



NADP-ME Subtype C₄ Grass *Saccharum spontaneum* L.: An Anatomical Study

Md. Mahabubur Rahman^{1*}, Mst. Shermin Khatun², Md. Shahriar Ahmed Sonet², Mst. Kaniz Fatema², Ayesha Siddika², Rubaiyat Sharmin Sultana³

¹Department of Crop Botany, EXIM Bank Agricultural University Bangladesh, Chapainawabganj-6300, Bangladesh

²Faculty of Agriculture, EXIM Bank Agricultural University Bangladesh, Chapainawabganj-6300, Bangladesh

³Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh

ARTICLE INFO	ABSTRACT
<p>Received date: January 15, 2021</p> <p>Accepted date: August. 16, 2021</p>	<p>Leaf blade of <i>Saccharum spontaneum</i> L. was scrutinized microscopically in the present study. Surface features and internal structures in leaf blades from plants grown in natural terrain were examined in peelings and transverse sections after staining with 1% aqueous solution of safranin. Different types of cell diagram were measured from the photographs. In both abaxial and adxial surfaces of the leaf blade, stomata, cork cells, micro hairs, long and short cells with sinuous wall were recognized. Epidermal cells (15.13µm) were found compactly arranged with oval shaped cells. Mesophyll layer composed of chlorophyllus cells, were 31.04 µm in diameter with irregular intercellular space and in between large air spaces were attended. Mesophyll cells surrounded chlorophyllus bundle sheath (14.17 µm in diameter) in a ring like fashion. The distance between vascular bundles were met up by two mesophyll cells, suggesting C₄ plant characteristics. Sclerenchymatous cells (12.71 µm) were recorded excessive in the region of large vascular bundle. In vascular bundle, phloem set with sieve tube and companion cell elements and xylem is composed of vessel, fibers and xylem parenchyma. A fiber cell was measured 10.58 µm in average diameter. The group vessel was observed, will be an identical feature for this grass studied. Lysigenous cavity in protoxylem region of large vascular bundle was a notable feature for this grass. The absence of thickened wall mestome sheath around vascular bundle and exclusive presence of bundle sheath cells layer, are the features of NADP-ME subtype C₄. The results of the present study are specifying that the experimented plant <i>S. spontaneum</i> is a NADP-ME subtype C₄ grass. The anatomical features of the grass studied here will help to resolve taxonomic problems of the Gramineae family.</p>

Keywords: C₄ grass, Kranz anatomy, Leaf blades, NADP-ME subtype, *Saccharum spontaneum*, Wild sugarcane

*CORRESPONDENCE

spmahabub@yahoo.com

Department of Crop Botany, EXIM Bank Agricultural University Bangladesh, Chapainawabganj-6300, Bangladesh

1. INTRODUCTION

Wild sugarcane (*Saccharum spontaneum* L.), a perennial grass, is native to the Indian subcontinent (Panje, 1970; Yadav et al., 2007). Biotypes have been recorded from Turkestan, Uzbekistan, Afghanistan, Bangladesh, Indonesia,

Iran, Israel, Japan, Jordan, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Syria, Thailand, Vietnam, and northern and eastern regions of Africa (Anon, 1972). This plant is common along the bank of rivers, lakes and ponds, beside highways and railways, and across waste ground (Holm et al., 1997).

To Cite: Rahman, M. M., Khatun, M. S., Sonet, M. S. A., Fatema, M. K., Siddika, A. & Sultana, R. S. (2022). NADP-ME subtype C₄ grass *Saccharum spontaneum* L.: an anatomical study. *EBAUB J.*, 4, 1-8.

Saccharum spontaneum is a coarse grass, thereby, not favored by cattle and is generally grazed only in times of scarcity. It has, however, been reported as good fodder for goats and camels (Thakur, 1984) and suitable for the production of silage (Komarov et al., 1963). Its extensive rhizome network makes it a very efficient binder of soils. It provides good thatching material and also used in production of ropes, mats and brooms. Pulp of *S. spontaneum* is suitable for wrapping, writing and printing, also for the production of grease proof paper. Its slow rate of decomposition makes it an excellent mulching material (Wapakala, 1966).

The common types of Gramineae are C_3 and C_4 grasses. C_3 plants are defined as the plants that exhibit the C_3 pathway and use the Calvin cycle in the dark reaction of photosynthesis. Approximately 95% of the plants are C_3 type. Based on the differences in leaf vascular systems, C_3 grasses are presented in three subgroups: bambusoid, panicoid and festucoid. The bambusoid and panicoid C_3 grasses have leaves with a denser vascular system than that of festucoid C_3 grasses. Interestingly, the festucoid C_3 grasses tend to have larger stomata in the leaf blade than bambusoid and panicoid C_3 grasses, but it shows a lower density of stomata (Ashida & Sugino, 1984).

The C_4 plants are defined as the plants that use the C_4 pathway or Hatch-slack pathway during the dark reaction. The leaves possess kranz anatomy, and the chloroplasts of these plants are dimorphic. About 5% of plants on earth are C_4 plants. In C_4 photosynthesis, atmospheric CO_2 is initially fixed in the mesophyll cells while, decarboxylation and refixation of CO_2 occur in the bundle sheath cells (Hatch, 1987). There are three subgroups in C_4 plants differing in the process of decarboxylation of C_4 acids i.e. the main enzyme used for decarboxylation: NADP-malic enzyme (NADP-ME), NAD-malic enzyme (NAD-ME) and phosphoenol pyruvate carboxykinase (PCK) (Hatch, 1987). The difference in biochemical function is associated with that in relation to the structural features of leaves. The layer of cells between the bundle sheath cells and the vascular bundle is absent in the NADP-ME subtype. Comparatively, the vasculature of the NAD-ME subtype is usually surrounded by a double sheath, consisting of the outer bundle sheath and the inner non-photosynthetic mesophyll sheath (Prendergast et al., 1987; Lundgren et al., 2014).

The bundle sheath cells of the C_4 subtypes also differ in the structure, intracellular position and amount of the chloroplasts and mitochondria (Hatch, 1987; Prendergast et al., 1987; Yoshimura et al., 2004). The quantitative balance of photosynthetic tissues (Hattersley, 1984; Dengler et al., 1996) and organelles (Yoshimura et al., 2004) between the mesophyll and bundle sheath cells reflects the difference in biochemical function of the photosynthetic subtypes.

Numerous reports on foliar anatomy and micro morphology are used for delimiting the deferent groups or tribes in the Poaceae family (Ellis, 1986; Ahmad et al., 2011). Epidermal cells, stomata and hairs in dermal tissue system have already been established as important tools in delimitation of taxa in many plant families (Stenglein et al., 2003; Gajendera et al., 2009). Anatomical features of leaf

can help to explain taxonomic relationships at different levels (Davila & Clark, 1990; Cai & Wang, 1994) and these leaf anatomical characters are of great value in grass systemization and characterization of broad groups within the grasses, particularly among the subfamilies and tribes. Sclerenchyma and bundle sheath (kranz sheath) amount, sclerenchyma width, leaf indumentums and length and frequency of epidermal cell are important features that can identify relationships among the genera of Poaceae (Jarves & Barkworth, 1992; Yousaf et al., 2008). The leaf thickness, number and arrangement of vascular bundles might be systematically useful and the distribution of prickles may be relatively stable or environmentally variable (Ellis, 1986). The position of vascular bundles in the blades appears to be a useful diagnostic character above the generic level (Ellis, 1976). To resolve the taxonomic problems within the tribe, anatomical studies could be an important tool.

The anatomy of grasses under the genus *Saccharum* has been described in species of *Saccharum officinarum* L. (Neufeld et al. 1992; Ferreira et al. 2007).

The reports of studied plant *S. spontaneum* L. have already been carried out in different contexts. Zhen et al. (2020) reported on genome-wide identification and expression profile analysis of WRKY family genes in the autopolyploid. Yang et al. (2020) discussed on enhanced sugar accumulation and regulated plant hormone signaling genes contribution to cold tolerance in hypoploid. A genetic linkage map of this grass was reported by Al-Janabi et al. (1993). Meng et al. (2020) developed the first set of oligonucleotide (oligo)-based probes based in the *S. spontaneum* genome and characterized the cytological features by the development of a complete set of chromosome-specific oligo probes. NAC transcription factors were accounted in autopolyploid (Wang et al. 2019). A few reports have been reported on anatomical studies of *S. spontaneum* (Ahmad et al. 2010; El-Gawad & El-Amier 2017; Chaudhari et al. 2014; Sukhova & Kazakov 1972).

The objective of the present study was to characterize the anatomy of the leaf blade of *S. spontaneum* aiming to find characteristics that could contribute to the recognition of identical characteristics in the demarcation of deferent groups or tribes in the Gramineae family.

2. MATERIALS AND METHODS

Naturally grown *S. spontaneum* plants at the road side were collected from the Barind region (Fig. 1). For the present study, healthy whole plants with stem, leaves and roots were brought together in the Crop Botany laboratory of EXIM Bank Agricultural University Bangladesh, Chapainawabganj. The free hand sections of leaf blade were used in this experiment which was prepared with stainless steel razor blade. The sectioning and staining procedures pursued the study of Rahman & Sultana (2019; 2020; 2021) and Sultana & Rahman (2020). The stained sections and peels of leaf blade were placed on fresh glass slide and mounted with a clean cover slip by putting a few drops of

glycerin and then observed under light microscope. The photographs were taken with digital camera and then diameter of different cell types was measured. Diameter was randomly accounted from ten cells for each cell type in a photograph. Ten photographs were used here which was performed from one hundred samples. Numbers of stomata were counted in a square unit area (per mm²).

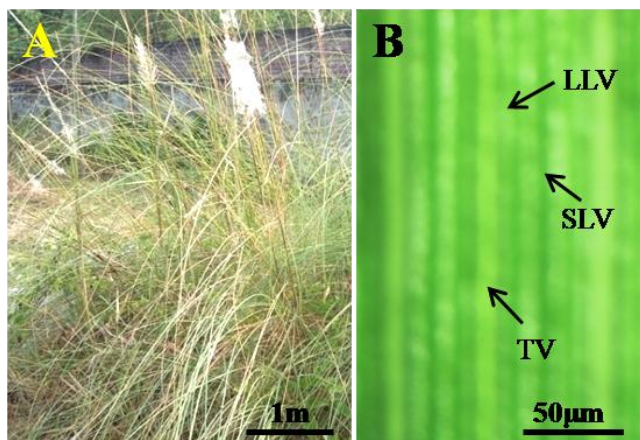


Fig. 1 Experimental plant *Saccharum spontaneum* L. (A) Growing plants in the field condition. (B) Paradermic impression of leaf blade of *Saccharum spontaneum* L. LLV, Long longitudinal vein; SLV, Small longitudinal vein; TV, Transverse vein.

3. RESULTS

3.1. Dermal Tissue System of Leaf Blade

Three types of veins, large longitudinal, small longitudinal and transverse of leaf blades were perceived in the leaf blade surface of *S. spontaneum* L. (Fig. 1A). Three rows of small longitudinal veins were present between two large longitudinal veins. Ligules (average 3 mm in length) were there within the connection of leaf blade and leaf sheath (Fig. 2A).

Both the adxial (upper) and abaxial (lower) epidermis in cross section were composed of one-layer of oval shaped cells. The average diameter of epidermal cell was 15.13 µm (Table 1). Bulliform cells on the upper epidermis were not discriminated from the epidermal cell although it is a common feature of grasses. In peeling of leaf blade, the epidermal cells were set in parallel rows for both surfaces with sinuous anticlinal walls. The both short and long cells were attended at the intercostals and costal zones. The long cells were elongated having no stomata.

The short cells having stomata were lower in number than the long cells. Number of cell row in intercostals zone was lower than the costal zone. Sometimes two large long cells were united by the cork cells (Fig. 3A). Prickles were present in costal zone (Fig. 2B). Some short cells were recognized within large long cells (Fig. 2B). In the intercostal zone i.e. between two stomatic cell rows, three rows of long cells were observed in abaxial surface of the leaf blade (Fig. 2B). Micro hairs were multicellular, subsisted

to the both intercostal and costal zones (Fig. 2B). The upper surface (adaxial side) features wide rounded ribs, separated by shallow V-shaped furrows (Fig. 2C).

The leaf blade of *S. spontaneum* was amphistomatic isobilateral and diameter of stomata was measured 26.46 µm (Table 1). Gramineous type stomata having two dumbbell shaped guard cells are surrounded by triangular subsidiary cells (Fig 2B). The diameter of the subsidiary cells was 58.33 µm (Table 1). The number of stomata in abaxial surface was counted 26/mm² (Table 1).

3.2. Ground Tissue System of Leaf Blade

Between two layers of epidermis in leaf blade a mesophyll layer was there, composed of parenchyma fortified with chloroplasts although it was not differentiated into palisade and spongy parenchyma. Mesophyll cells were hexagon and oval in shape with irregular intercellular space. Those cells were 31.04 µm in average diameter (Table 1). Some parenchyma cells were clustered around the bundle sheath cells in a ring-like fashion (Fig. 2D). The other mesophyll cells arranged compactly and a few small intercellular spaces were there. Two mesophyll cells were separated vascular bundles. Large air chambers were located just above the small vascular bundles in the leaf blade section (Fig. 2C, D).

Table 1 Diameter of different cells of leaf blade in *S. spontaneum* L.

Different plant cells	Diameter in µm ($\bar{x} \pm S.E.$)
Mesophyll cell	31.04 ± 4.52
Epidermal cell	15.13 ± 0.93
Bundle sheath cell	14.17 ± 1.27
Sclerenchyma cell	12.71 ± 1.3
Xylem fiber cell	10.58 ± 1.09
Meta xylem	24.16 ± 3.78
Stomata	26.46 ± 1.08

S. E., Standard error.

In cross section, sclerenchymatous cells were excessive in the region of large vascular bundle, extended from the vascular bundle towards lower epidermis, although it appeared some times in the location of small vascular bundles extended lower epidermis (Fig. 3D).

The recorded size of the sclerenchyma cells was 12.71 µm in average diameter (Table 1). These cells are located beneath the lower epidermal layer. In large vascular bundle at the side of phloem tissues, sheath cells were absent and were replaced by the sclerenchyma cells (Fig. 3D).

Bundle sheath cells are of round or oval shape with chloroplasts; cell walls were thin. Bundle sheath continuously surrounded small vascular bundle that was discontinuous in large vascular bundles (Fig. 3C). Bundle sheath cells were 14.17 µm in average diameter (Table 1). The number of chloroplasts in the bundle-sheath cells is more than that in the mesophyll cells.

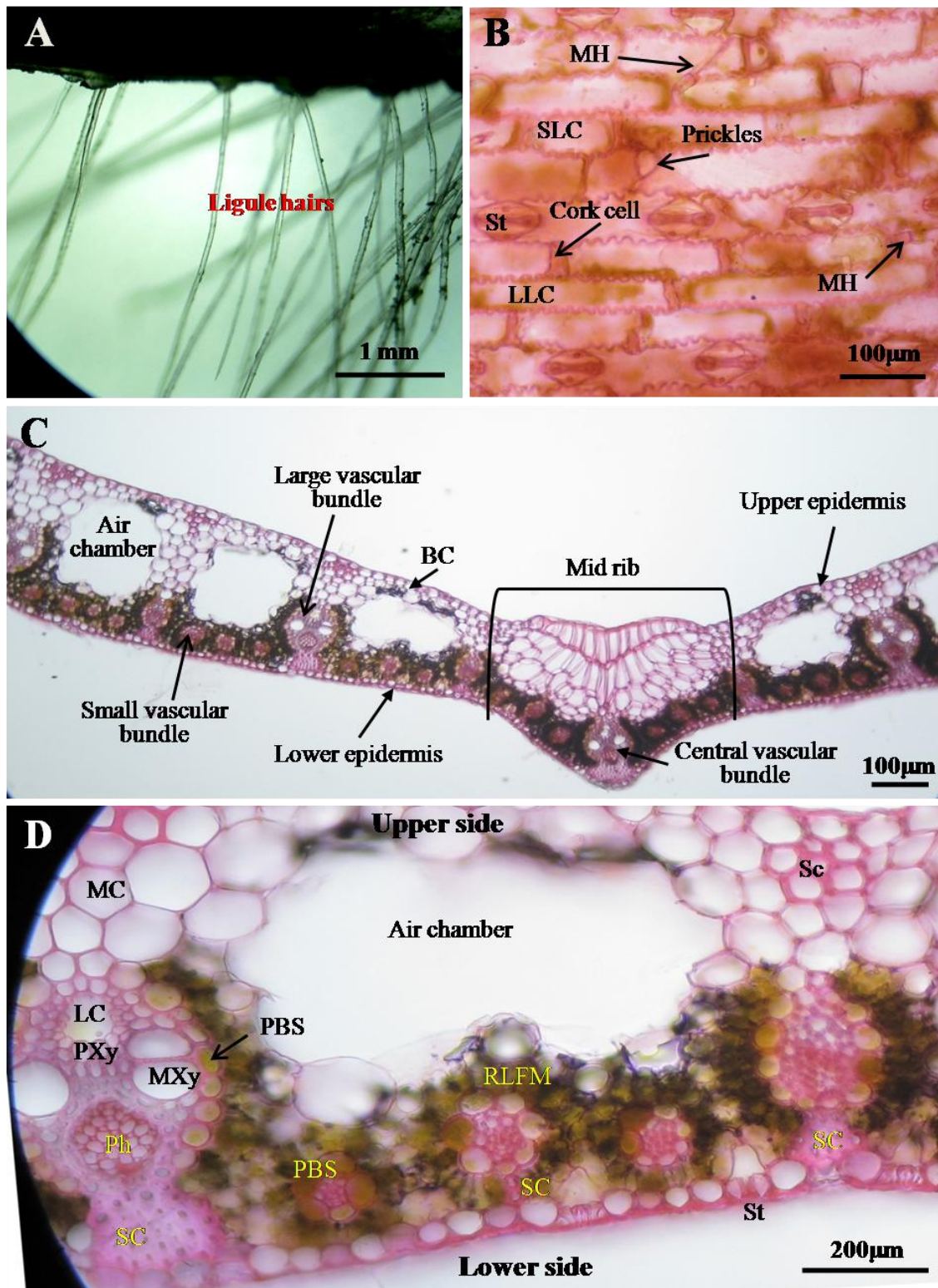


Fig. 2 Anatomy of experimental plant, *Saccharum spontaneum* L. (A) Ligule between leaf blade & leaf sheath. (B) Epidermal layer of leaf blade. (C) Transverse section of leaf blade showing different anatomical portions, (D) a close up view of transverse section of leaf blade having features of two large vascular bundles. BC, Bulliform cell; PBS, Parenchyma Bundle sheath; MC, Mesophyll cell; Ph, Phloem; MXY, Meta Xylem; PXY, Proto Xylem SC, Sclerenchyma, St, Stomata; SLC, Small longitudinal cell; LLC, Large longitudinal cell; MH, Micro hair; RLFM, Ring like fashion mesophyll; LC, Lysigenous cavity.

3.3. Vascular Tissue System of Leaf Blade

The vascular bundles of the leaf blade were associated with strands of hypodermal sclerenchyma in *S. spontaneum*. Vascular bundles were of two types, based on size. Central vascular bundle was similar to large vascular bundle. The chlorenchymatous mesophyll cells were surrounded radially with a ring like structure at vascular bundle. There was abundance of small bundles in comparison to large vascular bundle. Three small bundles were observed within two large bundles (Fig. 3C). All vascular bundles were enclosed with both xylem and phloem. Xylem was found towards the inner surface and phloem towards the outer surface. Xylem and phloem were present in the same radius where cambium was absent (Fig. 3C), therefore vascular bundle type is conjoint collateral closed. The xylem is composed of vessel elements (metaxylem, 24.16µm in average diameter), fibers (10.58µm in average diameter) and xylem parenchyma. Although fiber and xylem parenchyma were not clear, the meta and proto xylem were found in the large vascular bundle. Group vessel was found in large vascular bundle only. Interestingly, lysigenous cavity (protoxylem gap) was observed only in the large vascular bundle at proto xylems (Fig. 3C). The sieve tube and companion cells were observed as phloem elements. The size of the xylem cells were 10.58 µm in average diameter (Table 1). The vascular bundle was surrounded by bundle sheath (BS) cells (Fig. 3C). The vascular bundles were closely arranged and two mesophyll cells were present between two vascular bundles (Fig. 3C).

A chlorophyllous bundle sheath always surrounded around the xylem and phloem in both small and large vascular bundles (Fig. 3C). The non-photosynthetic mesophyll sheath was absent. The shape of midrib was estimated more or less conical towards the direction of abaxial (upper). A central large vascular bundle is present next to few small vascular bundles that stayed in touch of abaxial (upper) epidermis (Fig. 3C). The central region of the midrib showed colourless thin-walled large round shaped cells (Fig. 3C).

4. DISCUSSION

Large longitudinal veins, small longitudinal veins and transverse (central) veins in leaf blades of *S. spontaneum* were observed in the present study. The achievements were similar to the research of Chonan et al. (1974) and Rahman & Sultana (2021). The transverse (central) vein and large longitudinal veins in the cross section were similar in size. The contrast result was reported by Rahman & Sultana (2021), according to that observation; transverse vein was the largest among the veins in the cross section. The distance between small longitudinal veins in leaf blade was relatively short. Ueno et al., (2006) noticed that the distance between small longitudinal veins of NADP-ME and NAD-ME type was relatively shorter than PECK type. In the present study, three rows of long cells between two stomatal cell rows were present in an intercostal zone of abaxial surface. The result differing from the report of Nazir et al. (2013) also explained that four to eight rows of long cells are present between two

costal zones and two to three stomatal rows are present between two intercostal zones.

In grass systematics and characterization of broader groups within the grasses, particularly among the subfamilies and tribes, characteristics of leaf epidermal layer have a great impact. The epidermal features of leaf can help elucidate taxonomic problems (Cai & Wang, 1994; Sanchez, 1971). In the present study cork cell, prickles, micro hairs and stomata were identified as features of leaf epidermal layer. Cork cells were present between two long cells in the experimented plant. According to Scarpella & Meijar (2004), cork cells prevent the water loss from plant and also make them more resistant to fungal and bacterial infection. Prickles were perceived in the studied plant between two large cells. Three types of prickles were noticed in adaxial and abaxial epidermal surfaces of johnsongrass leaves (Mcwhorter et al. 1993). The present study revealed that microhairs features of the leaf surfaces have a considerable identifying characteristic of *S. spontaneum*. Rahman & Sultana (2021) reported that *E. indica* L. is characterized by microhairs. However, Ahmad et al. (2011) did not find microhairs, instead found saddle-shaped silica bodies. According to Nazir et al., (2013), bicelled microhairs are the characteristic of panicoid grass. According to Prat (1936), the types of microhairs and silica bodies are highly useful in systematic studies. The amphistomatic stomatal leaves showed in the experimented plant that is common feature of leaf for grass plants (Arber, 1923). It was greater in number in the lower surface than the upper surface of *S. spontaneum*. In the report of Anderson & Briske (1990), the greater proportion of stomata was recorded on the lower surface of C₄ plant but it was on the upper surface in the case of C₃ plant.

In the studied plant, mesophyll cells were appearing arranged around the bundle sheath cells in a ring-like fashion, which is an important structure for Kranz feature in C₄ grass. Dengler & Nelson (1999) reported that bundle sheath (BS) cells are surrounded by radially arranged mesophyll cells. Bundle sheath (Kranz) like cells appear lying in the mesophyll, separated from the parenchyma sheath, as single cells or in small groups (Dengler et al., 1996). Mesophyll cells were different in shape with irregular intercellular space, a rather common feature. The presence of large air space in the mesophyll zone is identical leaf feature for this grass species. According to Thorat et al., (2018), the presence of large air space indicates hydrophilic adaptation. Therefore, the experimented plant has hydrophilic adaptation features. In the present study, two mesophyll cells were present between two vascular bundles; therefore, vascular bundles were closely arranged in the leaf blades of studied plant. The result was supported by (Dangler et al., 1994) in C₄ grasses.

Sclerenchyma bundle were present in the region of large vascular bundle with abundant cells that was observed some times in the location of small vascular bundles made up of a few cells. It is developed in the leaf blade providing mechanical support mainly to erect leaf. The similar

observation was revealed in *E. indica* (Rahman & Sultana, 2021).

Chlorophyllous bundle sheath cells were made of thin wall. Those were present continuously around small vascular bundles and discontinuously around large vascular bundles. In the leaf veins of numerous angiosperms, the vascular bundles were surrounded, as a whole or in part, by a distinct bundle sheath comprising one or more layers of compact parenchyma cells (Metcalfe, 1979). Crookston & Moss (1970) provide an extensive review of many plants with leaves reported to have chlorenchymatous bundle sheaths. Most remarkable are plants with C_4 photosynthesis, which led Harberlandt (1914) to suggest the occurrence of co-operative photosynthesis in such plants.

Vascular bundles in leaf blade sections were of two sizes: peripheral small and large in size were abundant and few in number, respectively. Sima et al. (2015) pointed out the presence of small peripheral vascular bundles in large number. Two sizes of vascular bundle (large and small) were reported in *E. indica* (Rahman & Sultana 2021). The present observation was contrary to the outcome of Sima et al. (2017), Artschwager (1925) and Ellis (1976) who observed three types.

The vascular bundle encloses both xylem and phloem and conjoint collateral closed type in *S. spontaneum*. The bundles have distinct phloem towards the lower epidermis and xylem towards the upper epidermis. This is a common feature of vascular bundle in the Gramineae family (Ueno et al. 2006). The xylem bundles composed of vessel (metaxylem), fiber and xylem parenchyma. There were notable features in the present study that group vessel and lysigenous cavity at proto xylem region were observed only in the large vascular bundle. The phloem portion was clearly detected. The phloem elements like sieve tube and companion cells were identified in sugarcane (Colbert & Evert, 1982).

Fouracre et al. (2014) reported that Kranz structure varies as a consequence of the distinct evolutionary origins of C_4 plants. The mestome sheath layer between the bundle sheath cells and the vascular bundle is absent in the NADP-ME subtype and the bundle sheath cell wall was suberized. Grana are reduced bundle sheath arranged centrifugally (Gutierrez et al., 1974; Hattersley & Watson, 1976; Prendergast et al., 1987; Lundgren et al., 2014). Comparatively, in the NAD-ME subtype, the vascular bundle is usually surrounded by a double sheath, consisting of the bundle sheath and the non-photosynthetic mestome sheath (Prendergast et al., 1987; Lundgren et al., 2014). Suberin ubiquitously deposits in the mestome sheath rather than in bundle sheath cells and bundle sheath chloroplasts with developed grana are arranged centripetally (Hattersley & Watson, 1976; Nelson & Langdale, 1989; Fouracre et al., 2014; Mertz & Brutnell, 2014). Loss of one layer of mestome sheath cells in the NADP-ME type suggests differences in the origination of cell divisions. The single bundle sheath in C_4 NADP-ME type grasses is derived from the procambium and mesophyll cells develop from the ground meristem. In the double-sheath species of the NAD-

ME type, both the bundle sheath and mesophyll cells are derived from the ground meristem and the mestome sheath is derived from the procambium (Nelson & Langdale, 1989; Soros & Dengler, 2001). In the present study, both small and large vascular bundles were surrounded with only bundle sheath, suggested that experimented grass was NADP-ME subtype C_4 . At the midrib region, some large and thin wall cells were observed in the experimented plant that was similar to the results of Colbert & Evert (1982) in sugarcane and Sima et al. (2017) in Cogon grass.

5. CONCLUSION

In the studied plant, vascular bundles in leaf blade were very closely distributed and only two mesophyll cells were existent between two bundles. The vascular bundle was surrounded by bundle sheath cells and the bundle sheath cells were surrounded by mesophyll cells in a ring like fashion. Both bundle sheath cells and mesophyll cells contain chloroplasts, are suggested typical characteristics of C_4 plant. C_4 plants have been classified into three subtypes based on the enzymes used to decarboxylate C_4 acids in the bundle sheath cells, like as NADP-ME, NAD-ME and PEPCK subtypes. The layers surrounded to vascular bundles of PEPCK quite similar to NADP-ME or NAD-ME subtypes. The absence of mesotome sheath in the studied plant is suggestive of the *S. spontaneum* NADP-ME subtype. Moreover, group vessel and lysigenous cavity at proto xylem region were typical feature of vascular bundle in *S. spontaneum*. For more clarification of anatomical features of *S. spontaneum*, the present study will be useful.

ACKNOWLEDGEMENTS

Authors are also thankful to authorities of EXIM Bank Agricultural University Bangladesh (EBAUB) to support this study using laboratory of Crop Botany, Faculty of Agriculture, EBAUB.

REFERENCES

- Ahmad, F., Khan, M. A., Ahmad, M., Zafar, M., Arshad, M., Khan, A., Shah, G. M., & Awan, M. R. (2010). Taxonomic utilization of anatomical characters in tribe Andropogoneae (Poaceae) based on transverse sections of leaves. *Journal of Medicinal Plants Research*, 4(14), 1349-1358.
- Ahmad, F., Khan, M. A., Ahmad, M., Zafar, M., Iqbal, Z., & Khan, A. (2011). Leaf epidermal anatomy as an aid to the identification of genus *Setaria* weeds, tribe paniceae (Poaceae), from the salt range of Pakistan. *Journal of Medicinal Plants Research*, 5(15), 3500-3506.
- Al-Janabi, S. M., Honeycutt, R. J., McClelland, M., & Sobral, B. W. (1993). A genetic linkage map of *Saccharum spontaneum* L. 'SES 208'. *Genetics*, 134(4), 1249-1260.
- Anderson, V.J., & Briske, D.D. (1990). Stomatal distribution, Density and conductance of tree perennial

- grasses native to the Southern true Prairie of Texas. *The Americal Midland Naturalist*, 123(1), 152-159.
- Anon, F. (1972). *The wealth of India: Raw materials*. CSIR Publications & Information Directorate, Vol. IX. New Delhi, India.
- Arber, A. (1923). Leaves of the Gramineae. *Botanical Gazette*, 76(4), 374-388.
- Artschwager, E. (1925). Anatomy of the vegetative organs of sugarcane. *Journal of Agricultural Research*, 30, 197-221.
- Ashida, K., & Sugino, M. (1984). Studies on the stomata in the leaf blades of gramineous weeds. *Weed Research*, 29, 138-146.
- Cai, L. B., & Wang, S. J. (1994). Studies on the evolutionary trends and mechanism of the constituent cells of the leaf epidermis in Poaceae. *Acta Biologia Plateau Sinica*, 12, 13-27.
- Chaudhari, S. K., Arshad, M., Mustafa, G., Fatima, S., Amjad, M. S., & Yasmeen, F. (2014). Foliar epidermal anatomy of grasses from Thal desert, district Khushab, Pakistan. *International Journal of Biosciences*, 4(8), 62-70.
- Chonan, N., Kawahara, H., & Matsuda, T. (1974). Morphology of vascular bundles of leaves in gramineous crops. I. Observations on vascular bundles of leaf blades, sheaths and internodes in rice plants. *Proceedings of the Crop Science Society of Japan*, 43, 425-432.
- Colbert, J. T., & Evert, R. F. (1982). Leaf vasculature in sugarcane (*Saccharum officinarum* L.). *Planta*, 156, 136-151.
- Crookston, R. K., & Moss, D. N. (1970). The relation of carbon dioxide compensation and chlorenchymatous vascular bundle sheaths in leaves of dicots. *Plant Physiology*, 46, 564-567.
- Dangler, N. G., Dangler, R. E., Donnelly, P. M., & Hattersley, W. (1994). Quantitative leaf anatomy of C₃ and C₄ grasses (Poaceae). Bundle sheath and mesophyll surface area relationships. *Annals of Botany*, 73, 241-255.
- Davila, P., & Clark, L. G. (1990). Scanning electron microscopy survey of leaf epidermis of Sorghastrum (Poaceae) Andropogoneae. *Am. J. Bot.*, 77, 499-511.
- Dengler, N.G., Donnelly, P. M., & Dengler, R.E. (1996). Differentiation of bundle sheath, mesophyll, and distinctive cells in the C₄ grass *Arundinella hirta* (Poaceae). *American Journal of Botany*, 83, 1391-1405.
- Dengler, N. G., & Nelson, T. (1999). Leaf structure and development in C₄ plants. In: C₄ Plant Biology. Sage RF & Monson RK (eds.), San Diego: Academic Press, pp. 133-172.
- El-Gawad, A.M.A., & El-Amier, Y. A. (2017). Anatomical features of three perennial swampy plants of Poaceae, grown on the water stream banks in Nile Delta, Egypt. *Journal of Medicinal Botany*, 1, 58-64.
- Ellis, R. P. (1976). A procedure for standardizing comparative leaf anatomy of the Poaceae. I. The leaf blade as viewed in transverse section. *Bothalia*, 12, 65-109.
- Ellis, R. P. (1986). A review of comparative leaf blade anatomy in the Systematics of Poaceae. In: Grass systematics and evolution. The past twenty five years. Soderstorm TR & Hilu KH (Eds.), Smithsonian Institute, Washington D.C, pp. 3-10.
- Ferreira, E. A., Ventrella, M. C., Santos, J. B., Barbosa, M. H. P., Silva, A. A., Procopio, S.O. & Silva, E. A. M. (2007). Leaf blade quantitative anatomy of sugarcane cultivars and clones. *Plantadachina*, 25(1), 45-49.
- Fouracre, J. P., Ando, S., & Langdale, J. A. (2014). Cracking the Kranz enigma with systems biology. *J. Exp. Bot.*, 65, 3327-3339.
- Gajendera, S., Naithani, H. B., & Rawat, G. S. (2009). Observation on the flora of Mandal Forest, Garhwal Himalaya, India. *Indian Forester*, 135(2), 162-179.
- Gutierrez, M., Gracen, V. E., & Edwards, G. E. (1974). Biochemical and cytological relationships in C₄ plants. *Planta*, 119, 279-300.
- Harberlandt, G. (1914). *Physiological plant anatomy*. Macmillan and Co, London.
- Hatch, M. D. (1987). C₄ photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. *Biochimicaet Biophysica Acta*, 895, 81-106.
- Hattersley, P. W. (1984). Characterization of C₄ type leaf anatomy in grasses (Poaceae). Mesophyll: bundle sheath area ratios. *Annals of Botany*, 53, 163-179.
- Hattersley, P. W., & Watson, L. (1976). C₄ grasses: an anatomical criterion for distinguishing between NADP-malic enzyme species and PCK or NAD-malic enzyme species. *Aust. J. Bot.* 24, 297-308.
- Holm, L., Doll, J., Holm, E., Pancho, J., & Herberger, J. (1997). *World weeds: natural histories and distribution*. John Wiley and Sons, Inc., New York, USA.
- Jarves, J. K., & Barkworth, M. E. (1992). Morphological variations and genome constitution in some perennial Triticeae. *Bot. J. Linn. Soc.*, 103, 167-180.
- Komarov, V. L., Rozhevits, R. Y., & Shishkin, B. K. (1963). *Flora of the USSR*. The Botanical Institute of the Academy of Sciences of the USSR, Leningrad, USSR.
- Lundgren, M. R., Osborne, C. P., & Christin, P. A. (2014). Deconstructing Kranz anatomy to understand C₄ evolution. *J. Exp. Bot.* 65, 3357-3369.
- Mcwhorter, C. G., Ouzts, C., & Paul, R. N. (1993). Micromorphology of Johnson grass (*Sorghum halepense*) leaves. *Weed Sci.* 41(4), 583-589.
- Meng, Z., Han, J., Lin, Y., Zhao, Y., Lin, Q., Ma, X., Wang, J., Zhang, M., Zhang, L., Yang, Q., & Wang, K. (2020). Characterization of a *Saccharum spontaneum* with a basic chromosome number of $x=10$ provides new insights on genome evolution in genus *Saccharum*. *Theoretical and Applied Genetics*, 133, 187-199.
- Mertz, R. A., & Brutnell, T. P. (2014). Bundle sheath suberization in grass leaves: multiple barriers to characterization. *J. Exp. Bot.* 65, 3371-3380.
- Metcalf, C. R. (1979). The leaf: general topography and ontogeny of the tissues. In: Anatomy of the

- dicotyledons, Metcalfe CR & Chalk L (eds.), Vol. I., Oxford University Press, Oxford, pp. 63-75.
- Nazir, A., Khan, M. A., Ahmad, F., Ullah, K., & Shah, A. (2013). Foliar epidermal studies as an aid to the identification of grasses of tribe Andropogoneae (Poaceae) from Pothohar region of Pakistan. *Pakistan Journal of Botany*, 45, 235-241.
- Nelson, T., & Langdale, J. A. (1989). Patterns of leaf development in C₄ plants. *Plant Cell*, 1, 3-13.
- Neufeld, H. S., Grantz, D. A., Meinzer, F. C., Goldstein, G., Crisosto, G. M., & Crisosto, C. (1992). Genotypic variability in vulnerability of leaf xylem to cavitation in water-stressed and well-irrigated sugarcane. *Plant Physiology*, 10, 1002-1010.
- Panje, R. R. (1970). The evolution of a weed. *PANS*, 16, 590-595.
- Prat, H. (1936). La Systématique des Graminées. *Ann. Sci. Nat. Bot., Ser.*, 10(18), 165-257.
- Prendergast, H. D. V., Hattersley, P. W., & Stone, N. E. (1987). New structural/biochemical associations in leaf blades of C₄ grasses (Poaceae). *Aust. J. Plant Physiol.*, 14, 403-420.
- Rahman, M. M., & Sultana R. S. (2021). Anatomy on leaf blade of *Eleusine indica* L. (Gramineae): a Study on kranz grass. *EBAUB J.*, 3, 1-8.
- Rahman, M. M., & Sultana, R. S. (2020). Anatomy and developmental changes in stem of *Leucas aspera* (Wild.) Link. (Lamiaceae). *EBAUB J.*, 2, 1-8.
- Rahman, M. M., & Sultana R. S. (2019). Stem, leaf and root anatomy of *Eclipta alba* (L.) Hassk. (Asteraceae). *EBAUB J.*, 1, 1-7.
- Sanchez, E. (1971). Anatomia foliar de chlorideae (Gramineae) argentinas. *Kurtziana*, 6, 103-218.
- Scarpella, E., & Meijer, A. H. (2004). Pattern formation in the vascular system of monocot and dicot plant species. *Proceedings of the National Academy of Sciences*, 164(2), 209-242.
- Sima, S. N., Roy, A. K., & Joarder, N. (2015). Erect culm internodal anatomy and properties of sun ecotype of *Imperata cylindrica* (L.) P. Beauv. *Bangladesh Journal of Botany*, 44(1), 67-72.
- Sima, S. N., Roy, A. K., Akther, M. T., & Joarder, N. (2017). Cross-sectional anatomy of leaf blade and leaf sheath of Cogon grass (*Imperata cylindrica* L.). *J. Bio-Sci.*, 25, 17-26.
- Soros, C. L., & Dengler, N. G. (2001). Ontogenetic derivation and cell differentiation in photosynthetic tissues of C₃ and C₄ Cyperaceae. *Am. J. Bot.*, 88, 992-1005.
- Stenglein, S. A., Colares, M. N., Arambarri, A. M., Novoa, M. C., Vizcaino, C. E., & Katinas, L. (2003). Leaf epidermal microcharacters of the old world species of Lotus (Leguminosae: Loteae) and their systematic significance. *Aust. J. Bot.*, 51, 459-469.
- Sukhova, G. V., & Kazakov, I. F. (1972). Anatomical structure of the vegetative organs of *Saccharum spontaneum* L. and *Erianthus ravennae* (L.) Beauv. *Izvestiya Akademii Nauk Turkmenskoi SSR, Biologicheskikh Nauk*, 5, 25-31.
- Sultana, R. S., & Rahman, M. M. (2020). Anatomy of *Phyllanthus niruri* L. (Euphorbiaceae). *EBAUB J.*, 2, 24-29.
- Thakur, C. (1984). *Weed science*. Metropolitan Book Co. Ltd., New Delhi, India.
- Thorat, K. M., Kokate, P. S., & Pundkar, S.V. (2018). A report of petrified fossil monocot leaf from deccan intertrappean beds of mohgaonkalan, Dist. Chhindwara, M.P. India. *A Quarterly Journal of Life Sciences*, 15(1), 24-26.
- Ueno, O., Kawano, Y., Wakayama, M., & Takeda, T. (2006). Leaf vascular systems in C₃ and C₄ grasses: a two-dimensional analysis. *Annals of Botany*, 97, 611-621.
- Wang, H., Wang, Y., Xiao, N., Hua, X., Zhang, M., Ming, R., & Zhang, J. (2019). NAC transcription factors in autopolyploid *Saccharum spontaneum*: genome-wide identification, expression pattern and a 'Dry' orthologous gene. *Plant Molecular Biology and Genetics*, 1, 217-227.
- Wapakala, W. (1966). A note on the persistence of mulch grasses. *Kenya Coffee*, 31, 111-112.
- Yadav, A., Balyan, R. S., Malik, R.K., Malik, R. S., Singh, S., Banga, R.S., & Singh, S. (2007). Efficacy of glyphosate, MON-8793 and MON-8794 for general weed control under non-cropped situations. *Environment and Ecology*, 25, 636-639.
- Yang, H., Wang, T., Yu, X., Yang, Y., Wang, C., Yang, Q., & Wang, X. (2020). Enhanced sugar accumulation and regulated plant hormone signaling genes contribute to cold tolerance in hypoploid *Saccharum spontaneum*. *BMC Genomics*, 21, 152-172.
- Yoshimura, Y., Kubota, F., & Ueno, O. (2004). Structural and biochemical bases of photorespiration in C₄ plants: quantification of organelles and glycine decarboxylase. *Planta*, 220, 307-317.
- Yousaf, Z., Shinwari, Z. K., Asghar, R., & Parveen, A. (2008). Leaf epidermal anatomy of selected *Allium* species, family Alliaceae from Pakistan. *Pak. J. Bot.*, 40(1), 77-90.
- Zhen, L., Hua, X., Zhong, W., Yuan, Y., Wang, Y., Wang, Z., Ming, R., & Zhang, J. (2020). Genome-wide identification and expression profile analysis of WRKY family genes in the autopolyploid *Saccharum spontaneum*. *Plant and Cell Physiology*, 61(3), 616-630.