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LEAF BLADE AND MIDRIB ANATOMY OF TWO SUGARCANE CULTIVARS OF BANGLADESH

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Abstract

Context: Kranz anatomy of locally developed sugarcane cultivars were studied in relation to C4 vascular arrangement.

Objective: The objective of this study was to make gross cross-sectional anatomy and quantitative assessment of the anatomic traits of the leaf-blade and midrib of the sugarcane cultivars.

Materials and Methods: Leaf blade and leaf sheath of two sugarcane cultivars Ishurdi 20 and Ishurdi 32 were used as the materials. Free hand section with appropriate stain were used. Sections were studied using an advanced biological system microscope fitted with motic camera. Anatomic traits were studied through motic image plus J 1.0 software using Macintosh computer.

Results: Three sized vascular bundles and significant differences in distance between those vascular bundles were noted. Ishurdi 32 possessed two sized vascular bundles. Large vascular bundles characters by two large metaxylem vessels on either side of protoxylem. Phloem well developed. Intermediate and small bundles lack metaxylem vessels and protoxylem, but have metaphloem with thick and thin walled sieve tubes. Bundle sheaths have extended to upper and lower epidermis but for small bundle it is extended to abaxial epidermis. Vascular bundles are almost completely surrounded by chlorenchymatous bundle sheath and associated with hypodermal sclerenchyma on both abaxially and adaxially except small blade bundles which associated with the abaxial sclerenchyma. Bundle sheath cells were smaller in large and larger in other two types of vascular bundle. An inner mestome sheath with thickened walls is always present round the phloem and metaxylem around all or part of the xylem in large and intermediate bundles. In small bundles mestome sheath is altogether absent. Bulliform cells with varied area were present on the adaxial epidermis opposite to small vascular bundles. Midrib anatomy consists of central large vascular bundles lacking bundle sheath cells pushed deep inside parenchymatous hypodermis from abaxial hypodermal sclerenchyma girders. Lack of Kranz traits, and bundle sheath cells have transformed into sclerenchymatous bundle cover. Central mid-rib large bundle flanked by 3-10 small bundles on either side of midrib which have Kranz system of anatomy. Midrib region have continuous hypodermis consists of sclerenchyma cells and it is few layer (Ishurdi 32) to multilayer (Ishurdi 20).

Conclusion: Kranz system with well developed bundle sheath associated with Kranz mesophil in the leaf blade were observed but Kranz tissue absent in midrib region. Large and small vascular bundles alternate all alone the leaf blade. Bulliform cell well develop indicates zeric adaptation. Two cultivars differ in respect of quantitative expression of Kranz tissue.

Keywords: Sugarcane cultivar, Kranz tissue, bulliform cells, mestome sheath.

Introduction

Plant taxonomist considered anatomy of grass leaves as a part of taxonomic study and grass leaf-blade anatomy become an important component of taxonomic classification (Metcalf 1960, Ellis 1976). Most important discovery of C4 path way of photosynthesis in certain grasses and later realized faster transport of photosynthetic from source to sink were associated with C4 photosynthesis as compared to C3 photosynthesis system (Gallaher *et al.* 1975). This lead to intensive investigation of comparative grass leaf anatomy in relation to efficient photosynthesis was initiated. Though no strict relationship established between leaf structure and principal types of photosynthetic pathway but in C4 grasses Kranz structure of the leaf may be associated (Black *et al*. 1973, Bourdu 1976). Kranz anatomy consists of a sheath of tightly packed cells around the vascular bundles, giving more rapid export of photosynthetic products from the leaf (source) to region of storage (sink) in abundant light. They are characterized by walls thicker than wall of

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mesophyll cells; these walls have numerous pits and plasmodesmeta and the chloroplast are in some way different from mesophyll chloroplast, such as larger in size, higher in number per Kranz cells and specific position within the cells. The close association of chlorenchymatous tissues to the vascular bundles in the leaves of C4 species is thought to facilitate rapid transport of the photosynthesis to the sieve tubes (Gallaher *et al*. 1975, Lush 1976, Stephenson *et al*. 1976). Moreover special consideration gives on small vascular bundles, its number and distance from each other and from larger veins in C4 grasses compared to C3 grasses (Crookston and Moss 1974, Hattersley *et al.* 1976). Relation of phloem cross-sectional area and photosynthetic rates and rates of assimilate export from leaves of C3 and C4 are variable (Lush 1976). It is suggested larger vascular bundles with metaxylem vessels separated by protoxylem were responsible for bulk of longitudinal translocation while small vascular bundles were more important in collection of photosynthate and its movement over short distances (McDavid and Midmore 1980).

Kranz cells have been studied anatomically for one hundred years but have been reported in ten angiospermic families (Hatch *et al.* 1971, Johnson and Brown 1973, Brown 1975). In gramineae, Kranz anatomy basically consists of two concentric sheath of small, very thick walled, chloroplast-lacking cells; and an another parenchymatous sheath of thick or thin walled cells with few to numerous chloroplast and cell sizes from average to quite large. Schwendener (1890) discussed in detail the sheath of grasses and concluded that there is almost always a parenchyma sheath, but in some grasses the mestome sheath may not be present or evident. The present work considered leaf anatomy including the Kranz anatomy of two sugarcane genotypes, the nature and distribution of Kranz tissue in the leaf blade and midrib have been described and discussed.

Materials and Methods

The plants used in this study, *Saccharum officinarum* L., variety Ishurdi 20 and Ishurdi 32 were obtained from ten farmers field, five selected for Ishurdi-20 and five for Ishurdi-32. These plants were grown for commercial purpose by the farmers following optimum cultural practices. Ten healthy full grown leaves were collected randomly from each field. Tissue samples were collected from blades of collected leaves, approximately 2-4 cm tissue sample were excised from either side of the midrib, at a point midway along the length of the blade and fixed in FAA. Free hand sections were taken and stained in safranin and fast green. Thin cut sections were observed under a research microscope fitted with digital camera and attached with a Macintosh computer. Leaf blade gross cross-sectional anatomy was studied through taking micro-photographs. For quantitative measurements of various leaf-blade and midrib anatomical structures were measured with the aid of image analysis program, Motic J 1.0 software. Statistical analysis of the measurements was conducted through analysis of variance. Significance of the difference was tested using between plants within a field.

Results

Transverse section of the leaf-blade of the two sugarcane genotypes exhibited the Kranz anatomy characteristic of C4 grasses with mesophyll radially arranged around the chlorenchymatous bundle sheath. The layer of Kranz mesophyll encircling successive vascular bundles are separated from one another by a layer of non-Kranz cells, lacking chloroplast or containing few chloroplast. The colorless cells are mostly large and round in shape other resemble the bulliform cells of the adaxial epidermis and mostly in contact with them (Fig.1). Significant variation in leaf-blade vascular bundle size within a leaf and between genotypes was noted. Three types, or order, of longitudinal vascular bundles were recognized in Ishurdi 20 but in Ishurdi 32 it was two types (or orders). It was large (first order), intermediate (second order) and small (third order). The large vascular bundles were rhomboid or oval shaped (Figs. 1 & 2), the medium ones were oval shaped (Figs. 1 & 2) and small vascular bundles were round in shape (Figs.1 & 2). In Ishurdi 32 the second order vascular bundles were lacking.

As seen in transverse section, the pattern of longitudinal vascular bundles in the leaf-blades is some what variable. Commonly small bundles alternate with intermediate bundles and large bundles are flanked by small one (Fig. 1). The mesophyll cells are much branched and loosely arranged, having numerous intercellular air species among them (Fig. 1), together with sub-stomatal chambers.

Distance from large vascular bundles to small bundles is 111.86 ± 16.34 μ and 124.04 ± 12.69 μ; and that from small to small bundles are 120.16 ± 21.44 μ and 137.02 ± 11.76 μ respectively in Ishurdi 20 and Ishurdi 32 respectively. High standard error attached with the distance estimates reflected variation in bundle distances within a genotype and no genotypic difference noted. Two cultivars have different bundle distance which is due to difference in genotypes. Distance between intermediate to small bundles and large to intermediate bundles are 112.66 ± 12.07 μ and 240.77 ± 24.67 μ respectively in Ishurdi 20.

The large bundles of the leaf-blade (Figs. 2 & 3) characterized by a large metaxylem vessel on either side of the protoxylem or protoxylem lacunae. Protophloem is also visible but in mature bundles it is usually obliterated and the only conducting phloem in such bundles is metaphloem. The phloem of large bundles also consists of both thick and thin walled sieve tubes (Fig. 3) but metaphloem in large bundles also consists of both thick and thin walled sieve tubes The large bundles are more or less completely surrounded by chlorenchymatous bundle sheath (Figs. 2 & 3) which is often interrupted on the adaxial end/or abaxial surfaces by girders of hypodermal sclerenchyma. At least 2.5% and 6.3% of large vascular bundles respectively in Ishurdi 20 and Ishurdi 32 have no sclerenchymatous girders on the abaxial surface. An inner mestome sheath with thickened walls is always present around the phloem and may be present around all or part of xylem. Vascular bundle area (Table 1) of large type ranged from 178.02 to 231.09 μ^2 with a mean area 204.18 ± 14.16 μ^2 in Ishurdi 20; and that in Ishurdi 32, it ranged from 155.24 to 194.16 μ^2 with mean area 181.06±14.57μ^2. The size of first order bundles within genotypes are variable as the mean is attached with large standard error. Mean xylem and phloem area respectively 118.33±21.25 μ^2 and 79.11±9.55 μ^2 in Ishurdi 20; and 75.77±9.89 μ^2 and 69.96 ±7.42 μ^2 in Ishurdi 32.

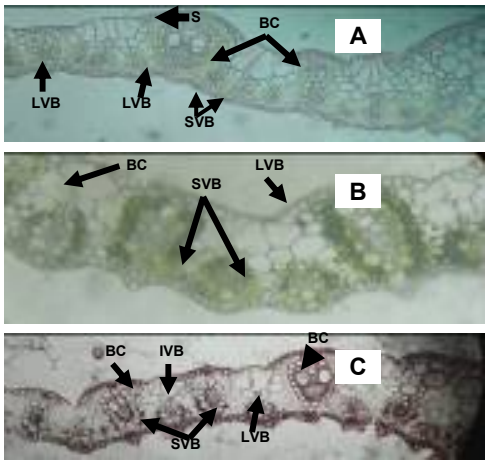

Fig. 1. Transverse section of leaf blade of sugarcane. Upper & Middle = Cultivar Ishurdi 32.; lower = Cultivar Ishurdi 20. S = hypodermal sclerenchyma; BC = bulliform cell; LVB = large Vascular bundle; SVB = small vascular bundle; IVB = intermediate vascular bundle.

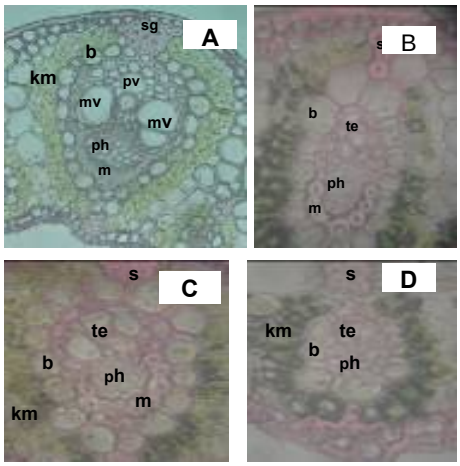

Fig. 2. Magnified view of leaf blade vascular bundle of the cultivar Ishurdi-20. Upper left: large vascular bundle with intact vessels. Upper right: = intermediate vascular bundle. Lower left and right: small vascular bundle. Sg = sclerenchymatous girder; b = bundle sheath; km = Kranz mesophyll; pv = protoxylem vessel; mv = metaxylem vessel; ph = phloem; m = mestome sheath; te = treachery elements.

Intermediate (second order) blade-vascular bundles were found only in Ishurdi 20. It was completely surrounded by a chlorenchymatous bundle sheath and is associated with hypodermal sclerenchyma both abaxially and adaxially (Figs. 1, 2 & 3). Unlike large bundles, intermediates lack large metaxylem vessels and protoxylem, although some may have protophloem. Protophloem observed in 2.64% vascular bundles. The chlorenchymatous bundle sheath cells are always bordered by Kranz mesophyll. An inner mestome sheath borders the phloem, although it may not be complete (Figs. 2 & 3). Only 1.67% of phloem of intermediates is completely surrounded by mestome sheath. Intermediate bundles are ranged from 121.06 to 165.12 μ^2 with a mean of 144.32 ± 12.44 μ^2. Quite a large range of variation in bundle area was noted. Xylem and phloem area respectively 89.24 ± 9.94 μ^2 and 41.25 ± 2.76 μ^2. On average, 61.83% and 28.58% of vascular bundle area consists of xylem and phloem tissue respectively. Phloem area was 46.22% of xylem area. Phloem well developed and consists of sieve tube and companion cells. Some metaxylem has sclerified cell wall.

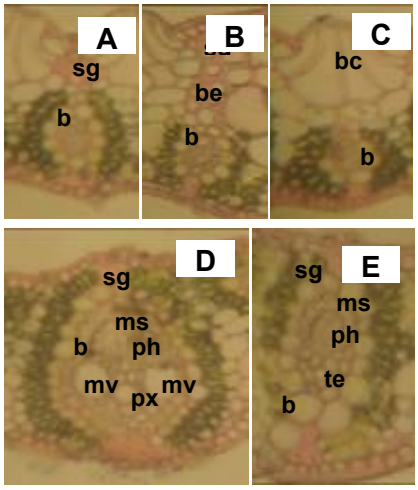

Fig. 3. Leaf blade vascular bundles of the cultivar Ishurdi-32. A, B & C = small vascular bundles. A = small bundle in-between two bulliform patch; B = small blade bundle nearest to midrib with strong bundle sheath extension touching adaxial sclerenchyma patch; C = small bundle topped bulliform patch. sg = sclerenchymatous girder; b = bundle sheath layer surrounded by Kranz mesophyll; ms = mestome sheath; ph = phloem; mv = metaxylem vessels; te = tracheary elements; D. large vascular bundle observed at certain interval; bundle sheath surrounds by Kranz sheath followed by empty sheath attached with Kranz mesophyll. Xylem & phloem tissue typical to monocot vascular bundle. E. Also a large bundle radially elongated lacking vessels member. bc = bulliform cell.

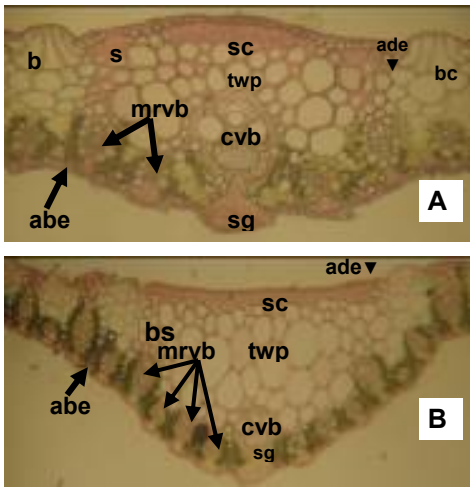

Fig. 4. Transverse section of sugarcane leaf through midrib of the cultivar Ishurdhi-32 (A) and Ishurdhi-20 (B). It consists of one central large vascular bundle flanked by few (A) to many small vascular bundle. Central bundle lacking Kranz characteristics and thin wall bundle sheath have transformed into fibrous sheath. Small bundle have typical Kranz tissue. ade = adaxial epidermis; abe = abaxial epidermis; bc = bulliform cell; sc = hypodermal sclerenchyma; twp = vascular bundle; cvb = central vascular bundle; sg = sclerenchyma girder.

Unlike large and intermediate blade vascular bundles, small (third order) bundles occupy only about half the thickness of the flat portions of the blade in both the genotypes. It is attached with abaxial epidermis and topped by large bulliform cells of the adaxial epidermis (Figs. 2 & 3). The small bundle consists entirely of metaxylem and metaphloem; and has thick walled sieve tubes. The vascular bundle area as determined is 71.24 ± 9.66 μ^2 and 90.75 ± 14.53μ^2 in Ishurdi 20 and Ishurdi 32 respectively. Small vascular bundle of Ishurdi 32 is larger than Ishurdi 20. Xylem and phloem area ranged from 36.66 to 51.34 μ^2 (mean 42.58 ± 3.44μ^2) and 12.81 to 22.66 μ^2 (mean 16.72 ± 1.09 μ^2) in Ishurdi 20 respectively; and those in Ishurdi 32 are 49.84 to 69.72 μ^2 (mean 60.62 ± 8.29 μ^2) and 14.77 to 27.62 μ^2 (mean 21.84 ± 3.44μ^2; Table 1). Almost 60-67% of vascular bundle area are consists of xylem tissue while 23-24% consists of phloem tissue in both the genotypes. Phloem

consists of 36-39% of xylem components per vascular bundle. Mestome sheath is almost absent or a few isolated thick walled cells were visible in 2.93% and 0.67% around phloem in Ishurdi 20 and Ishurdi 32 respectively. Metaxylem vessel area of large vascular bundles ranged from 3.24 – 5.16 μ^2 (mean 4.61 ± 0.24 μ^2) and 2.94 – 3.44 μ^2 (mean 3.27 ± 0.44μ^2) respectively in Ishurdi 20 and Ishurdi 32; reflected a clear genotypic difference in metaxylem vessel area. Bundle sheath cell number in first and third order leaf blade vascular bundle ranged from 21–25 and 6–8; and 25–31 and 8–11 number per vascular bundle respectively in Ishurdi 20 and Ishurdi 32. The second order vascular bundles (only recorded in Ishurdi 20) have 10–14 cells in the bundle sheath. Bundle sheath cell size ranged from 0.47–1.19 μ^2, 1.97–2.84μ^2 and 1.61–2.44μ^2 respectively in large, intermediate and small vascular bundles of Ishurdi 20 and these in Ishurdi 32 are 0.41–1.04 μ^2 and 1.21–2.14 μ^2 .Bundle sheath cells are smaller in size in large bundles compared to intermediate and small bundles and gradual increase in cell size noted from large to small bundles (Figs. 2 & 3).

Midrib anatomy differs from leaf blade anatomy. The ground tissue of midrib is composed of large colorless parenchymatous cells (Figs. 4, 5 & 6) which account for most of the thickness of the leaf blade midrib. The region have a very large central longitudinal vascular bundle in the centre much deep inside the ground tissue from abaxial surface. The size of the central vascular bundle is 297.06 ± 21.14 μ^2 (range 244–331 μ^2) in Ishurdi 20 and 264.44 ± 21.91 μ^2 (range 204–295μ^2) in Ishurdi 32. Central bundle is flanked by at least 4–7 small vascular bundles (Figs. 4, 5 & 6) in Ishurdi 20 and 3–4 in Ishurdi 32. Small midrib bundle is very similar in size to those described in leaf blade small vascular bundle. Vascular bundles in the midrib region is associated with longitudinal patches or strands of hypodermal sclerenchyma formed beneath the abaxial epidermis which is almost 2/3rd of the vascular bundle size in Ishurdi 20 and 1/5th in Ishurdi 32. It provides strong hypodermal girder of the midrib to withstand any mechanical damage of the leaf. In addition, in the midrib region, a continuous sub epidermal layer of sclerenchyma is formed beneath the adaxial epidermis separating it from the colorless cells below (Figs. 4, 5 & 6). Adaxial sub-epidermal sclerenchyma consists of few larger in Ishurdi 20 and several larger in Ishurdi 32. The central large midrib vascular bundle is a maize type bundle having large hard bast over the phloem extended up to sub epidermal (abaxial) patch of sclerenchyma. Vascular bundle completely surrounded by sclerenchyma bundle sheath with well developed phloem and xylem like those of large vascular bundles of the leaf-blade. But it differs from large leaf blade bundles in respect of complete lack of a chlorenchymatous bundle sheath and associated Kranz mesophyll. The chlorenchymatous bundle sheath has been replaced by a sclerenchyma sheath which merges with the hypodermal sclerenchyma girders on its abaxial surface.

The small midrib vascular bundles are round in shape surrounded by a large chlorenchymatous bundle sheath without any visible mestome sheath. Kranz mesophyll located on two sides of the bundle sheath, the abaxial and adaxial ends lack Kranz tissue. Vascular bundle consists of metaphloem and metaxylem elements only. The small bundles are attached with each other with little visible space between them.

When the epidermal thickness of the two genotypes was compared, it was observed that Ishurdi 32 presented highest thick epidermis (9.32 ± 0.42 μ) on the adaxial surface but in Ishurdi 20 it is 7.16 ± 0.15μ. The outer wall thickness of adaxial epidermal cell is very thick in Ishurdi 32 and measured 4.21 ± 0.11μ.This genotype also has heavy deposit of cuticle. Bulliform cells also occupy a considerable area in the blade anatomy. It is always situated in the adaxial epidermis and topped by small vascular bundles. The genotype Ishurdi-20 has 91.66 ± 11.55μ^2 near the midrib and 99.24 ± 14.67μ^2 far away from the midrib. In Ishurdi 32 similar pattern emerge but have more cross sectional area covered by bulliform cells (112.33 ± 21.64 μ^2 and 127.73 ± 24.44 μ^2).

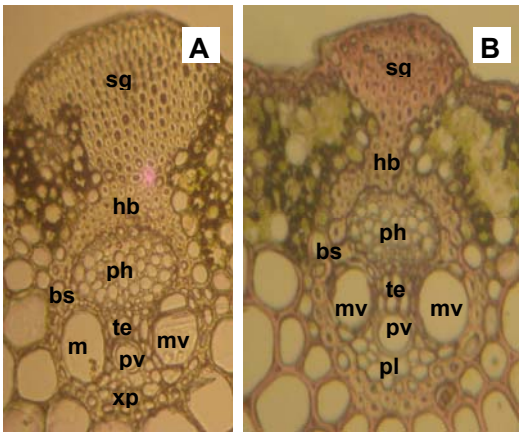

Fig. 5. Magnified view of central midrib vascular bundle of the cultivar Ishurdi-20 (A) and Ishurdi-32. Bundle sheath cells have been transformed into thick walled fibrous tissue and lacking Kranz structure. Vascular bundle looks typical monocot bundle in tissue composition and arrangement sg = sclerenchyma girder; hb = hard bast; bs lignified fibrous bundle sheath; p = phloem; mv = metaxylem vessel; pv = protoxylem vessel; pl = protoxylem lacuna; te = tracheary elements.

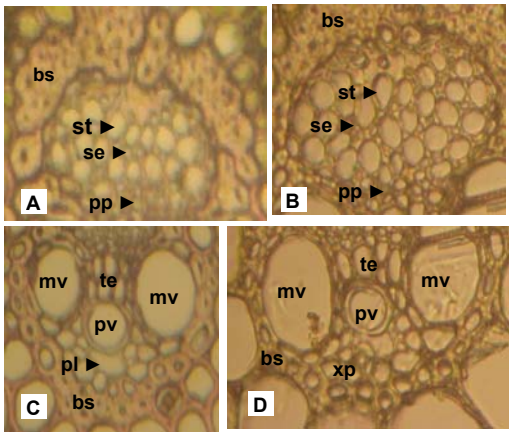

Fig. 6. Magnified view of midrib central vascular bundle showing hloem (A & B) and Xylem (C&D). bs = bundle sheath highly lignified and fibrous in nature; st = sieve Tube thick walled; se = sieve elements; pp = obliterating protophloem; mv = Metaxylem vessel; pv = protoxylem vessel; pl = protoxylem lacuna; te = tracery Elements; xp = xylem parenchyma.

Discussion

In most of the leaf-blade studied the maximum lateral cell count is two i.e. there are two Kranz mesophyll cells between chlorenchymatous bundle sheath as observed by others (Hatersley and Watson 1975, Colbert and Evert 1982). Three order vascular bundles in leaf-blades of sugarcane have been reported (Colbert and Evert 1982, Elahi and Ashraf 2001) and in grasses (Ellis 1976). Some times, however, as seen in Ishurdi 32 two or three small bundles may occur successively or a large bundle may be flanked by an intermediate one as observed by Colbert and Evert (1982).

The net work of intercellular air spaces in the leaf-blade occupies a considerable area. It is almost 14-16% in sugarcane (Colbert and Evert 1982) but Byott (1976) reported to be 2.8% only. Middle of bundle to middle of bundle distance on average 0.122 mm as reported by Colbert and Evert (1982); and bundle to bundle distance ranged from 6-20 μ (Elahi and Ashraf 2001) in sugarcane leaf-blade. The thick walled sieve tubes in sugarcane like maize are undignified (Walsh 1974, Evert *et al*. 1978).

Lower proportion of phloem in sugarcane cultivar has been reported with significant variation among genotypes (Ferreira *et al.* 2007). When xylem and phloem area were expressed in percentage over total vascular bundle area, it is greater in Ishurdi 20 compared to Ishurdi 32. But percentage of phloem area over xylem area is high in Ishurdi 32 which is 83.09%. It implies that Ishurdi 32 have greater proportion of phloem complex compared to xylem. Higher proportion of phloem is related with transportation of herbicide (Ferreira *et al.* 2007).

Presence of protophloem in large and intermediate bundles has been reported by Colbert and Evert (1982). Isolated thick-walled cells were also found in an inner sheath position next to xylem. Isolated thick walled cells of the type noted in vascular bundles have been reported in several sugarcane genotypes (Colbert and Evert 1982). Lack of mestome sheath in small bundles have also been reported (Colbert and Evert 1982, Elahi and Ashrat 2001).

Mesophyll chlorenchyma has been noted in Ishurdi 20 over the phloem area of the vascular bundle. Lack of parenchymatous bundle sheath and Kranz tissue in midrib central bundle with sclerenchymatous bundle sheath and multilayered hard bast has been observed in sugarcane by Colbert and Evert (1982). The abaxial epidermis of both the genotypes has moderate thick cells and cell wall variation in adaxial epidermal cells has been reported (Ferreira *et al.* 2007). Adaxial epidermal cell thickness is more important in herbicide interception and penetration (Hess and Falk 1990). Significant varietals differences reported in respect of area bulliform cells occupy (Elahi and Ashrat 2001).There is modified cells of adaxial epidermis with very thin cell wall (Ferreira *et al.* 2007).

Wakkar (1875) found that the band formed by motor cells were widest in the neighborhood of the midrib. Ferreira *et al.* (2007) found strong lignifications on walls of epidermal cells but absent of signification on walls of bulliform cells. Moreover bulliform cells are more radially elongated than epidermal cells. Ishurdi 32 is more sensitive to water loss from the leaves and start curling when their moisture content are reduced compared to Ishurdi 20. Evan (1939) reports sensitive varieties have greater motor cells than non-sensitive sugarcane. Dunlop (1913) correlated the anatomical traits of the leaf blade of sugarcane in respect of curling and varieties with prominent motor cells are sensitive to water lose. Van Dallewijn (1962) reports existence of certain relationship between the anatomy of the leaf and curling habit in sugarcane.

Conclusion

Kranz system with well developed bundle sheath associated with Kranz mesophil in the leaf blade were observed but Kranz tissue absent in midrib region. Large and small vascular bundles alternate all alone the leaf blade. Bulliform cell well develop indicates zeric adaptation. Two cultivars differ in respect of quantitative expression of Kranz tissue.

References

Black CC, Campbell WH, Chen TM and Dittrich P. 1973. The monocotyledons: their evolution and comparative biology. III. Pathway of carbon metabolism related to net carbon dioxide assimilation by monocotyledons. *Q Rev Biol* 48, 299-313. http://dx.doi:10.1086/407592

Bourdu R. 1976. Discussion sur les caracteristiques structurales et infra-structurales des feuilles en function de l'appartenance aux trois types metaboliques. *Physiol Veg* 14, 551-561.

Brown WV. 1975. Variation in anatomy, associations and origin of Kranz tissue. *Am J Bot* 62, 395-402. http://dx.doi:10.2307/2442093

Byott GS. 1976. Leaf air space systems in C3 and C4 species. *New Phytol* 76, 295-299. http://dx.doi:10.1111/j.1469-8137.1976.tb01464.x

Colbert JT, Evert RF. 1982. Leaf structure in sugarcane (*Saccharum officinarum* L.) *Planta* 156, 136-151. http://dx.doi:10.1007/BF00395428

Crookston RK, Moss DN. 1974. Intermeinal distance for carbohydrate transport in leaves of C3 and C4 plants. *Crop Sci* 14, 123-125. http://dx.doi:10.2135/cropsci1974.0011183X001400010038x

Dunlop WR. 1913. Stomatal characteristics of varieties of sugarcane. *West Indies Bull* 13, 314-323.

Elahi NN, Ashraf M. 2001. Study of various sized leaf vascular bundles and surrounding tissues of six sugarcane varieties. *Pak J Biol Sci* 4, 1078-1082. http://dx.doi:10.3923/pjbs.2001.1078.1082

Ellis RP. 1976. Procedure for standardizing comparative leaf anatomy in the Poaceae. I. The leaf-blade as viewed in transverse section. *Bothalia* 12, 65-109.

Evan H. 1939. Some aspect of the problem of drought resistance in sugarcane. *Proc. Int. Soc. Sugarcane Technol* 6, 802-808.

Evert RF, Eschrich W, Heyser W. 1978. Leaf structure in relation to solute transport and phloem loading in *Zea mays* L. *Planta* 138, 279-294. http://dx.doi:10.1007/BF00386823

Ferreira EA, Ventrella MC, Santos JB, Barboso MHP, Silva AA, Procopio SO, Silva E AM. 2007. Leaf blade quantitative anatomy of sugarcane cultivars and clones. *Planta Daninha* 25, 25-34. http://dx. http://dx.doi:10.1590/S0100-83582007000100003

Gallaher RN, Ashley DA, Brown RH. 1975. 14 C- photosynthesis translocation in C 3 and C 4 plants as related to leaf anatomy. *Crop Sci* 15, 55-59. http://dx.doi:10.2135/cropsci1975.0011183X001500010016x

Hatch MD, Osmond CB, Slatyer RO. 1971. *Photosynthesis and photorespiration*. Wiley- Intersience, John Wiley and Sons, New York 565p.

Hatersley PW, Watson L. 1975. Anatomical parameters for predicting photosynthetic pathways of grass leaves: the maximum lateral cell count and the maximum cells distance count. *Phytomorphology* 25, 325-333.

Hattersley PW, Watson L, Osmond CB. 1976. Metabolite transport in leaves of C4 plants: specification and speculation. Transport and Transfer Processes in Plants; Proceedings of a Symposium. pp. 191-201.

Hess FD, Falk RH. 1990. Herbicide deposition on leaf surface. *Weed Sci* 38, 280-288.

Johnson SC, Brown WV. 1973. Grass leaf ultrastructural variations. *Amer J Bot* 60, 727-735. http://dx.doi:10.2307/2441166

Lush WM. 1976. Leaf structure and translocation of dry matter in a C3 and C4 grass. *Planta* 130, 235-244. http://dx.doi: 10.1007/BF00387827

McDavid CR, Midmore DJ. 1980. 14 C fixation and translocation in sugarcane clones with contrasting weights of leaf per unit weight of cane and storage cell volumes. *Ann Bot* 46, 479-483.

Metcalfe CR. 1960. *Anatomy of the monocotyledons. I. Gramineae*. Clarendon Press. Oxford.

Schwendener S. 1890. Die Mestomscheiden der Gramineen blatter. *Konig Preuse Akad Wiss (Berlin)* 405-426.

Stephenson RA, Brown RH, Ashley DA. 1976. Translocation of 14 C-labled assimilate and photosynthesis in C3 and C4 species. *Crop Sci* 16, 285-288. http://dx.doi:10.2135/cropsci1976.0011183X001600020033x

Van Dallewijn C. 1962. *Botany of sugarcane*. Waltham: The Chronica Botanica co.: Book Departament. 371 p.

Wakkar JH. 1875. De stand der suikerriet bladen bij vocht en drought. *Archief java-suikerind* 3, 41-48.

Walsh MA. 1974. Late-formed metaphloem sieve-elements in *Zea mays* L. *Planta* 121, 17-25. http://dx.doi:10.1007/BF00384002.