

amount of water is determined from preliminary trails to get the required amount of water. The plants are thinned to 6 per m² day after emergence. Root growth is restricted. To prevent the sidewall of the cylinder from getting heated, it could be coated with white paint.

8. **Technique for seedling drought resistance in hydroponics:** The screening for seedling drought resistance is generally done in soil of flats, pots or beds in greenhouse or growth chamber. When the seedlings are established, water is simply withheld until they show severe wilting. At this stage, stress is released with water and survival counts taken. The problem with this method is that it is very difficult to control the level of stress to which the plants are exposed, particularly with repeatability of subsequent experiments, and water extraction may differ markedly in the vicinity of individual plant roots. The technique adopted by Sullivan and Ross (1979) overcomes these defects.

Methodology: seedlings are grown hydroponically in rows of plastic dishes. The seeds are first germinated in wet paper towels and then transferred to the dishes or 3 days to the dish pans. An acrylic plastic tray is cut to fit the pans. Rows of holes (about 2 mm diameter) are drilled and spaced about 2 cm, with 4 cm between rows. A countersink is made above each hole. The seedling is placed in the hole when the seedling is transplanted with the seed being in the countersink area. The solutions need to be aerated for growth of sorghum seedlings. When the seedling are 7 to ten days old, Carbowax 600 is added to the solutions in increments over a 3 day period until a stress of 15 bars is reached. The plants are then evaluated for drought resistance after exposure to the stress for several days. For a small number of entries, the height, leaf number, leaf area, maximum root length and dry weight are measured and compared to nonstressed controls. When many genotypes are evaluated, they are visually ranked from 1 - 5. One is assigned to green, two to plants with few, or no symptoms of drought injury. A rating of 5 is given to dead plants.

9. **Visual scoring of seedling vigor:** The objective of this technique is to estimate rapidly and efficiently the seedling vigor of a large number of lines. The visual scoring system used is a relative one, based on the range of variability in seedling size in the material being scored. The individual 15 ratings (1 = most vigorous, 5 = the least vigorous) are based on individual plots within an experiment which serves as a reference for scoring all entries. The following factors enter into the assessment of seedling size: height, pseudostem thickness, spread of leaf canopy and/or the length of breadth of the individual leaves. The restriction to a limited number of classes may be a limitation to the use of visual scoring for some types of studies (e.g. parent-progeny comparisons).



LEAF MORPHOLOGY

Each sorghum leaf consists of a thin flat lamina with a definite midrib and a thicker rigid leaf sheath clasping the pseudostem internode. The midrib may be strong or weak, white or green in color. Leaves may be erect, semi-erect to drooping; the leaf blade and sheath meet at a point called collar at different angles to the stem which may vary from almost vertical to near horizontal. At the base of the lamina ligules project from the leaf base. Leaf length becomes gradually shorter and smaller towards the tip. The terminal leaf is called flag leaf. The length of leaf may be as long as 1 m and the width 10 to 15 cm. The number of leaves in well adapted genotypes vary from 14 to 17, whereas in less adapted ones there may be as many as 30 leaves. The leaves are arranged in 2 ranks alternatively at an angle along the stem and each node. The sheath is attached to the node, and surrounds the internode, and often the node above it. In some cultivars the leaf sheath is covered with a waxy bloom. Leaves are glabrous except on the inside, just above the membranous ligule and on the cuticle near the junction with the sheath. The leaf margins are smooth or scabrid (Fig. 4.1; House, 1980).

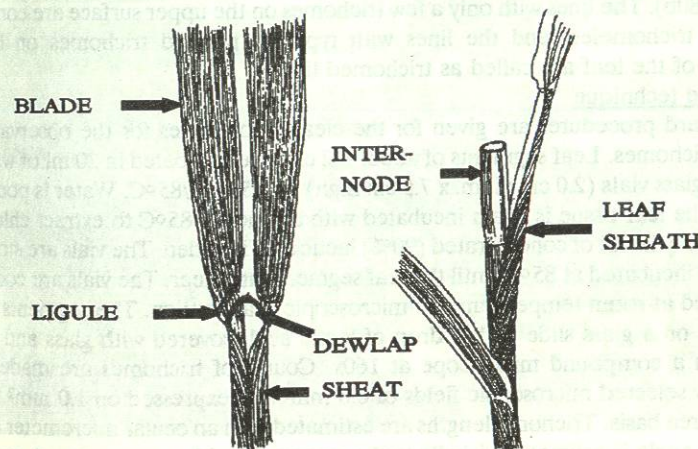


Figure 4.1 Morphology of a leaf showing its parts and its attachment with stem. a) A portion of a leaf; b) Attachment of leaf sheath with stem.

LEAF ANATOMY

The development of sorghum is typical of grass leaves described by Dale (1961). It begins development as a single ridge which is oriented laterally on the shoot meristem. The crescent-shaped primordium grows into a hood-shaped structure which envelopes the apical dome.

Leaf surface

Leaf epidermal cells are of various sizes with wavy outline and stomata between 2 adjacent stomates (Fig. 4.2). After a 30 hr treatment with cold temperature (10°C) one third of the upper mesophyll were badly swollen while in the rest of the leaf, no such contraction and reduction in starch grain size occurred. This affected the pattern of photosynthetic radiocarbon exchange (Brooking and Taylor 1977).

Preliminary studies by Yadav (1976) indicate that in lines resistant to periderm fungus, the leaves have a thickened cuticle and hypodermis in the midrib. Epidermal pattern showed higher number of stomata with shorter length and wider guard cells in resistant forms.

The epidermis of leaves of many of the glossy lines (85%) show the presence of microscopic hairs called trichomes. Trichomes are single-celled projections, easily visible at 160x magnification on the epidermis of the leaf. They are often pointed at the tip. The size and morphology of the trichomes vary in different genotypes (Plate 4.1; Fig. 4.2), and they are directed towards the leaf base, more being present on the upper (adaxial) surface (Tables 4.1-4.2).

Trichomes are dense at the tip, intermediate at midportion, and less at the base of the leaf (Fig. 4.3). It is difficult to distinguish genotypes on the basis of trichome density as some trichomes are not visible under a low-power (X160) microscope. Their length varies from 20 to 55 μm and these glandular trichomes can be observed under a high-power microscope or a scanning electron microscope (SEM). These trichomes are bicellular and have a rounded tip. Lines with typical trichomes on the abaxial surface are recognised as trichomed lines (Figs. 4.4-4.7; Maiti *et al.*, 1980b). The lines with only a few trichomes on the upper surface are considered as trichomeless and the lines with typically pointed trichomes on both surfaces of the leaf are called as trichomed lines.

Trichome technique

Standard procedures are given for the clearing of leaves for the observation of leaf trichomes. Leaf segments of about 1-2 cm^2 are incubated in 20 ml of water in small glass vials (2.0 cm diam. x 7.5 cm high) for 15 min./85°C. Water is poured off and the leaf tissue is again incubated with alcohol at 85°C to extract chlorophyll; finally 20 ml of concentrated (90%) lactic acid is added. The vials are sealed and incubated at 85°C until the leaf segments are clear. The vials are cooled and stored at room temperature for microscopic examination. The segments are mounted on a glass slide with a drop of lactic acid, covered with glass and observed in a compound microscope at 160x. Counts of trichomes are made in randomly selected microscopic fields of 0.8 mm^2 and expressed on 1.0 mm^2 surface area basis. Trichome lengths are estimated with an ocular micrometer. Trichome angle is estimated visually to the nearest 5° with a protractor. A strong relationship between trichomes on the lower surface and shootfly resistance has been found (Maiti and Bidinger, 1979).

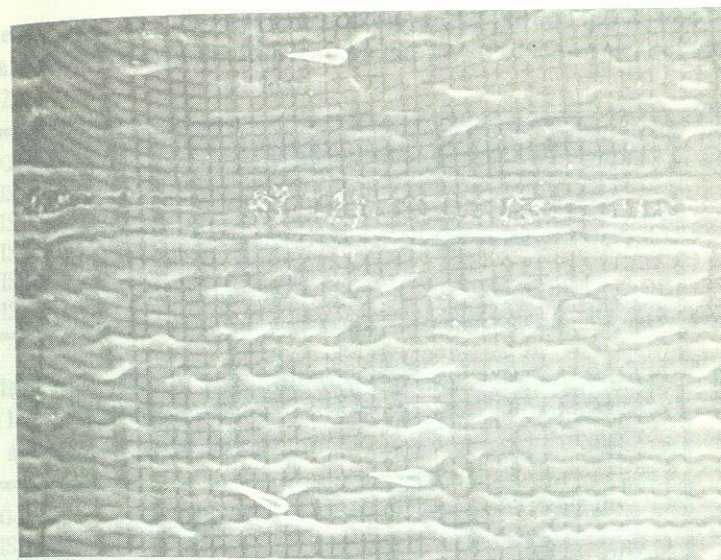


Figure 4.2 Leaf surface of IS 4664 showing morphology of epidermal cells and attachment of trichomes, under the light microscope.

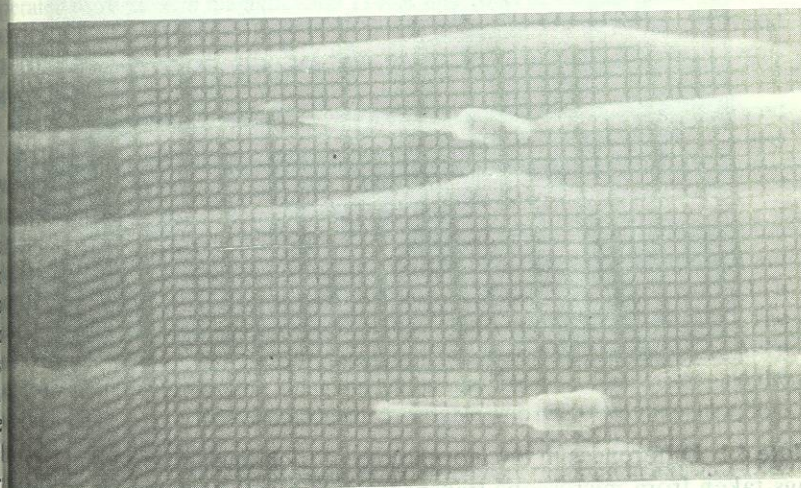


Figure 4.3 Scanning electron micrograph of the surface of a leaf of IS-844, showing glandular bicellular trichomes.

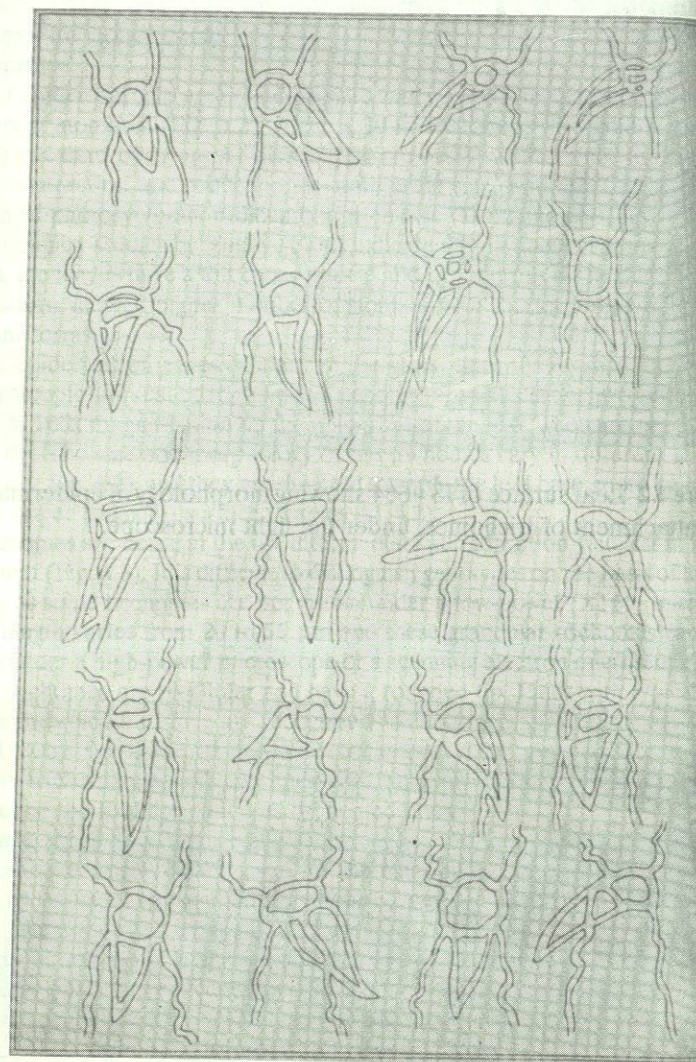


Plate 4.1 Differences in the morphology of trichomes of sorghum. Drawings taken from microscope transparencies of the abaxial surface of leaves.

Table 4.1 Trichome density (mm^2) on the center portion of the adaxial and abaxial surfaces of the fifth leaf. Means are of 10 leaves per cultivar and 2 microscope fields per leaf.

Genotype	Adaxial surface	Abaxial surface
IS 1054	17 ± 1.3	6 ± 1.0
IS 2146	45 ± 3.8	27 ± 4.4
IS 2314	22 ± 1.6	4 ± 0.6
IS 5604	28 ± 1.6	9 ± 2.2
IS 5484	37 ± 5.1	22 ± 4.1

Table 4.2 Range of trichome density on the abaxial leaf surface of the fifth leaf in selected sorghum cultivars. Means are of 10 leaves per cultivar and 2 microscope fields per leaf.

Genotype	Trichomes / mm^2	Genotype	Trichomes / mm^2
IS 1119	46 ± 4.9	IS 5613	45 ± 4.7
EN 3342-4	45 ± 4.1	IS 2146	45 ± 4.4
IS 2205	30 ± 4.1	IS 18588	28 ± 2.5
IS 5622	28 ± 4.4	IS 1054	8 ± 0.9
NCL-3	4 ± 1.3	IS 5067	4 ± 0.9

Electron microscopy of glossy and nonglossy lines (Maiti *et al.*, 1983b)

For the SEM of the leaves of glossy and nonglossy lines, a JOEL SEM was operated at 18 kv with magnification 1100X and 16 kv at magnification 15000X, 100X. The lower surface of each cultivar was photographed at 200X, 1500X and 1000X. Silica deposits, trichomes and epicuticular wax were the main features of sorghum leaf surface.

Silica: Dumbbellshaped deposits, with either 2 or 3 bumps are regularly spaced along the veins. Along major veins, sometimes 2 or 3 rows of silica bodies are also present (Fig. 4.4-4.5). All cultivars show the presence of these lines of silica, irrespective of their glossy or nonglossy nature.

Trichomes: All sorghum genotypes show trichomes under SEM. Two types are observed, prickly hairs and microhairs. Prickly hair have pointed tips and are elongated, but micro hair is bicellular and looks glandular (Fig. 4.6-4.7). In a few cases, both types of hair are present (e.g., cultivars Swarna and IS 4405). The majority of glossy lines have prickly hair. The density of hair varies between cultivars and the distribution along the leaf is very irregular. Shape, size and the orientation to leaf surface of pricklyhair is variable with hair of different morphologies occurring together.

Wax: Protruded wax filaments on sorghum leaves were reported by Sánchez-Díaz *et al.* (1972). Clusters of filaments were more scattered further away from the midrib on leaf and groups of 2 to 5 filaments occurred at random between the larger clusters. The wax filaments reflect some radiation, lowering the net radiation



Figure 4.4 SEM of the surface of a leaf showing bilobed and trilobed silica crystals.



Figure 4.5 SEM of the surface of a leaf showing trilobed silica crystals.

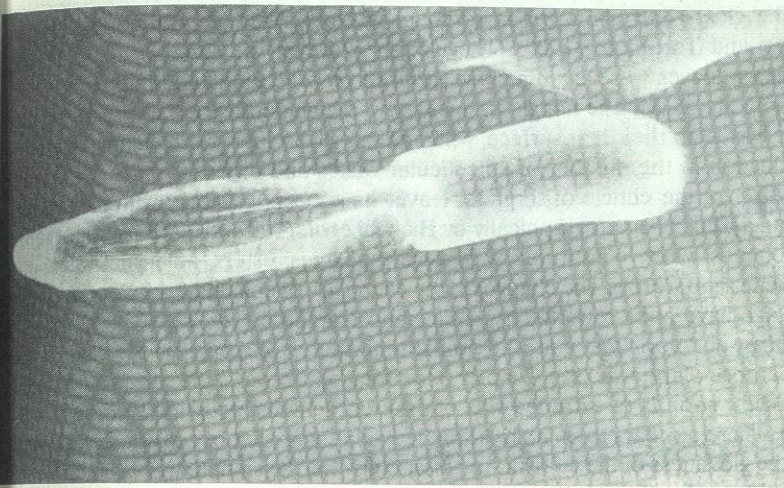


Figure 4.6 SEM of the surface of a leaf of IS-4846, showing a view of a unicellular trichome.

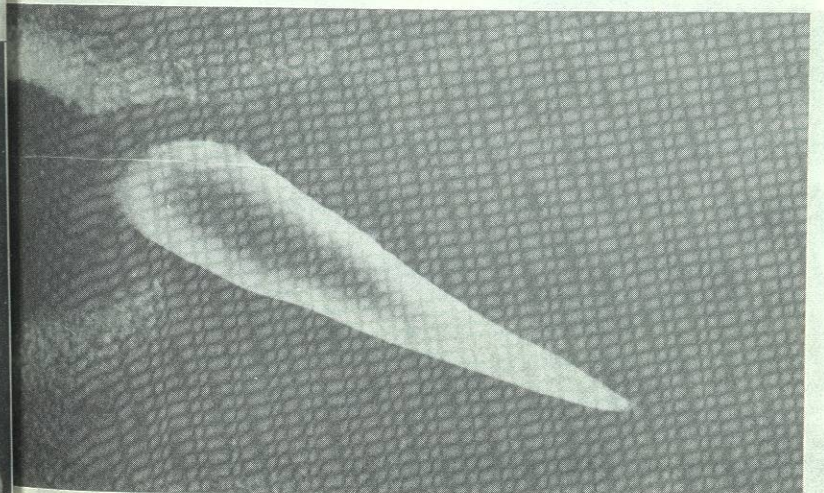


Figure 4.7 SEM of the surface of a leaf of IS-4777, showing an elongated view of a pointed trichome.

and will thicken the boundary layer next to the leaf, thereby increasing resistance to diffusion of water, oxygen and carbon dioxide in and out of the leaf (Haberlandt, 1928).

The glossy leaf character in sorghum was described by Maiti and Biding (1979) and Tarumoto (1980). The ultrastructure of the surfaces of glossy and nonglossy leaves were examined by Tarumoto *et al.* (1981) and Maiti (1986) with the help of a SEM. The nonglossy lines showed high density of starshaped epicuticular waxes on their leaf surface, whereas the glossy lines were characterized by a reduction in the number of epicuticular waxes and different shapes of waxes.

Waxes of the cuticle of sorghum leaves have been extracted with chloroform and analyzed chromatographically by Bianchi *et al.* (1977). The classes of organic compounds which constituted wax were n-alkanes, esters, aldehydes, alcohols, n-alkenes and sterols. The changes in the chemistry of epicuticular wax of sorghum with age have been reported by Atkins and Hamilton (1982a).

Glossy and nonglossy cultivars can be separated in 2 groups on the basis of the appearance of the wax (see Chapter 3 for details). Between the silica bodies, both lines show strands of extruded wax of different lengths along the veins (Fig. 4.10). There appears to be no relation between the extent of extrusion of the wax and the glossiness of the cultivar. In glossy cultivars, the smooth wax layer covering the epidermal cells and the cuticle shows patchy aggregations of large, irregular shaped crystals (Fig. 4.11). The density of these aggregations differs with cultivar, but areas of smooth wax are always visible, and silica bodies are rarely covered to any extent by these wax crystals.

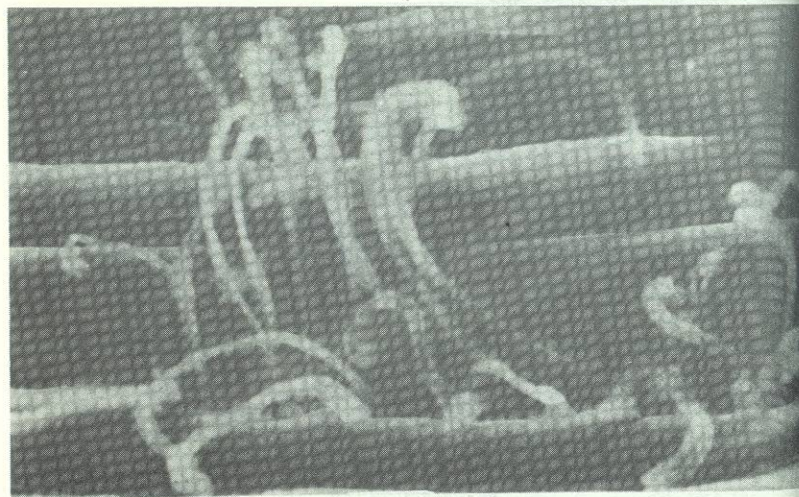


Figure 4.8 SEM of the surface of the surface of a leaf of IS-4776, showing long wax strands.

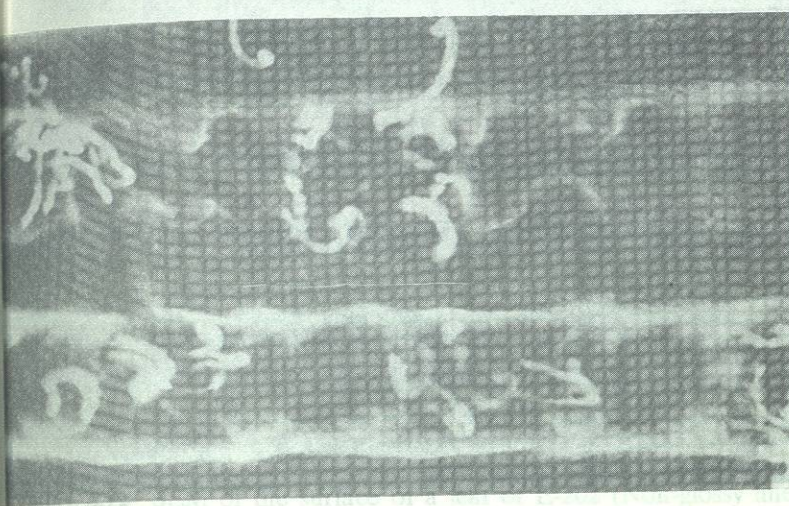


Figure 4.9 SEM of a leaf of IS-4664, showing groups of medium length wax strands partially covering the silica crystals.

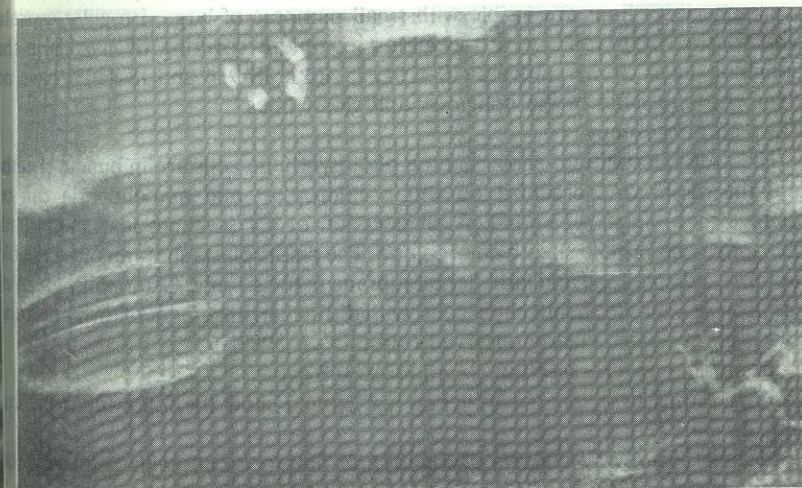


Figure 4.10 SEM of the surface of the surface of a leaf of IS-5031, showing short wax strands.