MATERIALS AND METHODS

SOURCE OF PLANT MATERIAL

The present investigation was carried out in five different varieties of Sorghum bicolor (L.) Moench. namely IS 18164, IS 32265, IS 5487, M 35-1 and E 36-1. Of these, IS 18164 is sweet stalk sorghum, IS 32265 and IS 5487 are salt tolerant and M 35-1 and E 36-1 are drought resistant. Seed samples were obtained from germplasm maintained at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhrapradesh, India.

Seedlings were raised in the field without addition of organic or inorganic fertilizers under irrigated condition. Samples were collected only from the healthy fruiting stalks. The third internode of each variety was collected, cut into small segments of uniform size and was used in the anatomical and analytical studies. For studies on fibre macerates and wall substances, the rind and core from the third internodal segments, separated by mechanical means were used.

Studies were carried out on the stalk anatomy with special reference to i) structure and distribution of fibro-vascular bundles, ii) fibre characteristics from macerates, iii) localisation of major cell wall substances such as cellulose and lignin and iv) cytospectral analysis of cell wall substances at the rind and core regions of the stalk. Histometrical parameters of fibro-vascular bundles,
and morphometric studies on fibres were statistically analysed following standard procedures (Snedecor and William, 1967; Scheffler, 1969).

ANATOMICAL STUDIES

Anatomical studies were carried in transections of the stalk internode. Microtome sections were taken using the standard paraffin embedding technique (Johansen, 1940). Six samples of third internode from six different plants in each of the variety were used in this study. Fresh segments of the internode with rind and core portions were cut into small pieces (5 x 5 x 10 mm) and subjected to alternate boiling and cooling in water to remove the air and to soften the materials. Subsequently the samples were fixed in formalin acetic - alcohol (FAA) for 48 hrs. and stored in 70% alcohol prior to sectioning. Materials were dehydrated in tertiary butyl alcohol (TBA) series (Sass, 1958; Berlyn and Miksche, 1976) following the schedule outlined below:

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>95% Ethyl alcohol</th>
<th>100% Ethyl alcohol</th>
<th>100% TBA</th>
<th>Distilled Water</th>
<th>Duration of dehydration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>50 ml</td>
<td>-</td>
<td>10 ml</td>
<td>40 ml</td>
<td>3 hrs</td>
</tr>
<tr>
<td>2.</td>
<td>50 ml</td>
<td>-</td>
<td>20 ml</td>
<td>30 ml</td>
<td>24 hrs</td>
</tr>
<tr>
<td>3.</td>
<td>50 ml</td>
<td>-</td>
<td>35 ml</td>
<td>15 ml</td>
<td>3 hrs</td>
</tr>
<tr>
<td>4.</td>
<td>-</td>
<td>-</td>
<td>50 ml</td>
<td>-</td>
<td>3 hrs</td>
</tr>
<tr>
<td>5.</td>
<td>-</td>
<td>25 ml</td>
<td>-</td>
<td>-</td>
<td>3 hrs</td>
</tr>
<tr>
<td>6.</td>
<td>-</td>
<td>-</td>
<td>100 I</td>
<td>-</td>
<td>3 hrs</td>
</tr>
<tr>
<td>7.</td>
<td>-</td>
<td>-</td>
<td>100 II</td>
<td>-</td>
<td>3 hrs</td>
</tr>
<tr>
<td>8.</td>
<td>-</td>
<td>-</td>
<td>100 III</td>
<td>-</td>
<td>3 hrs</td>
</tr>
</tbody>
</table>
After dehydration, specimens were infiltrated with paraffin wax at 50-60°C. Blocks were made with a mixture of paraffin and bee wax in the proportion of 50:1 by weight. The blocks were stored in refrigerator prior to microtome sectioning.

Transverse sections were taken at a thickness of 10-14μm and mounted onto slides with Haupt's adhesive, using 4% formalin as flooding solution.

Besides microtome sectioning, freehand sections were also taken using fine razor blade and hand sledge microtome at 20μm thickness.

Sections were stained with toluidine blue 'O' (Feder and O'Brien, 1968). After staining, paraffin sections were air dried, dewaxed by passing through two changes of pure xylene and mounted in DPX. Freehand sections were also stained in 0.05% toluidine blue 'O' in benzoate buffer at a pH of 4.4 for 15 minutes. After removing excess stain, if any, the sections were mounted in water and sealed with glue prepared by dissolving thermocole in xylol for observations.

The following data were collected from transectional view of stalk sections. As many as one hundred observations were made from the six samples in each of the variety investigated and the mean was taken.

The observations were confined to:

i. Structure and frequency of occurrence of fibro-vascular bundles at the rind and core regions of the stalk
ii. Size of fibro-vascular bundles, radial height (R) and tangential width (T) of bundles and radial-tangential (R/T) ratio (Grosser and Liese, 1971)

iii. Percentage of fibres and parenchyma cells in the cross sectional areas of rind and core regions of the stalk. Percentage of these cell elements were calculated employing the standard methods of Brown et al. (1949) and Sulthoni (1990) using cut outs of microphotographs of the cross section of stalk with a magnification of 50% of cell elements (P).

\[ P = \frac{\text{Weight of cell image}}{\text{Weight of whole image}} \times 100 \]

HISTOCHEMICAL LOCALISATION OF WALL SUBSTANCES

The major cell wall substances viz., cellulose and lignin in transections of internode were localised using histochemical reagents and fluorescent dyes.

i. For histochemical localisation of lignin, sections were stained in 0.05% toluidine blue ‘O’ in benzoate buffer at 4.4 pH for 15 minutes and mounted in water (Feder and O'Brien, 1968).

ii. Phloroglucinol method was also employed for lignin localisation (Johansen, 1940; Siegel, 1953). Sections were placed in aqueous solution of phloroglucinol in 20% HCl for 1-2 minutes and observed.

iii. Cellulose was localised following calcofluor white M₂R method (Hughes and McCully, 1975). Sections were stained in 0.01% aqueous solution of
calcofluor white M₄R for 1 minute and examined under fluorescence microscope using UV (410nm).

Transsections were also stained using Acridine orange 0.1% in phosphate buffer at pH 6.0 for one minute (Armstrong, 1956) and 0.1% Coriphosphine O for one minute (Harada, 1973; Harris and Oparka, 1993). These fluorescent dyes were used to stain the cell wall.

MACERATION STUDIES

Six internodal segments of stalk samples from each of the variety were split and the outer rind and inner core portions were separated. Thin slivers of 1mm thickness were cut, boiled in water and then cooled to get rid of the air. Slivers obtained from rind and core portions were processed separately for maceration.

The processed samples were macerated using

i. Jeffrey's fluid consisting of 10% nitric acid and 10% chromic acid at 60°C for 24 hrs. (Johansen, 1940).

ii. Nitric acid (40%) at 50°C for 30 minutes (Abdul Latif et al. 1990).

The macerated fibres were washed several times in distilled water and stained using

i. 0.05% toluidine blue ‘O’ for 20 minutes, washed and mounted in water for observations.
ii. 1% Saffranin ‘O’ (Johansen, 1940) for 24 hrs, and then dehydrated using graded series of alcohol and mounted in DPX.

MORPHOMETRIC MEASUREMENTS

For morphometric measurements such as length, width, wall thickness and lumen width of macerated fibres of rind and core regions of each variety were pooled separately. For each sample, one hundred fibres were randomly chosen for measurements and the mean was taken. Morphometric measurements were made using an ocular micrometer under a magnification of 10 X and 45 X.

Based on wall thickness and lumen width, the fibres were classified into four types (Metcalfe, 1968).

i. Very thick-walled  lumen almost completely closed
ii. Thick-walled    lumen less than the thickness of wall
iii. Thin-walled    lumen more than the thickness of wall
iv. Very thin-walled lumen much greater than the thickness of wall

The following derived values were calculated from the data of fibre dimensions as suggested by Tamalong et al. (1957).

i. Slenderness ratio (SR) = \[
\frac{\text{Length of fibre}}{\text{Diameter of fibre}}
\]

ii. Flexibility ratio (FR) = \[
\frac{\text{Lumen width of fibre} \times 100}{\text{Diameter of fibre}}
\]
iii. Runkel ratio (RR) \[ = \frac{2 \times \text{Wall thickness}}{\text{lumen width}} \]

Besides the observation on dimensional data such as length, breadth, wall thickness and lumen width, pitting, wall lamellation, septation and tip characters of fibres were studied.

**STATISTICAL ANALYSIS**

Data on fibre dimension of the rind and core regions were subjected to statistical analysis. Arithmetic mean (X), standard deviation (SD) and coefficient of variation (C.V.) were calculated for all the data pertaining to the two regions of the stalk.

**Test of Significance**

Comparison of fibre dimensions between the rind and core in different varieties were made taking the respective sample means at 0.05 level of significance, as suggested by Schefler (1969).

**Analysis of Variance (ANOVA)**

Comparison of the fibres of different varieties with reference to their characteristics was made using the ANOVA method (Schefler, 1969). Studentized range, the least significant difference at 0.05 level between any two means was calculated for each parameter (Snedecor and William, 1967).
CHARACTER WEIGHTAGE

In order to evaluate the efficiency of a variety in relation to their use, character weightage was given using the following formula and subsequent ranking of varieties.

\[
\text{Character weightage (CW)} = \frac{\text{Ranking position of a variety for a particular character}}{\text{Total number of investigated varieties (n)}}
\]

The flexibility ratio is inversely proportional to the strength properties and accordingly the weightage values were given. Similar weightage values were given for fibre percentage and bundle frequency to evaluate the varieties in relation to the use.

PHOTOGRAPHY

Nikon Labophot II research microscope was used for taking microphotographs pertaining to histological studies. Microphotographs were also taken under Nikon microscope equipped with a polariser and analyser in cross position. A first order red plate was placed over the polariser at an angle of 45° to create a red background (Bennet, 1950; Dayanandand and Pon Samuel, 1979). 100 ASA konica colour negative films were used for documentation. Negatives were processed and printed at local commercial laboratories.
CYTOSPECTRAL ANALYSIS OF CELL WALL SUBSTANCES

The major cell wall substances viz., cellulose and lignin in the stalk internode were analysed using Fourier Transform Infra-red spectroscopy (FTIR).

Small fragments of the rind and core regions of each variety were dried, crushed and powdered separately using a homogenizer. The homogenate were sieved using a muslin cloth of fine mesh. The powder thus obtained was ground with potassium bromide in the ratio of 1:80 using a mortar and pestle. From the sample ground, pellets of 0.2 mm thickness were made (William Kemp, 1991). The pellets were used in Bruker IFS 66V spectrophotometer and the spectrum was recorded as percentage of transmittance over a wave number range of 4000-400 cm⁻¹.

Percentage of transmittance was expressed as the ratio of radiant power transmitted by the sample to the radiant power incident on the sample. Absorbance is the logarithm to the base 10 of reciprocal of transmittance, i.e.,

\[ A = \log_{10} \frac{I}{T} \]