration. Hand sections taken from the other half were also stained with Safranin and made into permanent mounts. Only two characters of the epidermis, namely the number of Silica cells per unit area and the thickness of outer wall of long cells, which were likely to impart hardness to the midrib were considered. Other components of the epidermis, such as the stomata and cork cells which were soft, and therefore, not likely to offer any resistance to the entry of a top borer larva were left out of consideration.

Silica cells were counted in 10 microscope-fields of the epidermis of each midrib, the diameter and area of the field being 470 μ, and 0.17 sq. mm. respectively. Thirty measurements of the thickness of the outer wall of the long cells in the neighbourhood of the central vascular bundle were taken from transverse sections, ten from each midrib.

The hardness of a midrib was expressed in terms of the weight required to puncture it on its convex side at 3" from the transverse mark with a fine but blunt needle (0.6 mm. in diameter at its piercing end). The needle was fixed to a rind hardness testing apparatus designed by Puri and Venkatraman (1929) and improved upon (Plate XXVIII) by one of the authors (Khanna, 1935). Each time a 50 gm. weight was added till the midrib was punctured. To the weight actually put on the circular plate, the weight of the latter along with that of the plunger which came to 510 gm. was added. Twenty such determinations were made for each variety.

Also anatomical structure as detailed below for four varieties, Co 285, P.O.J. 2725, Chin and Saraitha (Saraitha group) together with that of Co 213 and Co 513 was studied to understand the erratic behaviour of the former group discovered when data on the originally selected three characters were statistically analysed:—

1. (a) The number and size of vascular bundles.
   (b) The size of sclerenchymatous cushions on which medium-sized vascular bundles were situated.
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2. Thickness of sclerenchymatous cell walls.
3. Area of the midrib in transverse section.

The size of a vascular bundle was found out by measuring its radial and tangential axes and multiplying the two (Khanna and Sharma, 1947) and from each midrib five of them were measured to determine its average size for a variety. Similarly the product of the base and height gave the size of sclerenchymatous cushions which were more or less columnar in shape. In this case also, five of them were measured from each midrib. Ten measurements of thickness of two adjoining sclerenchymatous cell walls were taken from each midrib at the phloem end of the vascular sheaths situated in the middle portion of the convex surface. The area of midribs was determined from camera lucida drawings (linear magnification, 25 ×) with the help of a planimeter.

III. EXPERIMENTAL RESULTS

The detailed examination of the data (Table I) showed that Sewari (Nargori group) which had almost the maximum number of Silica cells per unit area (Plate XXIX, Fig. 1) was characterised by soft midrib. On the other hand, P.O.J. 2725 and Co 299 which were not significantly different from the

<table>
<thead>
<tr>
<th>Variety</th>
<th>No. of Silica cells per unit area</th>
<th>Thickness of outer wall</th>
<th>Puncture-weights (gm.)</th>
<th>Percentage of the average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>(1)</td>
</tr>
<tr>
<td>Co 210</td>
<td>18.1</td>
<td>19.3</td>
<td>885</td>
<td>78</td>
</tr>
<tr>
<td>Co 213</td>
<td>17.4</td>
<td>20.6</td>
<td>1010</td>
<td>75</td>
</tr>
<tr>
<td>Co 285</td>
<td>24.0</td>
<td>20.0</td>
<td>1273</td>
<td>104</td>
</tr>
<tr>
<td>Co 299</td>
<td>36.4</td>
<td>22.5</td>
<td>1035</td>
<td>171</td>
</tr>
<tr>
<td>Co 313</td>
<td>24.0</td>
<td>20.2</td>
<td>1038</td>
<td>104</td>
</tr>
<tr>
<td>Co 331</td>
<td>14.5</td>
<td>23.1</td>
<td>1110</td>
<td>83</td>
</tr>
<tr>
<td>Co 421</td>
<td>20.3</td>
<td>29.5</td>
<td>1085</td>
<td>88</td>
</tr>
<tr>
<td>Co 513</td>
<td>13.7</td>
<td>19.5</td>
<td>998</td>
<td>59</td>
</tr>
<tr>
<td>Saretha</td>
<td>18.8</td>
<td>18.2</td>
<td>773</td>
<td>81</td>
</tr>
<tr>
<td>Chin</td>
<td>13.3</td>
<td>16.5</td>
<td>598</td>
<td>58</td>
</tr>
<tr>
<td>Hemia</td>
<td>16.0</td>
<td>18.1</td>
<td>810</td>
<td>69</td>
</tr>
<tr>
<td>Sewari</td>
<td>39.9</td>
<td>14.5</td>
<td>723</td>
<td>173</td>
</tr>
<tr>
<td>P.O.J. 2725</td>
<td>41.4</td>
<td>16.4</td>
<td>1065</td>
<td>179</td>
</tr>
<tr>
<td>G.M.</td>
<td>28.15</td>
<td>19.57</td>
<td>955.2</td>
<td>..</td>
</tr>
<tr>
<td>S.E.</td>
<td>1.29</td>
<td>0.49</td>
<td>39.49</td>
<td>5.57</td>
</tr>
<tr>
<td>C.D. at 5%</td>
<td>3.87</td>
<td>1.35</td>
<td>94.60</td>
<td>..</td>
</tr>
</tbody>
</table>

Note.—The thickness of cell walls is given in divisions of eye-piece micrometer each of which is equal to 0.22μ.
abovementioned variety in this respect (Plate XXIX, Figs. 2 and 3) had fairly hard midribs as shown by puncture-weights. Other varieties having hard midribs such as Co 331 and Co 421 (Plate XXIX, Figs. 4 and 5) did not have per unit area, even half their number found in Sewari. Co 210 (Plate XXIX, Fig. 6) which was found to possess a midrib softer than that of Co 513 (Plate XXIX, Fig. 7), had significantly greater number of these cells than the latter variety. Similarly in Co 213 and Co 313 (Plate XXIX, Figs. 8 and 9) in which the midrib was softer than in Co 331, Silica cells occurred in significantly greater number per unit area than in the latter variety.

There was thus a very poor correlation between the number of Silica cells and the hardness of midrib as shown by puncture-weights (Table II, Text-Fig. 1).

![Text-Fig. 1](image)

**Table II**

*Showing coefficients of correlation between the three characters*

<table>
<thead>
<tr>
<th>Character</th>
<th>B</th>
<th>C</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-0.3285</td>
<td>+0.1388</td>
<td>Not significant</td>
</tr>
<tr>
<td>B</td>
<td>..</td>
<td>+0.6377</td>
<td>Significant at 5% level</td>
</tr>
</tbody>
</table>

where A = No. of Silica cells.

'' B = Thickness of cell walls.

'' C = Puncture-weights.
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Thuljaram Rao (1947) also came to a similar conclusion on the basis of his study of eight varieties. But the number of Silica cells found to occur per sq. mm. did not exceed 14 in any of the varieties selected by him for these investigations; also it was not stated how many observations he had taken for this feature. The authors on the other hand, only once out of the 390 observations recorded, came across a microscope-field (0·17 sq. mm. in area) having only 3 Silica cells in Hemja, or 18 per sq. mm. even for this field. The maximum number of these cells in the same field was found to be 75 in Co 299 or nearly 450 per sq. mm. The microphotographs of peelings of epidermis (Plate XXIX) cover an area of 0·68 sq. mm. approximately.

As regards the thickness of outer cell-wall of long cells, these varieties were significantly different from one another and formed more or less compact groups. Co 421, Co 331 and Co 299 had significantly thicker cell walls than the rest. Co 213, Co 313, Co 285 and Co 513 formed an intermediate group. Of the remaining six varieties, Co 210, Saraitha and Hemja formed one sub-group and Chin, P.O.J. 2725 and Sewari the other.

Thus roughly they could be grouped as follows:

(1) With hard midribs: Co 421, Co 331 and Co 299.
(3) With soft midribs: (a) Co 210, Saraitha and Hemja.
(b) Chin, P.O.J. 2725 and Sewari.

On the basis of puncture-weights, the arrangement of these varieties was slightly different as given below:

(i) With hard midribs: Co 285, Co 331, Co 421, P.O.J. 2725, Co 313 and Co 299.
(iii) With soft midribs: Hemja, Saraitha, Sewari and Chin.

When puncture-weights and thickness of cell walls of different varieties expressed as percentages of their respective general means (Table I), were taken into consideration it was found that in nine out of thirteen varieties, there was such a close correspondence between these two characters, that the disparity between their percentages did not exceed 7 (Text-Fig. 1). In Saraitha and Chin, the hardness of midrib was found to be 12 and 21 units less than that warranted by the thickness of their cell walls, while in the case of P.O.J. 2725 and Co 285 it was respectively 29 and 31 units harder than that arrived at, on the basis of the latter character. In spite of the erratic behaviour, on the part of these varieties which were found to be abnormal in one way or the other, when their anatomical structure was studied in this
connection, the correlation coefficient between the thickness of cell walls and the puncture-weights was found to be +0.6377 which was significant at 5% level, and the former might therefore be taken as a fairly good index of the latter.

Co 285 and P.O.J. 2725 as already stated had a midrib much harder than what it ought to be on the basis of the thickness of cell walls whereas the reverse was the case with Chin and Saraitha. To understand this apparent departure from the general behaviour, their internal structure was studied in detail and compared with that of Co 213 and Co 513 which were selected not only because the disparity between the percentage values for these characters was less than 4, but also because they were all within 6% of the general mean for thirteen varieties (Table I). Co 331 and Sewari in which the magnitude of puncture-weights was almost equal to that of the thickness of cell wall, also could have been used for comparison. But they were far removed from the general average.

**TABLE III**

*Showing the size and anatomical structure of midribs of six varieties*

<table>
<thead>
<tr>
<th>Variety</th>
<th>% of the average</th>
<th>Area of midrib sq. cm.</th>
<th>Vascular bundles</th>
<th>Size of sclerenchymatous cushions</th>
<th>Thickness of sclerenchymatous cell walls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Puncture-weights</td>
<td>Thickness of cell walls</td>
<td>No.</td>
<td>Size</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>------------------</td>
<td>------------------------</td>
<td>-----</td>
<td>------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Co 213</td>
<td>106</td>
<td>105</td>
<td>80-5</td>
<td>14-0</td>
<td>3441</td>
</tr>
<tr>
<td>Co 513</td>
<td>104</td>
<td>100</td>
<td>82-0</td>
<td>15-0</td>
<td>3198</td>
</tr>
<tr>
<td>Co 285</td>
<td>133</td>
<td>102</td>
<td>55-0</td>
<td>15-3</td>
<td>2539</td>
</tr>
<tr>
<td>P.O.J. 2725</td>
<td>113</td>
<td>84</td>
<td>68-5</td>
<td>10-3</td>
<td>3747</td>
</tr>
<tr>
<td>Saraitha</td>
<td>81</td>
<td>93</td>
<td>55-5</td>
<td>11-0</td>
<td>2820</td>
</tr>
<tr>
<td>Chin</td>
<td>63</td>
<td>84</td>
<td>38-5</td>
<td>10-0</td>
<td>2212</td>
</tr>
</tbody>
</table>

*Note.*—1. The area given in Column 4 is for a camera lucida drawing of a midrib, having a linear magnification of 25.

2. *(a)* Size of characters in Cols. 6 and 7 is given in sq. divisions of eye-piece micrometer each of which is equal to 4.5 sq. \( \mu \), and *(b)* for the thickness in Col. 8. 1 division = 0.22 \( \mu \).

From Table III, it would appear that almost identical puncture-weights for Co 213 and Co 513 was due to the close resemblance of their anatomical structure. The larger size of vascular bundles in Co 213 was balanced by their smaller numbers, and poorer lignification of sclerenchymatous cell walls to give the same hardness to the midrib as that of Co 513 in which the vascular bundles were smaller in size but greater in number and having more
highly lignified sheaths. The sclerenchymatous cushions were more or less equal in size in both the varieties. It would thus appear that for more or less the same size of the midrib, the strengthening tissue in both the varieties was almost equal in quantity.

Now turning to defaulting varieties, one would find that in Chin, the midrib was smallest in area having at the same time the least amount of supporting tissue which was more or less equally lignified as that in Co 213. The number and size of both the vascular bundles and sclerenchymatous cushions were reduced by nearly 30% each, as a result of which one need not be surprised, if the puncture-weight was 21 units less than its expected value on the basis of the thickness of epidermal cell wall. Disparity between these two characters in Saraitha was just 12 and was easily explained when its anatomical character was compared with that of Co 513 in which the lignification of tissues was more or less of the same order as that in the variety mentioned above. The strengthening frame in Saraitha was much stronger than that of Chin. Hence although the percentage values for the thickness of cell walls in these varieties differed by only 9, those for puncture-weights had a difference of 18 points.

In the case of Co 285, there was reduction only in the size of vascular bundles and in the lignification of tissues when compared with Co 513. When judged with Co 213 as the standard, the decrease in the size of vascular bundles was enhanced whereas that in the thickness was greatly narrowed down. Slight increase in the size of sclerenchymatous cushions and in the number of vascular bundles was introduced to balance the resultant weakness in the frame, which it would thus appear, was more or less of equal strength in all the three varieties, viz., Co 213, Co 285 and Co 513 as indicated by the thickness of the epidermal cell walls. But this frame in the case of Co 285 supported a midrib which was nearly \( \frac{2}{3} \) the size of those of Co 213 and Co 513, and hence the puncture-weight for this variety went up by 31 units. P.O.J. 2725 had unusually large vascular bundles and sclerenchymatous cushions which were almost 50% more in size than those of Co 285. The thickness of sclerenchymatous cell walls being more or less the same in both the varieties, the effect of increase in size was offset to a very great extent by reduction in their number and increase in the area of midrib. Consequently the puncture-weight for P.O.J. 2725 was less than that of Co 285. It is interesting to note that difference between the percentages of thickness of cell wall in these two varieties was 18 as against 20 for the puncture-weights.

A fuller contribution on the method of estimation of hardness of midrib by the study of anatomical structure will follow in due course.
IV. SUMMARY

1. (a) Number of Silica cells per unit area in the lower epidermis of leaf midribs of thirteen varieties of sugarcane, covering a wide range of hardness of leaf midribs was noted. P.O.J. 2725, Sewari (Nargori group) and Co 299 had on the average, 39.4 to 41.4 of them in a circular field which was 470 µ in diameter, whereas in Co 331, Co 513 and Chin (Saraitha group) the number was as low as 13.3 to 14.5 in the same area.

(b) Silica cells were found to have no consistent association either with the weight required to puncture the midrib on its convex side or with the thickness of the outer cell walls, both the correlation coefficients being too low to be significant even at 10% level.

2. (a) The range of the thickness of outer walls of long cells in the epidermis as expressed in the divisions of the eye-piece micrometer was from 14.5 in Sewari (Nargori group) to 23.5 in Co 421 (1 division = 0.22 µ).

(b) The varietal differences were significant at 5% level.

3. (a) Weights required to puncture a midrib varied from 598 gm. in Chin to 1273 gm. in Co 285 and these for the varieties studied were found to be significantly different at 5% level.

(b) The correlation coefficient between this character and the thickness of cell walls was found to be +0.6377 which was significant at the same level.

(c) The thickness of outer wall of long cells of lower epidermis could, therefore, be used as a fairly reliable indicator of the puncture-weight and, therefore, of the hardness of a leaf midrib.

4. The erratic behaviour of four varieties, namely Co 285, P.O.J. 2725, Chin and Saraitha (Saraitha group) in which the puncture-weights were found to be higher or lower than those warranted by the thickness of cell walls was explained by the size and number of vascular bundles and the sclerenchymatous cushions together with the thickness of sclerenchymatous cell walls when these characters were considered in relation to the size of a midrib in cross-section. All the four varieties were found to be abnormal for one character or the other.

V. ACKNOWLEDGEMENT

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VI. REFERENCES


4. ————. Ibid., 1941, 18-19.


EXPLANATION OF PLATES

PLATE XXVIII

A rind hardness testing apparatus with midrib placed in position for the determination of puncture-weight. The midrib is kept on a block of hard paraffin so as to ensure that the piercing point is the same for all the midribs. If it strikes a hard surface, it will be altered in respect of its diameter and sharpness of the piercing surface, and puncture-weights will not be comparable.

PLATE XXIX

Peelings of lower epidermis of leaf midribs of sugarcane. Fig. 1, Sewari; Fig. 2, P.O.J. 2725; Fig. 3, Co 299; Fig. 4, Co 331; Fig. 5, Co 421; Fig. 6, Co 210; Fig. 7, Co 513; Fig. 8, Co 213; Fig. 9, Co 313; Figs. 1, 2 and 3 show a much greater density of Silica cells than Figs. 4, 5, 6, 7 and 8. Fig. 9 occupies an intermediate position in this respect. (Magnification × 90).