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Study of Various Sized Leaf Vascular Bundles and Surrounding Tissues of Six Sugarcane Varieties

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Abstract: Three sized vascular bundles and significant difference in the distance between these vascular bundles and different types of motor cells in the leaf blades of all six sugarcane varieties (i.e., CP84-1198, CP85-1491, CP88-1165, CP89-846, TCP86-3368, CP77-400) were noted and these results indicated the existence of relationship between leaf cytology and morphological characters like curling habit.
Large vascular bundles were characterized by the presence of large metaxylem vessels on either side of the protoxylem. The cellular composition of large vascular bundles indicated that the phloem loading in large vascular bundle is an apoplastic step. In some varieties large motor cells were observed. Cytological differences in leaf blade indicated the difference in morpho-physiological characters in all the varieties.

Key words: Motor cells, curling habit, vascular bundle, C_4 plant, phloem loading.

Introduction

Structural diversity among plant species precludes a universal pathway for assimilate movement from the photosynthetic tissues to the phloem. The distribution of plasmodesmata, the cellular composition of the veins involved in phloem loading and the structural relationships between these veins and adjacent photosynthetic tissues have been shown to be species specific (Fisher, 1986; Schmitz *et al.*, 1987; Botha and Evert, 1988; Gamalei, 1989; McCauley and Evert, 1989). Sugarcane is a large tropical grass and is regarded as the most efficient of all storers of the sun's energy (Ledon and Gonzales, 1950). It is a C_4 plant and can use solar radiation to the maximum without being light saturated (Nayyar, 1989). Sugarcane has been the subject of many studies on sugar transport and accumulation (Hartt *et al.*, 1963; McDavid and Midmore, 1980) and both a description of the leaf vasculature (Colbert and Evert, 1982). While some studies have been made on the photosynthetic tissues (Laetsch, 1974) and the phloem of sugarcane (Singh, 1980).
The principal objective of the present study was to determine the cellular composition of different sized vascular bundles and surrounding tissues of the leaf blade of different sugarcane varieties under study.

Materials and Methods

Leaf samples of six sugarcane varieties i.e. CP84-1198, CP85-1491, CP88-1165, CP89-846, TCP86-3368 and CP77-400 were collected from the experimental field area of National Agriculture Research Centre, Islamabad, Pakistan. Special care was taken to select the materials that had reached the same stage of development. On the average, 10 individual samples of each variety were tested. Tissue samples were collected from blades of mature fully expanded leaves. Approximately, 2-4 mm^2 tissue samples were excised from either side of the midrib, at a point midway along the length of the blade and fixed in 5% glutaraldehyde in phosphate buffer, pH 7.2, containing 0.5% tannic acid for eight hours at room temperature.
Sugarcane leaf tissues exhibited excellent preservation when treated with 0.5% tannic acid (Robinson-Beers and Evert, 1991).
Post-fixation in 2% Osmium tetroxide was carried out overnight at 4 °C. The tissue samples were then dehydrated in a graded acetone series and embedded in Epon 812 resin. Blocks were then trimmed with common blades under Bausch and Lomb dissecting microscope. The semithin sections were cut with ultratom, LKB-Bromma 2088, and stained with 1% aqueous Toluidine blue and studied under NIKON optiphot research microscope and photographs were taken on Kodak 100 VX film.
Statistical analysis of the mean distances from large vascular bundles to small vascular bundles and from intermediate vascular bundles to small vascular bundles were conducted using analysis of variance technique (Steel and Torrie, 1980). Then Duncan's multiple range tests at 5% probability level was performed to determine the significance of means.

Results

Three sizes or orders of longitudinal vascular bundles were observed in mature leaf blade of six sugarcane varieties. These were large (First order), intermediate (second order) and small (third order).
The large vascular bundles were rhomboid or oval shaped (Fig. 1), the medium ones were oval shaped (Fig. 2), and small vascular bundles were round in shape (Fig. 4). The small vascular bundles were situated near the lower epidermis, while the large and medium ones were found in the center of the blade. The large vascular bundles were always flanked by two small ones (Fig. 3).
The small and medium sized vascular bundles occurred between the large bundles alternately (Fig. 3).
It was noted that the distances between various sized vascular bundles were different within different sugarcane varieties.

Distances from large vascular bundles to small vascular bundles: Among six different sugarcane varieties significant difference for the distance from large vascular bundles to small vascular bundles were noted. Its maximum value was noted in the variety CP89-846 (60 μm) and the minimum value was found in the variety CP88-1165 (20 μm). The difference in the distance from large vascular bundle to small vascular bundles was significant for all the varieties (Table 1).

Distances from intermediate vascular bundles to small vascular bundles: Variety CP85-1491 and CP89-846 had similar and maximum values of distance from intermediate to small vascular bundles with the value of 60 μm. The varieties CP84-1198, CP88-1165, TCP86-3368 and CP77-400 had minimum

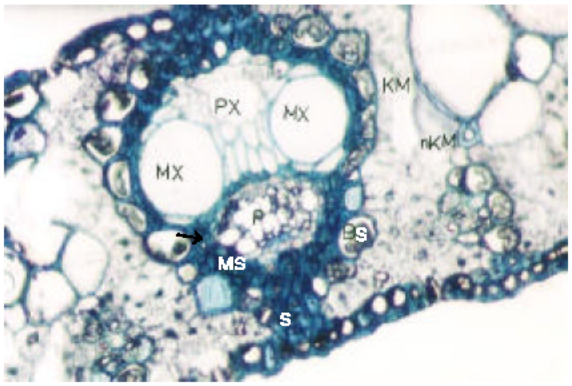

Fig. 1: Photomicrograph of transverse section from mid portion of leaf blade of sugarcane variety CP88-1165, showing large vascular bundle (X416), (BS, chlorenchymatous bundle sheath; KM, Kranz mesophyll; nKM, non-kranz mesophyll; MS, mestome sheath; P, phloem; PX, protoxylem; MX, metaxylem; S, hypodermal schlerenchyma; Unlabeled arrow point to mestome- sheath-like cells.

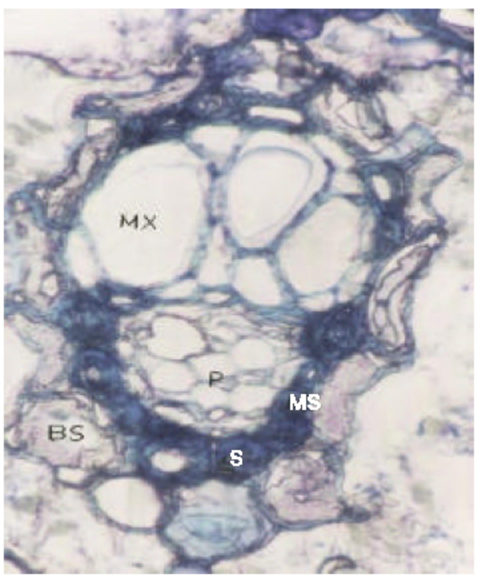

Fig. 2: Photomicrograph of transverse section from portion of leaf blade of sugarcane variety CP85-1491, showing intermediate vascular bundle (X416), (BS, chlorenchymatous bundle sheath; MS, mestome sheath; P, phloem; MX, metaxylem; S, hypodermal schlerenchyma).

and similar distance from intermediate to small vascular bundles with the values of 50 μm (Table 1).

Other characters of vascular bundles and surrounding tissues: The large vascular bundles were characterized by the presence of large metaxylem vessels on either side of protoxylem. Such bundles also posses both protophloem and metaphloem, but the former was usually obliterated in mature bundles.

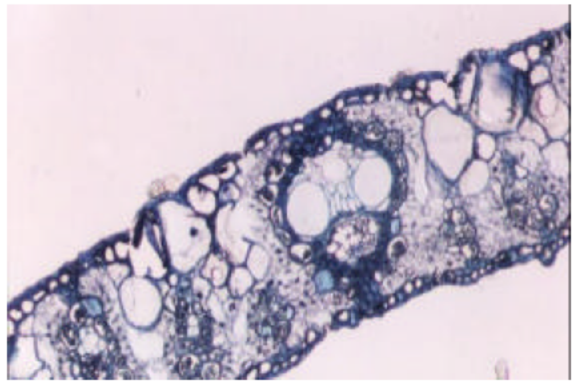

Fig. 3: Photomicrograph of large vascular bundle (Centre) and two small vascular bundles (right and left), from the transverse section of leaf blade of the sugarcane variety CP88-1165 (X208).

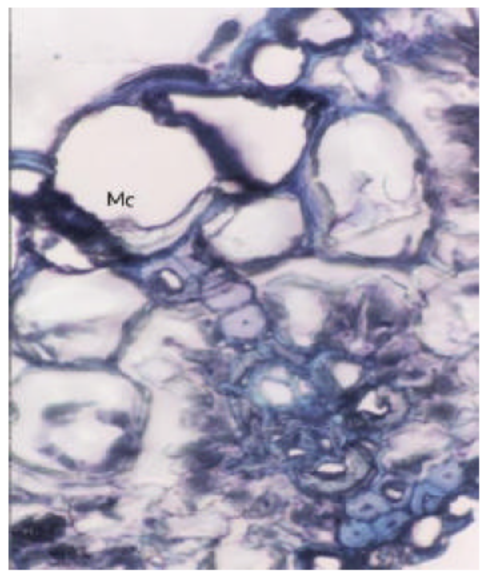

Fig. 4: Photomicrograph of the small vascular bundle from the transverse section of leaf blade of the sugarcane variety CP89-846 (X832), (Mc, motor cell).

Mestome sheath like cells sometimes occurred at the interface between the xylem and phloem of the large vascular bundles (Fig. 1).

Intermediate vascular bundles, like the large bundles were extended from the lower to the upper epidermis, but lack protoxylem and large metaxylem vessels typical of large bundles (Fig. 2). Intermediate bundles also contain vascular parenchyma cell, most of which abut both the xylem and phloem.

Small vascular bundles occupied only half of the thickness of the flat portion of the blade, being overlapped by large bulliform cells of the upper epidermis (Fig. 4).

The small bundles consist entirely of metaxylem and metaphloem, like the intermediate bundles. Phloem intermediate bundle (Fig. 2).

Table 1: Distances from large to small vascular bundles and from intermediate to small vascular bundles of six sugarcane varieties.

Name of the variety	Mean distances from large vascular bundles to small vascular bundles (μm)	Mean distances from intermediate vascular bundles to small vascular bundles (μm)
CP84-1198	40.0 c	50.0 b
CP85-1491	50.0 b	60.0 a
CP88-1165	20.0 e	50.0 b
CP89-846	60.0 a	60.0 a
TCP86-3368	50.0 b	50.0 b
CP77-400	30.0 d	50.0 b

Any two means of varieties, not sharing a letter in common, differ significantly at 5% probability by using Duncan's Multiple Range Test.

In many of the large bundles the mestome sheath also bordered the xylem laterally, with mestome sheath cells, commonly intervening between the large metaxylem vessels and the chlorenchymatous bundle sheath.

The degree of differentiation of cells in the mestome sheath position, or of suberized elements bordering the chlorenchymatous bundle sheath, also varied with bundle size. Cells, similar in appearance to those of the mestome sheath of large and intermediate bundles also occur along the periphery of the phloem of small vascular bundles, but did not form a sheath. So the extent of the "mestome sheath" vary with bundle size.

The mestome sheath cells associated with large and intermediate bundles were readily discernible with the light microscopy (Figs. 1,2). It was noted that in some large bundles, more than one layer of mestome sheath cells might occur on the phloem side. In contrast to the large and intermediate bundles, the small bundles rarely exhibit distinct thick walled mestome sheath cells (Fig. 4).

Discussion

In all the varieties of sugarcane there were three types of longitudinal vascular bundles in their mature leaf blades as noted by (Artschwager, 1925; Ellis, 1976; Colbert and Evert, 1982).

The distances between various sized vascular bundles, structure and arrangement of motor cells in sugarcane leaf blade varied in number and sizes in all the varieties. Wakkar (1875) found that in Black Charibon the bands formed by motor cells were widest in the neighbourhood of the midrid, about midway between base and tip of the leaf blade.

Evan (1939) stated that some varieties were very sensitive to water loss from the leaves; they start curling when their moisture contents reduced by 1.5 or 2.0 percent. Other varieties are not so sensitive and even continuing to loose water at a fast rate, they do not show any outward sign of distress. Dunlop (1913) correlate the anatomical characters of the leaf blade of some sugarcane varieties with curling and indicated that in the varieties where the motor cells are not prominent and cells immediately interior to the motor cells are generally comparatively large and thin-walled, can readily curl involutely and in the varieties where the motor cells are prominent, large and are firmly attached to the vascular bundles and also where in the leaf the vascular bundles are situated close together shows revolute curling. Van Dellewijn (1952) stated that involute curling is a habit of most varieties, that differ widely as to the extent of curling, and there are varieties which do not curl at all. These observations indicate the existence of certain relationships between the anatomy of the leaf and its curling habit. Keeping in view the above information and results obtained from present study, it is stated that varieties CP84-1198, CP88-1165, CP86-3368 have less curling ability as compared to the varieties CP89-846, CP77-400, CP85-1491.

In sugarcane leaf blade two suberized bundle sheaths were observed. Evert *et al.* (1977); Hattersley and Browning (1981), Botha and Evert (1988), indicated that the walls of the bundle-sheath cells of sugarcane, like those of other NADP-malic enzyme C_4 grasses, contain continuous suberin lamellae in their outer tangential and radial walls.

Robinson-Beer and Evert (1991) reported that in sugarcane, the suberin lamellae are continuous in all walls of bundle-sheath cells bordering the xylem and the suberin lamellae in the radial walls between adjacent bundle-sheath cells do not merge, so the potential exists for apoplastic movement of substances by way of the compound middle lamellae. By the use of space marker studies on maize by Evert *et al.* (1977) and on sugarcane by Both and Evert (1986) indicated that apoplastic movement of water and solutes does occur along this pathway. Using fluorescent tracers, Peterson *et al.* (1985) and Eastman *et al.* (1988) have also demonstrated that neither suberized mestome sheath nor parenchymatous bundle sheath function in a restricted sense as an endodermis, but allow the free passage of apoplastic water and solutes from the veins to the mesophyll. Evert *et al.* (1977) and Canny (1986) have further proposed that the function of the suberin lamellae is to separate the outward flux of water from the inward flux of photoassimilates.

In addition to a suberized chlorenchymatous bundle sheath, the large vascular bundles of the sugarcane leaf blade also possess a suberized mestome sheath, which in some bundles consists of more than one layer of cells on the phloem side. A partial mestome sheath, usually consisting of a single layer of cells; border the phloem of the intermediate vascular bundles. Some mestome - sheath cells are interposed between the outer bundle sheath and the phloem of the small vascular bundles. Thus, in sugarcane, more than one layer of suberized cells must often be traversed by photoassimilates during their passage from the chlorophyllous cells to the sieve tubes.

Brown (1975) proposed that the outer chlorenchymatous bundles sheath of some C_4 grass species, known today to be NADP-malic enzyme types, is homologous with the mestome sheath of other C_4 species and all C_3 grass species at least with regard to the large bundles.

Fritz *et al.* (1989) indicated that the large vascular bundles in the maize leaf are primarily transport bundles, it seems likely that at least some photosynthate enters the large bundle sieve tubes from the surrounding photosynthetic tissues.

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 is very low plasmodesmatal frequencies between all components of the large-bundle phloem in sugarcane. These observations show that phloem loading in the large vascular bundles in the leaf blade of sugarcane surely involves an apoplastic step.

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