Study of anatomical parameters of leaf and brix content of some promising commercial Indian varieties of sugarcane

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ABSTRACT
The structural and quantitative diversity of Kranz anatomy of nineteen Indian varieties of sugarcane was studied in relation to brix content or yield of sugar. The nature and distribution of Kranz tissue and types of vascular bundle in the leaf blade have also been described and discussed. In hand made transverse sections of leaf blade of sugarcane varieties, two different sizes (large and small) of vascular bundles were found. The large vascular bundles were characterized by the presence of metaxylem vessels on the either side of protoxylem. The small vascular bundles entirely consist of metaxylem but lack of protoxylem. Variation of Brix content of the basal, middle and top part of Culm of nineteen varieties was also observed. Statistically, average Brix content was positively correlated with the total area of the photosynthetic cells as well as bundle sheath cells and the linear regression curve followed the straight line.

Key words: Brix content, Kranz anatomy, Sugarcane.

INTRODUCTION
Sugarcane is a large perennial grass belonging to the genus Saccharum L. which is an important component of the grass family Poaceae and the tribe Andropogoneae. Sugarcane is an important commercial crop worldwide (Misbah et al. 2017; Kumawat et al. 2016; Hasija et al. 2003), producing 80% of the world’s raw sugar and is increasingly used as bio-fuel. Physiologically, sugarcane is a C₄ plant (Gulati et al. 2015) and the variation of leaf anatomy of such grass has been the subject of many studies on sugar transport and accumulation (McDavid and Midmore, 1980; Hartt et al. 1963). Some anatrophic studies have also been made on the photosynthetic tissue (Laetsch, 1974) and the phloem of sugarcane leaf blade (Singh, 1980). The most important well established relationship between the C₄ grass leaf anatomy and its principal pathway of photosynthesis is the 'Kranz' anatomy (Bourdu, 1976; Black et al. 1973).

The main objective of the present study was to determine the cellular composition of different sized vascular bundles and surrounding tissues of the leaf blade of nineteen promising commercial varieties of Indian sugarcane under study. The nature and distribution of Kranz tissue in the leaf blade and midrib have also been described and discussed in relation to brix content (% sugar by mass). A secondary objective was to develop a method of describing anatomically and photo-micro-graphically representing the differences among the selected sugarcane varieties that facilitated comparisons.

MATERIALS AND METHODS
Nineteen commercial varieties of Saccharum were collected from the National Hybridization Garden (NHG) Sugarcane Breeding Institute, Coimbatore (11°1'6"N76°58'21"E) elevation = 411.2 m (1,349.1 ft above mean sea level), Tamil Nadu, India and also used as experimental materials for studying leaf anatomy. Name of the commercial varieties are: Co B 94164 (Madhuri), Co 775, Bo 91, Co Se 92423 (Rajbhog), Co Se 01424, Co 453, Co Se 96436 (Jalpari), Co 62198, Co 658, Co 62175, Co 740, Co 7527, Co 449, Co A 88081, Co C 671, Co 8371 (Bhima), Co 6304, Co 6806 and Co 7219 (Sanjeevani).

Free-hand transverse section cutting of sugarcane leaf for anatomical study: The youngest or uppermost leaf (preferably 3rd leaf from the top) was collected from five plants of each variety. Tissue samples were collected from blades of collected leaves, approximately 2-4 cm tissue sample was excised from either side of the midrib, at a point midway along the length of the blade and fixed in FAA (formalin- Aceto-Alcohol) and stored in 70% alcohol. Free hand transverse sections were taken and stained in Safranin and Light green technique. Thin cut sections were observed under a research microscope (Olympus Ch20i microscope) fitted with CCTV camera (IS 500, 5.0MP) and attached to a computer. Microphotographs were taken using 10X objective of the microscope. For quantitative measurements of various leaf-blade and midrib, anatomical structures were measured with the aid of VIEW 7 image analysis software to obtain data on the area and linear means.

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**Determination of sugarcane juice brix:** Sugarcane Juice Brix refers to the total sugar content present in the juice expressed in percentage. Brix can be measured in the field itself in the standing sugarcane crop using a Hand Refractometer or Hand Refractometer Brix or HR Brix. In the field using a Pierce or d dibbler, juice samples were collected from several canes from the top, middle and the bottom portion of culm. Then 2-3 drops of juice were applied onto the prism surface of the Hand Refractometer (ERMA, Japan and Model No. RHB-32) and the daylight plate was closed. The HR Brix meter has graduations from 0 to 32 percent. Looking through the eye piece, the boundary line where the blue and white color met on graduation lines could easily be read and the reading value would indicate the Brix (%).

**RESULTS AND DISCUSSION**

In transverse section, the main cells and tissues present in the sugarcane leaf blade are presented in Fig-1. The sugarcane leaf consists of two parts: the sheath, which represents about 20% of the total leaf length. In hand made transverse sections of leaf blade of nineteen sugarcane varieties in the present study, two different sizes of vascular bundles were found. These were large and small. No intermediate size of vascular bundles was found. Large and small vascular bundles alternate all along the leaf blade. Large vascular bundles were rhomboidal or oval in shape and the small vascular bundles were spherical in shape. The small vascular bundles were situated near the lower epidermis, while the large ones were found in the center of the leaf blade. It was also noted the distances between various sized vascular bundles were different within different sugarcane varieties. Among nineteen varieties of sugarcane, the significant difference for the distance from large vascular bundles to small vascular bundles or interbundle distance was noted. Its maximum value was noted in the variety Co...
Table 1: Tissue proportion: Vascular bundles (VB); bundle sheath cells (BS); mesophyll cells and leaf thickness (LT) and Brix content in nineteen sugarcane varieties

<table>
<thead>
<tr>
<th>Sugarcane Variety</th>
<th>TOTAL AREA OF A MESOPHYLL CELL (in µm²)</th>
<th>TOTAL AREA OF A BUNDLE SHEATH CELL (BS) (in µm²)</th>
<th>LT ARROUND LARGE V.B (in µm²)</th>
<th>Distance (in µm)</th>
<th>LT ARROUND LARGE V.B (in µm²)</th>
<th>*BRIX CONTENT (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bo 91</td>
<td>39.143 ±1.766</td>
<td>304.90 ± 0.090</td>
<td>137.23 ±2.0768</td>
<td>48.705 ±0.27577</td>
<td>117.87667 ±0.3421</td>
<td>114.26667 ±0.41259</td>
</tr>
<tr>
<td>Co 6806</td>
<td>34.0715 ±0.94108</td>
<td>279.61 ± 2.10</td>
<td>138.05 ±1.84643</td>
<td>44.845 ±0.43134</td>
<td>58.2 ±1.3164</td>
<td>60.88667 ±0.6787</td>
</tr>
<tr>
<td>Co 7219(Sanjeevani)</td>
<td>10.5369 ±1.279</td>
<td>332.31 ± 1.11</td>
<td>94.88667 ±0.54903</td>
<td>47.333 ±0.52814</td>
<td>48.2733 ±2.42743</td>
<td>60.91333 ±1.26718</td>
</tr>
<tr>
<td>Co 8371(Bhima)</td>
<td>34.4422 ±1.72859</td>
<td>273.95 ±3.59</td>
<td>158.1133 ±0.34005</td>
<td>40.55 ±0.65826</td>
<td>47.11 ±1.25742</td>
<td>63.80667 ±2.94436</td>
</tr>
<tr>
<td>Co 62198</td>
<td>9.41283 ±1.03696</td>
<td>312.12 ± 2.58</td>
<td>200.0133 ±2.52967</td>
<td>81.125 ±0.23335</td>
<td>151.83 ±1.37886</td>
<td>188.905 ±2.77893</td>
</tr>
<tr>
<td>Co 7527</td>
<td>47.3598 ±1.36532</td>
<td>245.44 ±1.27</td>
<td>158.1133 ±0.34005</td>
<td>40.55 ±0.65826</td>
<td>47.11 ±1.25742</td>
<td>63.80667 ±2.94436</td>
</tr>
<tr>
<td>Co A 88081</td>
<td>21.3551 ± 1.699</td>
<td>313.01 ±0.88</td>
<td>62.81 ±0.35355</td>
<td>47.05 ±1.18072</td>
<td>43.24667 ±2.00301</td>
<td>46.145 ±0.30406</td>
</tr>
<tr>
<td>Co B 94164 (Madhuri)</td>
<td>19.42317 ± 0.1099</td>
<td>351.63 ± 0.56</td>
<td>118.8951 ±1.18087</td>
<td>63.07 ±1.43931</td>
<td>67.66 ±2.39435</td>
<td>66.24 ±2.18515</td>
</tr>
<tr>
<td>Co C 671</td>
<td>16.92803 ± 0.65574</td>
<td>313.01 ±0.88</td>
<td>62.81 ±0.35355</td>
<td>47.05 ±1.18072</td>
<td>43.24667 ±2.00301</td>
<td>46.145 ±0.30406</td>
</tr>
<tr>
<td>Co 740</td>
<td>12.97353 ±1.40572</td>
<td>297.47 ±3.99</td>
<td>144.66 ±0.7767</td>
<td>54.885 ±1.37866</td>
<td>70.845 ±1.01116</td>
<td>82.855 ±0.99702</td>
</tr>
<tr>
<td>Co 62175</td>
<td>16.1381 ± 0.84197</td>
<td>307.56 ±1.18</td>
<td>116.3663 ±1.10546</td>
<td>97.29 ±0.21213</td>
<td>97.315 ±1.08187</td>
<td>57.88 ±0.6364</td>
</tr>
<tr>
<td>Co 449</td>
<td>14.18747 ±0.94247</td>
<td>355.87 ±3.96</td>
<td>120.985 ±0.12021</td>
<td>122.3433 ±1.5151</td>
<td>83.37 ±0.72125</td>
<td>92.44667 ±2.00241</td>
</tr>
<tr>
<td>Co 453</td>
<td>21.81447 ±1.17304</td>
<td>279.88 ±1.85</td>
<td>157.7 ±2.2865</td>
<td>50.33 ±0.86267</td>
<td>46.28 ±0.42426</td>
<td>56.145 ±1.2021</td>
</tr>
<tr>
<td>Co 658</td>
<td>53.21017 ±0.94718</td>
<td>317.26 ±2.22</td>
<td>132.305 ±0.27577</td>
<td>64.83 ±1.13137</td>
<td>117.115 ±0.40305</td>
<td>95.565 ±0.5869</td>
</tr>
<tr>
<td>Co 775</td>
<td>56.20723 ±1.06923</td>
<td>295.40 ±4.63</td>
<td>100.83 ±1.2028</td>
<td>70.425 ±0.2192</td>
<td>48.44 ±1.27279</td>
<td>53.7333 ±1.34441</td>
</tr>
<tr>
<td>Co 6304</td>
<td>26.91893 ±0.623</td>
<td>291.52 ±5.41</td>
<td>106.81 ±1.0049</td>
<td>61.975 ±0.28991</td>
<td>73.1 ±1.38592</td>
<td>62.83 ±2.28059</td>
</tr>
<tr>
<td>Co Se 01424</td>
<td>15.75873 ±0.24638</td>
<td>350.34 ±3.76</td>
<td>100.885 ±0.4879</td>
<td>72.795 ±0.92631</td>
<td>66.325 ±0.4879</td>
<td>26.77 ±2.05061</td>
</tr>
<tr>
<td>Co Se 92423 (Rajbhog)</td>
<td>9.82357 ±0.38847</td>
<td>310.55 ±1.18</td>
<td>73.085 ±0.2192</td>
<td>70.9 ±0.29304</td>
<td>35.11 ±2.55693</td>
<td>42.3 ±0.28284</td>
</tr>
<tr>
<td>Co Se 96436 (Jalpari)</td>
<td>11.02463 ±0.09386</td>
<td>355.93 ±3.73</td>
<td>153.78 ±0.7638</td>
<td>56.53 ±0.49497</td>
<td>110.11 ±1.75143</td>
<td>113.065 ±1.05359</td>
</tr>
</tbody>
</table>

*Degrees Brix (symbol °Bx) is the sugar content of an aqueous solution. One degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by mass.
449 (122.34 µm) and the minimum value was found in the variety Co 7527 (40.55 µm). The difference in the distance from large vascular bundle to small vascular bundle was significant for all varieties. Perimeters of the large vascular bundle (mid-vein) varied from 42.46 µm (Co C 671) to 63.31 µm (Co 8371). In the case of small vascular bundles (small vein), the value of perimeter varied from 13.91 µm (Co 7527) to 35.12 µm (Bo 91). The lamina thickness (LT) around the large vein, interbundle area and around small bundle varied significantly. The value of LT around large bundle ranged from 200.01 µm (Co A 88081) to 62.19 µm (Co 62198). The total area of the primary vascular bundle in nineteen varieties of sugarcane varied from 71101.3194 µm² in Co 8371 to 11512.16 µm² in Co 740. Similarly, the total area of a bundle sheath cell (bsc) ranged from 245.44 µm² in Co 7527 to 383.24 µm² in Co 62198 (Table 1). The chlorenchymatous bundle sheath cells are always bordered by Kranz mesophyll. The large vascular bundles were characterized by the presence of metaxylem vessels on the either side of protoxylem. Mesotome sheath like cells sometimes occurred at the interface between xylem and phloem of large vascular bundles. The small vascular bundles entirely consist of only metaxylem but lack protoxylem.

Variation of Brix content (% sugar by mass) of the basal, middle and top part of Culm of nineteen varieties of sugarcane was also noted. Its average maximum value was found in the variety Co 62198 (19.06%) and the minimum value was noted in the variety of Co 7527 (12.53%).

In the present study, it was also found statistically that the variation of average Brix content has an impact on some anatomical characters of leaf blades of sugarcane. Average Brix content was positively correlated (r = 0.9811, P < 0.0001) with the total area of photosynthetic cells (mesophyll cells + bundle sheath cells) (Fig-2). Similarly, the total area of bundle sheath cells was positively correlated with average Brix content (r = 0.8972, P < 0.0001) (Fig-3).

The functioning of the C₄ photosynthetic system is directly related to the anatomical features of the leaves where it exists (Furbank et al. 2000). Anatomically, leaf of nineteen varieties of sugarcane differed significantly in lamina thickness at the large (primary) vascular bundles, the perimeter of large vascular bundles, the distance between the large and small vascular bundles, total area of large vascular bundle, total area of bundle sheath cell, number of bundle cells surrounding bundle sheath, total area of mesophyll cell and also average Brix content of the Culm. Bulliform cells are more radially elongated than epidermal cells and it well develops in all varieties indicates their xeric adaptation. Judged from the relatively brief description provided by Lush (1976), vascularization of the Panicum maximum leaf resembles that of sugarcane. Lush recognized only two orders (major and minor) of the vascular bundle, noted that only one major vascular bundle between successive minor vascular bundle. This observation supports the presence of two types of the vascular bundle in the leaf blade of present study materials of sugarcane, since all C₄ plants share a number of characteristics in terms of leaf anatomy. Our results also show that despite a decrease in bundle number in the region of the blade joint, a corresponding decrease in sieve-tube or tracheary element

![Figure 2](image2.png)

**Fig-2:** Relationship between the total area of photosynthetic cells and Brix content (%) of nineteen varieties of sugarcane represented by linear regression line.

![Figure 3](image3.png)

**Fig-3:** Relationship between the total area of bundle sheath cells and Brix content (%) of nineteen varieties of sugarcane represented by linear regression line.
cross-sectional area does not take place in sugarcane. It was suggested that the large veins were responsible for the bulk of longitudinal translocation, while the minor veins (all other bundles) were more important in the collection of and its photosynthetic movement over short distances. Robinson-Beer and Evert (1991) showed that in sugarcane leaf, there is a lack of thick-walled sieve tubes and vascular parenchyma cells in the large vascular bundles and there are very low plasmodesmata frequencies between all components of the large bundles phloem in sugarcane. These observations show that phloem loading in the large vascular bundles in the leaf blade of sugarcane surely involves in the apoplastic pathway.

Sugarcane, primarily used for sugar production, has a unique source-sink system. Its stem (Culm) sinks store photosynthesize as a soluble di-saccharide, sucrose, which can reach exceptionally high concentrations, up to 650 mM (Welbaum and Meinzer, 1990) or 18% of stem fresh weight in commercial sugarcane varieties (Inman-Bamber et al. 2011). Despite numerous studies, the factors affecting the quantitative sucrose accumulation are still poorly understood (Rohwer and Botha, 2001). However, the relationship between source leaf photosynthetic activity and sucrose accumulation in the culm sink of sugarcane is not well understood (McCormick et al. 2009). In the past several studies have demonstrated that the differences in leaf photosynthetic rates related to the quantity/total area of photosynthetic tissue and quantity/total area of bundle sheath cells and the maturity of the culm (Allison et al. 1997; Amaya et al. 1995; Bull and Tovey, 1974; Hartt and Burr, 1967). In the present study, it is noted that average Brix content of the Culm was positively correlated with the total area of photosynthetic cells and the linear regression curve followed the straight line. However, similar type result was also observed in the case of the total area of bundle sheath cells and average Brix content of the Culm.

**CONCLUSION**

The variation of Kranz system with well developed bundle sheath associated with Kranz mesophyll in the leaf blade (source) of nineteen sugarcane varieties were studied in relation to the accumulation of sugar content in the Culm (sink). The result shows that there is a strong positive correlation between average Brix content and the total area of photosynthetic cells and also the total area of bundle sheath cells. Bulliform cell well develop indicates its xeric adaptation. It is concluded that the varieties under study differ in respect of quantitative expression of Kranz tissue.

**REFERENCES**


