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# Stem and Root Anatomy of *Cynodon dactylon* (L.) Pers. from Different Habitats of Bangladesh

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## Abstract

The present study was conducted with the stems and roots of 24 accessions of *Cynodon dactylon* was collected from different habitats of Bangladesh. Anatomical features of this grass species include different tissues and tissue systems, which were studied through qualitative and quantitative measurements. Stem anatomy exhibited epidermis, hypodermis, ground tissue system and vascular bundles through transverse section of the respective parts. Both epidermis and hypodermis were found to be thick-walled, but composed of parenchyma and sclerenchyma cells, respectively. Their thickness was always found to be lowest for epidermis (380 µm) in the accessions of Cox's Bazar and sclerenchyma thickness (0.07 cm) in the accession of both Cox's Bazar and Saint Martin's Island collected from the saline area. The ground tissue system did not show distinct cortex, endodermis, pericycle, pith, and pith rays; it was made up of parenchyma cells occupying the whole stem interior. Here also, cortex thickness was lowest (0.26 cm and 0.31 cm) in the case of Saint Martin's Island and Cox's Bazar, respectively, in accessions of the saline area compared to that of other habitats. Vascular bundles were conjoint, collateral, but closed. In case of the root anatomy, one remarkable feature, such as intensive clarification in outer cortex, vascular region and endodermis layer was observed in the accessions collected from Barguna, Cox's Bazar and Saint Martin's Island. Another distinctive feature was observed in root anatomy, and that was lysigenous aerenchyma, which is created by the formation of gas spaces as a result of death and subsequent lysis, causing collapse of the files of cells, i.e, root cortical cells. This was very prominent in the accessions of the saline area. Vascular bundles were found to be arranged in a ring of differential radials. Their quantitative characters, such as metaxylem area, phloem area, pith area, and pith thickness, showed differences among accessions from saline and non-saline habitats, with the highest and lowest values observed in the saline and non-saline habitats, respectively. ANOVA confirmed significant variation among accessions ($p \leq 0.001$). These findings suggest that anatomical modifications, particularly aerenchyma formation and tissue sclerification, contribute to the ecological resilience of *C. dactylon* in saline and drought-prone environments.

***Keywords:*** Accessions, Anatomy, Bermudagrass, Quantitative characters, Root, Stem.



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## Introduction

*Cynodon dactylon* (L.) Pers. commonly known as Bermudagrass, is a typical warm season turfgrass that belongs to the family Poaceae, and this grass species is widely adapted to various environments of tropical and sub-tropical regions around the world (Zheng et al. 2017). It spreads by scaly rhizomes and flat stolons. Many grass species succeed from their tolerance of grazing herbivores and some other disturbances, but they survive by different means of reproduction and by their versatility in photosynthesis. In case of flowering plants, where new growth starts in the aerial plant body, occurs at the shoot tips only. Anyhow, if the tip is removed, buds in the axils of lower leaves may start growing, but at the same time the original shoot stops growing. Meristems of grasses die at the base of each stem between the leaves, and thus, regrowth may be possible even after the removal of the tip by any means. *Cynodon dactylon* is a creeping grass, and it exhibits a tough and rough texture. Its root grows, wherever; a node touches the ground and forms a thick mat (Nitu et al. 2019a). The stems are slightly flattened, with a hard, longitudinally furrowed surface. Asthana et al. (2012) stated that it had a sweet gelatinous taste and stems of this grass ranged from erect to prostrate.

*C. dactylon* is distributed abundantly in tropical and warm temperate areas (Chaudhary 1989). Natural population of this grass species may have considerable genetic variation for tolerance to soil temperatures, salinity and drought (Speranza 1995, Nitu et al. 2019b). It can tolerate relatively high salinities (Mass and Hoffman 1977). Morphological and anatomical modifications in the plant body act to minimize various detrimental effects of stress (Poljakoff-Mayber 1988, Grigore and Toma 2017, Çitak and Dural 2020). Salt tolerant species shows a range of anatomical adaptive features due to increased succulence, thick cuticle and deposition of wax, salt secretory trichomes, thick and many layered epidermis and well-developed water storing tissues in the cortex, widening of casparian bands and enhanced development of root endodermis (Akram et al. 2002, Wahid 2003). The effect of salinity on anatomical structures was discussed by Gadalla (2009) and Younis et al. (2014). Stem and root anatomy of *C. dactylon* as seen through transverse section includes descriptions, distributions and arrangements of different tissues and tissue systems along with qualitative and quantitative measurement of different anatomical traits. From these points of view, the present study deals with the measurement of ecotypic variability and the degree of adaptation in *C. dactylon* collected from different ecological habitats.

## Materials and Methods

### Materials

Roots and stems of 24 accessions of *C. dactylon,* sharp blade, soft camel hairbrush, forceps, petri-plates, glass slides, cover slips, needles, forceps, safranin, glycerin, FAA solution, acetic-alcohol solution, fast green, watch glass, dropper, distilled waters, blotting paper, tissue paper, light microscope, ocular meter, stage micrometer etc.

### Preparations of slides

Plants were removed from the soil; their stems and roots were separated and washed with distilled water to remove soil dust and other contaminants. For anatomical study, freehand transverse sections were cut, and thin sections were separated with the help of a research microscope. Transverse sections of the stem and root of 10 individual plants per accession were made using a sharp blade from the fresh materials. For the root, some potato blocks were used. The root pieces were put inside the block, then freehand transverse sections were cut, and at least three thin sections were selected for each root with the help of a microscope. The materials were fixed in FAA solution (formalin 10%, acetic acid 5%, ethyl alcohol 50% and distilled water 35%) for 48 h and then transferred to an acetic-alcohol solution (1:3) for root and stem anatomical studies. Double staining dehydration procedure (safranin and fast green) was adopted for preparing permanent slides to study various cells and tissues of root and stem in the Laboratory of Ecology, University of Rajshahi. The stained sections were observed under a light microscope at 10× and 15× magnification and photographed. The anatomical characters observed under microscopes were recorded. Measurements were taken with a light microscope, using an ocular micrometer.

### Microscopic analysis

The stained transverse sections of root and stem were examined under a compound microscope (SWIFT INSTRUMENTS INTERNATIONAL, S.A No.760090). The best micro-photographs of the mounted materials were taken using a digital camera (model: C-B5, Brand: Optica) and mounted on the electrical light microscope (Model: XSZ-107T, Brand: Novel) with total magnification of 15×10X in the Laboratory of Phycology and Limnology, University of Rajshahi, Bangladesh. These micro-photographs were useful for the identification and differentiation of various cells and tissues of the root and stem on the basis of microscopic features.

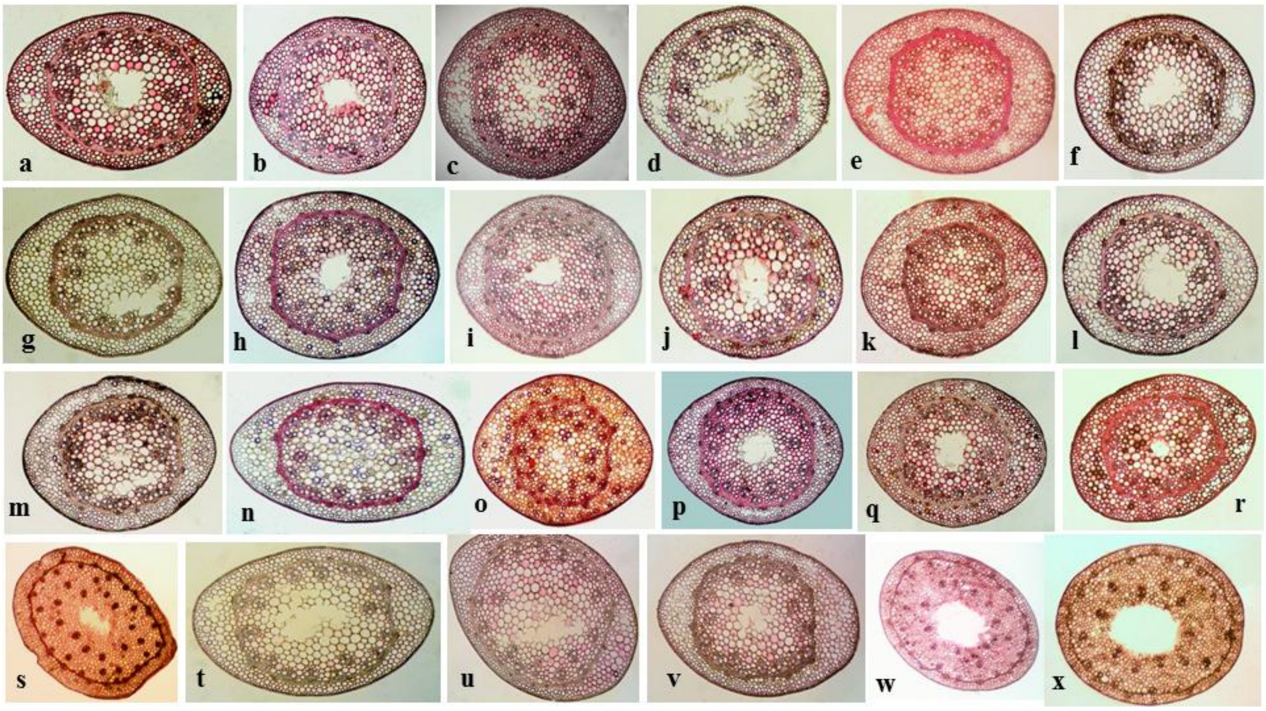
### Statistical analysis

A statistical comparison by means of Duncun's Multiple Range Test (DMRT) was made. The quantitative anatomical characters of the root and stem of different accessions were statistically analyzed by analysis of variance (ANOVA). Significance level was set at $p \leq 0.001$. The data analysis was done using SPSS version 20.0 for Windows.

## Results

### Stem anatomy

Anatomical attributes are illustrated here based on the transverse section of the stem as mentioned in the Figs. 1 (a-x) and recorded data in Table 1 as follows:




**Figs. 1 (a-x):** Stem anatomy of *C. dactylon* collected from different habitats of Bangladesh, a) Rangpur, b) Lalmonirhat, c) Dinajpur, d) Thakurgaon, e) Panchagarh, f) Gaibandha, g) Rajshahi, h) Naogaon, i) Pabna, j) Gazipur, k) Narsingdi, l) Sherpur, m) Mymensingh, n) Khulna, o) Jessore, p) Jhenaidah, q) Faridpur, r) Shariatpur, s) Barguna, t) Khagrachari, u) Bandarban, v) Rangamati, w) Cox's Bazar, x) Saint Martin

### Epidermis

The transverse section (TS) of epidermis reveals that the structure was comprised of a single layer of epidermis with a thick cuticle. The TS of all the stems looked like an oval shape. Epidermis, the outermost layer of the stem, was found to be made up of compactly arranged transparent, elongated and rectangular-barrel-shaped living parenchyma cells. The cells of the epidermis were radially broader than in the tangential direction. The outer periclinal wall was thicker compared to the inner periclinal wall. Due to the presence of cuticles, the epidermal cells looked much thicker on the outside of the outer wall. No visual differences were found in the length of epidermal cells of all the internodes. Hairs were found to be absent. The highest value (2110 µm) for epidermis thickness of the stem was found in the accession collected from Lalmonirhat, and the lowest value (380 µm) of it was found in the accession of Cox's Bazar.

### Hypodermis

The hypodermis was found to be composed of thick-walled lignified sclerenchyma cells. Cell size was small, acting as a heat screen and providing a heat shield, rigidity, and mechanical strength to the stem. The overall transverse sections were oval-shaped but wavy in appearance due to the formation of small ridges, where small-sized vascular bundles were found. Greatly increased sclerification outside vascular bundles was observed in the stem of Barguna, Cox's Bazar, and Saint Martin's Island. In other accessions, low sclerification outside the vascular bundles of the stem was observed. Sclerenchyma thickness of the stem was highest (0.71 cm) in accession of Rangpur, and that was lowest (0.07 cm) in the case of both Cox's Bazar and Saint Martin's Island.

**Table 1:** Stem anatomical attributes of different accessions of *Cynodon dactylon* collected from different habitats of Bangladesh.

| Acc. No. | Name of the habitats | Epidermis thickness (µm) Mean±SE | Cortex thickness (cm) Mean±SE | Sclerenchyma thickness (cm) Mean±SE | Vascular bundle area (µm²) Mean±SE | Vascular bundle number Mean±SE | Metaxylem area (µm²) Mean±SE | Phloem area (µm²) Mean±SE |
|---|---|---|---|---|---|---|---|---|
| 1. | Rangpur | 1480±0.58f | 0.89±0.06fgh | 0.71±0.01a | 5660±0.43l | 21±0.14gh | 1160±0.36p | 3330±0.06d |
| 2. | Lalmonirhat | 2110±0.29a | 1.36±0.03ab | 0.22±0.02bc | 6880±0.07d | 18±0.20jk | 1930±0.06d | 4310±0.11b |
| 3. | Dinajpur | 1390±0.14i | 0.85±0.03gh | 0.15±0.03bc | 5770±0.09h | 24±0.01f | 1530±0.43k | 4140±0.15c |
| 4. | Thakurgaon | 1230±0.58k | 0.59±0.03i | 0.19±0.01bc | 4520±0.13s | 19±0.05ij | 1150±0.07q | 3270±0.54e |
| 5. | Panchagarh | 870±0.46q | 1.11±0.01cde | 0.24±0.03bc | 5070±0.16q | 27±0.13d | 1650±0.57i | 1600±0.07q |
| 6. | Gaibandha | 1560±0.20d | 0.86±0.01gh | 0.15±0.06bc | 4980±0.17r | 17±0.33k | 1210±0.07o | 3200±0.33f |
| 7. | Rajshahi | 1470±0.09g | 1.45±0.08a | 0.33±0.06b | 8690±0.18a | 20±0.41hi | 2320±0.07a | 4620±0.53a |
| 8. | Naogaon | 1650±0.35c | 1.25±0.05bc | 0.22±0.07bc | 5850±0.20g | 30±0.34c | 1570±0.11j | 2660±0.15h |
| 9. | Pabna | 1360±0.29j | 0.97±0.02efg | 0.24±0.02bc | 5170±0.23o | 30±0.23c | 1450±0.22k | 1660±0.15p |
| 10. | Gazipur | 840±0.06r | 0.41±0.03j | 0.17±0.00bc | 4480±0.24t | 19±0.57ij | 1070±0.40t | 1810±0.40m |
| 11. | Narsingdi | 1120±0.14l | 1.24±0.06bc | 0.22±0.08bc | 5490±0.28m | 22±0.03g | 1270±0.08n | 1770±0.19n |
| 12. | Sherpur | 1790±0.10b | 1.05±0.02def | 0.35±0.02b | 6430±0.06f | 18±0.04jk | 1800±0.08f | 3010±0.41g |
| 13. | Mymensingh | 1550±0.34e | 1.22±0.04bc | 0.30±0.12b | 8630±0.58b | 23±0.52f | 1730±0.12h | 2000±0.14l |
| 14. | Khulna | 1400±0.32h | 1.20±0.05bcd | 0.32±0.10b | 6790±0.08e | 19±0.05ij | 2120±0.11c | 2120±0.42k |
| 15. | Jessore | 890±0.07p | 1.01±0.03efg | 0.18±0.05bc | 3370±0.58u | 21±0.44gh | 1270±0.45n | 1600±0.46q |
| 16. | Jhenaidah | 670±0.03u | 0.95±0.05efg | 0.19±0.09bc | 5760±0.47i | 24±0.37f | 1890±0.15e | 1670±0.15o |
| 17. | Faridpur | 670±0.19u | 0.96±0.09efg | 0.23±0.02bc | 5740±0.55j | 20±0.41hi | 1080±0.56s | 1520±0.57t |
| 18. | Shariatpur | 910±0.13n | 0.84±0.02gh | 0.22±0.11bc | 5230±0.13n | 19±0.45ij | 1090±0.50r | 1530±0.14s |
| 19. | Barguna | 690±0.26s | 0.72±0.03hi | 0.15±0.05bc | 5120±0.27p | 35±0.50b | 1440±0.44l | 2160±0.31j |
| 20. | Khagrachari | 900±0.15o | 0.76±0.16h | 0.19±0.05bc | 480±0.59x | 21±0.37gh | 1400±0.09m | 1590±0.51r |
| 21. | Bandarban | 990±0.34m | 0.99±0.05efg | 0.32±0.01b | 7280±0.50c | 21±0.32gh | 2180±0.55b | 2000±0.16l |
| 22. | Rangamati | 680±0.16t | 0.83±0.05gh | 0.24±0.10bc | 5670±0.25k | 20±0.28hi | 1760±0.39g | 2210±0.07i |
| 23. | Cox's Bazar | 380±0.06w | 0.31±0.06j | 0.07±0.03c | 3120±0.06v | 43±0.44a | 800±0.15u | 1270±0.06u |
| 24. | St. Martin's Island | 480±0.17v | 0.26±0.03j | 0.07±0.01c | 2270±0.17w | 26±0.35e | 480±0.14v | 990±0.41v |

*Mean values followed by same lower-case letters (a, b etc.) are not significantly different (p<0.005) among different areas of cultivars as determined by Duncan's Multiple Range Test (DMRT, by SPSS 25).*

## Ground tissue system

The ground tissue is made up of parenchyma cells and occupies the whole stem interior. Two types of cells were found in the stem of *C. dactylon* . Outer cells were small and angular towards the hypodermis, but became large and oval in the inner region. The pith region was large and consisted of parenchyma cells. Abundant intercellular spaces were present in the ground tissue. These spaces communicate with the exterior through the stomata present in the epidermis. Down to the hypodermis, vascular bundles show some pattern of arrangement. Outer vascular bundles were small and arranged in a complete circle. But the inner vascular bundles had a ring-like arrangement somewhat scatteredly. Vascular bundles were found to be distributed scatteredly in the cortical areas, and those are smaller but more numerous toward the outside than towards the center. The vascular bundles of center were much larger than those next to hypodermis. The parenchyma cells around the vascular bundles were the smallest, while the pith cells were larger. Vascular bundles nearest to the epidermis were thickly arranged, whereas the cortical bundles were thinly distributed. Hypodermal vascular bundles were of two different sizes, large and small, arranged almost alternately with equal spacing. These vascular bundles were always attached to thick-walled cells, with rare exceptions. Twin vascular bundle found at the hypodermis layer in *C. dactylon* was an exceptional feature.

Cortex was parenchymatous, usually having thin-walled cells, and these cells surrounding a vascular bundle gradually sclerified into thick-walled cells. These sclerified cells looked like bundle sheath extensions compared to distal internodes from the ground. In basal internodes chlorenchyma cells gradually transformed into thick wall cells of the chlorenchyma were frequently irregular in shape and arrangement, and exhibited no characteristic pattern. The highest value (1.45 cm) for cortex thickness of the stem was found in the accession collected from Rajshahi, and the lowest value (0.26 cm) was found in the accessions of Saint Martin's Island, and the 2nd lowest value (0.31 cm) was found in the case of Cox's Bazar. Cortical cells around the vascular bundles were smaller and became sclerified as the culm internode aged. Basal internodes had greater amount of sclerified cells compared to distal internodes. The pith region was limited to the centre of the culm, where usually no vascular bundles were found. The cells of the pith were usually large and had intercellular spaces in them.

**Vascular bundles**

The vascular bundles were rounded in outline, and they contained both phloem and xylem. Phloem lies towards the outside, and the xylem on the inner side. Cambium was absent as the whole pro-cambium was consumed in the formation of vascular tissues. The vascular bundles were, therefore, conjoint, collateral but closed. Each vascular bundle was surrounded by a bundle sheath. The bundle sheath was more developed on the outer and the inner sides. Hypodermis and bundle sheaths were found to be coalesced in some of the outer vascular bundles. Phloem consisted of sieve tubes, companion cells and a few phloem fibres. Phloem parenchyma was absent. Vascular bundle area of stem was highest (8690 $\mu m^2$) in accession of Rajshahi and the lowest (480 µm) in accession of Khagrachari. Vascular bundle number per stem was highest (43) in accession of Cox's Bazar, 2nd highest value (35) in Barguna, and lowest value (17) in accession of Gaibandha.

Xylem was observed in the form of letter Y and it is termed as endarch, i.e., protoxylem lies towards the center of the stem. Xylem was made up of vessels, tracheids, xylem parenchyma and a few xylem fibers. Metaxylem generally consisted of two large oval or rounded vessels lying at the upper two angles of the xylem. The metaxylem vessels had pitted walls. The two vessels were connected by polygonal tracheids with pitted thickenings. The individual vascular bundles were usually more or less circular or oblong to elliptical in transverse section. The phloem was found to make up of sieve elements and phloem parenchyma, where the sieve elements were remarkable for its diameter. Companion cells were frequent and sieve tubes had thick walls.

Protophloem was visible in many cortical vascular bundles. In most of the cortical and large hypodermal vascular bundles, the metaxylem was characterized by two vessels, of which the vascular bundle was composed. On rare occasions, more than two metaxylem vessels were noticed, but with a smaller diameter. The protoxylem, lying at the opposite pole of the bundle to that at which the phloem was situated, usually had a solitary vessel (true for hypodermal bundles) or a short radial row of vessels (true for cortical bundles) that had a much shorter diameter than the other vessels. There were many vascular bundles scattered in this heavily sclerified area in accessions of Barguna, Cox's Bazar and Saint Martin's Island. Metaxylem area of stem was highest (2320 $\mu m^2$) in accession of Rajshahi and lowest (480 $\mu m^2$) in accession of Saint Martin's Island and $2^{nd}$ lowest in case of Cox's Bazar. Phloem area of stem was highest (4620 $\mu m^2$) in accession of Rajshahi and lowest (990 $\mu m^2$) in accession of Saint Martin's Island (Figs. 2a-c). Analysis of variance (Table 2) reveals that all the anatomical features of stem showed significant differences of 0.001% level among the 24 accessions of *C. dactylon* collected from different habitats of Bangladesh.

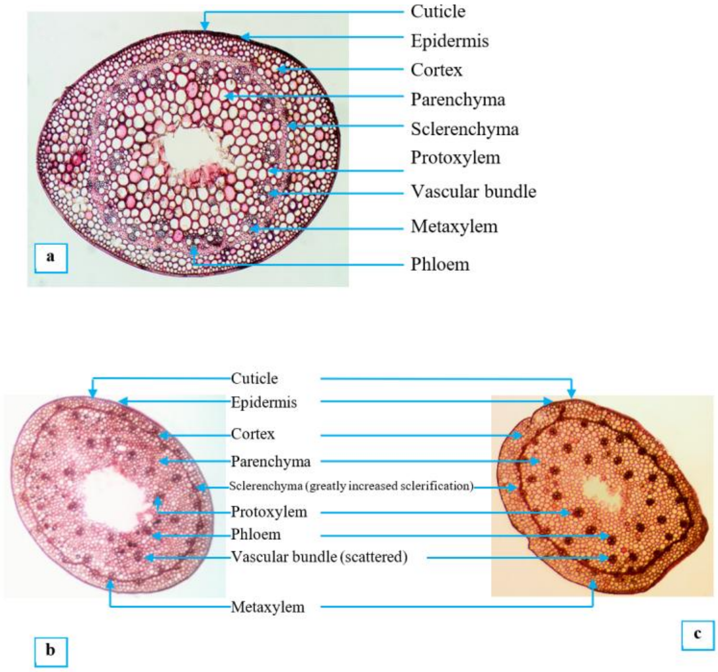



**Fig. 2(a-c):** Stem anatomy of *C. dactylon* accession collected from saline and non-saline habitat of Bangladesh; a) Lalmonirhat (non-saline habitat), b) Cox's Bazar (saline habitat), and c) Barguna (saline habitat).

Phloem and xylem were found to be separated from each other either by fibrous cells or by tracheary elements. Xylem parenchyma and phloem parenchyma with sclerified walls were noticed in most of the bundles. Protoxylem lies at a lower angle than xylem. Xylem parenchyma and a few fibers were found just outside them. Some of the protoxylem vessels and xylem parenchyma cells were found to be dissolved or separated during the rapid growth of the stem to form a cavity called the protoxylem cavity or lacuna.

**Table 2:** Analysis of variance (ANOVA) subjected to variation of different anatomical attributes of stem of *C. dactylon* on the basis of 24 habitats of Bangladesh.

| Data source | Source of variation | DF | Mean sum of square | F-value |
|---|---|---|---|---|
| Epidermis thickness | Habitat | 23 | 600486.194 | 2.646*** |
| | Error | 48 | 0.227 | |
| Cortex thickness | Habitat | 23 | 0.286 | 31.715*** |
| | Error | 48 | 0.009 | |
| Sclerenchyma thickness | Habitat | 23 | 0.047 | 4.493*** |
| | Error | 48 | 0.010 | |
| Vascular bundle area | Habitat | 23 | 9918495.109 | 3.153*** |
| | Error | 48 | 0.315 | |
| Vascular bundle number | Habitat | 23 | 112.429 | 331.756*** |
| | Error | 48 | 0.339 | |
| Metaxylem area | Habitat | 23 | 597560.326 | 1.998*** |
| | Error | 48 | 0.299 | |
| Phloem area | Habitat | 23 | 3033156.522 | 9.848*** |
| | Error | 48 | 0.308 | |

*** = significant at $p \leq 0.001$.

## Root anatomy

Anatomical attributes are illustrated here based on the transverse section of the root, as mentioned in the Figs. 3(a-x) and recorded data in Table 3 as follows. The highest value (4500 µm) of epidermis thickness of the root was found in the accession collected from Rajshahi, and the lowest value (1150 µm) was in the accession of Barguna. But an exceptional result was found in accessions of Cox's Bazar and Saint Martin's Island, where the epidermis thickness of the root was high (3240 µm and 3400 µm, respectively) compared to that of the other twenty-one accessions. Lysigenous aerenchyma was observed in roots of *C. dactylon* in the present study. The highest value (2.48 cm) for cortex thickness of the root was found in the accession collected from Cox's Bazar, and the 2nd highest value (1.93 cm) was found in the accession of Saint Martin's Island, and the lowest value (0.38 cm) was found in Gaibandha.

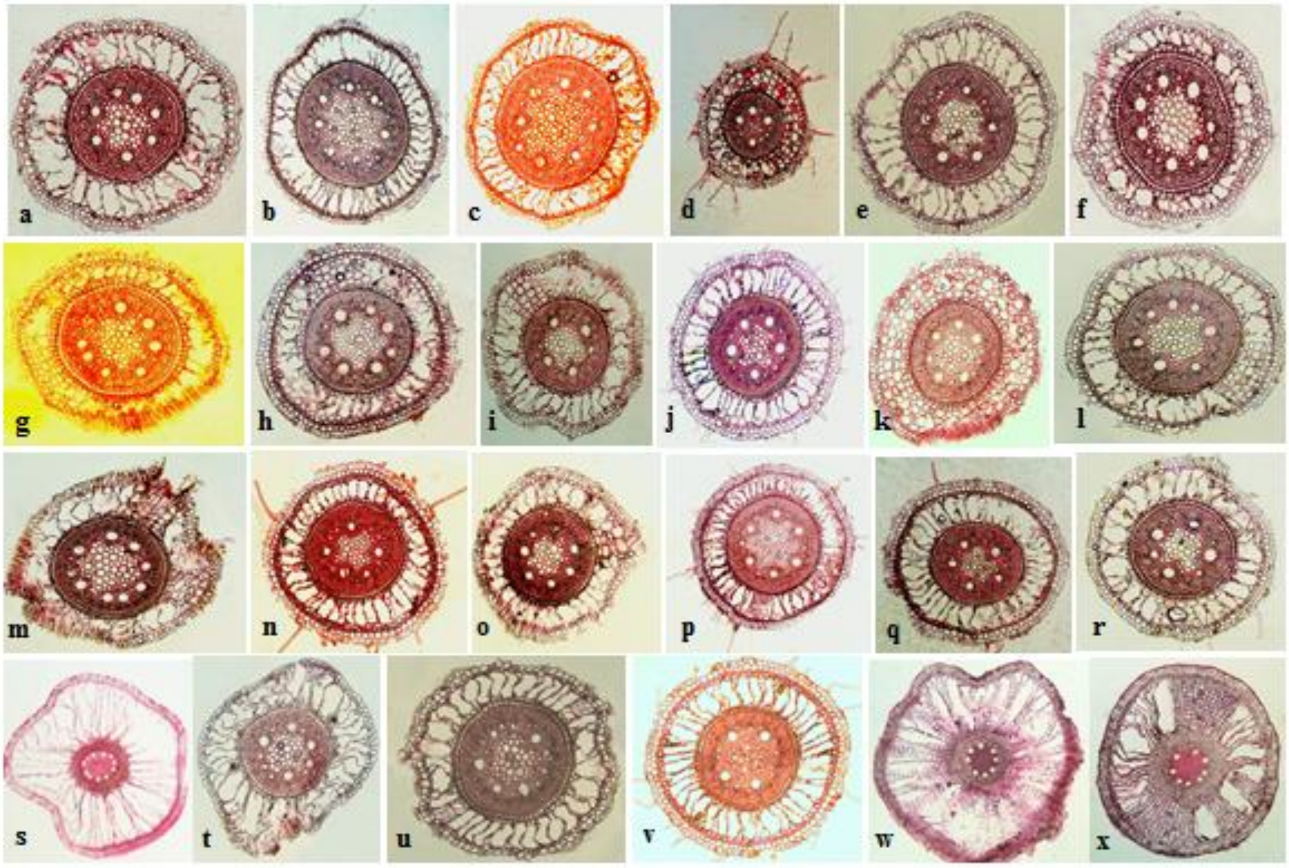


**Fig. 3 (a-x):** Root anatomy of *Cynodon dactylon* accessions collected from different habitats of Bangladesh; a) Rangpur, b) Lalmonirhat, c) Dinajpur, d) Thakurgaon, e) Panchagarh, f) Gaibandha, g) Rajshahi, h) Naogaon, i) Pabna, j) Gazipur, k) Narsingdi, l) Sherpur, m) Mymensingh, n) Khulna, o) Jessore, p) Jhenaidah, q) Faridpur, r) Shariatpur, s) Barguna, t) Khagrachari, u) Bandarban, v) Rangamati, w) Cox's Bazar, and x) Saint Martin's Island.

**Table 3:** Root anatomical attributes of different accessions of *Cynodon dactylon* collected from different habitats of Bangladesh (Mean±SE).

| Acc. No. | Name of the habitats | Epidermis thickness (µm) | Cortex thickness (cm) | Sclerenchyma thickness (cm) | Endodermis thickness (µm) | Metaxylem area (µm²) | Phloem area (µm²) | Pith thickness (cm) | Pith cell area (µm²) |
|---|---|---|---|---|---|---|---|---|---|
| 1. | Rangpur | 2790±0.12l | 0.89±0.04efg | 0.13±0.03abc | 2240±0.14f | 2710±0.53k | 2080±0.28i | 1.36±0.08efgh | 1660±0.14m |
| 2. | Lalmonirhat | 3430±0.53c | 0.93±0.01efg | 0.19±0.02abc | 1960±0.40l | 2950±0.50g | 2620±0.23d | 2.49±0.08bc | 2130±0.23e |
| 3. | Dinajpur | 2880±0.21k | 0.96±0.10def | 0.18±0.01abc | 2210±0.47g | 3090±0.08d | 3310±0.14b | 2.64±0.04ab | 2040±0.14f |
| 4. | Thakurgaon | 2660±0.48p | 0.60±0.03jk | 0.16±0.03abc | 1970±0.53k | 2880±0.15i | 2470±0.50f | 1.06±0.04ij | 1100±0.37s |
| 5. | Panchagarh | 2757±0.09m | 1.00±0.01cdef | 0.15±0.01abc | 1640±0.37t | 2480±0.33o | 2060±0.24j | 1.48±0.05e | 2333±0.45c |
| 6. | Gaibandha | 2720±0.39o | 0.38±0.04l | 0.11±0.05bc | 1680±0.14s | 2730±0.42j | 2500±0.35e | 1.22±0.02ghi | 1960±0.42g |
| 7. | Rajshahi | 4500±0.05a | 1.16±0.03c | 0.24±0.05a | 4243±0.10b | 5148±0.38a | 3350±0.32a | 2.43±0.01cd | 3300±0.49a |
| 8. | Naogaon | 2980±0.16i | 0.71±0.03hijk | 0.13±0.03abc | 1480±0.38u | 2580±0.27m | 1620±0.35s | 1.29±0.02efgh | 1820±0.15i |
| 9. | Pabna | 2630±0.30q | 0.88±0.07efg | 0.18±0.05abc | 2600±0.14e | 3150±0.46c | 2160±0.38h | 1.46±0.03ef | 1720±0.38l |
| 10. | Gazipur | 2610±0.27s | 0.60±0.10jk | 0.11±0.06bc | 1690±0.37r | 2440±0.23r | 1750±0.44n | 1.05±0.03ij | 1590±0.39q |
| 11. | Narsingdi | 2620±0.48r | 0.84±0.04fgh | 0.12±0.02abc | 1890±0.51o | 2470±0.18p | 1740±0.39o | 1.39±0.05efg | 1790±0.32j |
| 12. | Sherpur | 2400±0.11u | 0.69±0.09higk | 0.13±0.07abc | 1930±0.16n | 2560±0.26n | 1890±0.12l | 1.33±0.06efgh | 1820±0.36i |
| 13. | Mymensingh | 3640±0.45b | 1.13±0.07cd | 0.19±0.01abc | 2610±0.50d | 3980±0.34b | 2770±0.19c | 2.74±0.03a | 3110±0.32b |
| 14. | Khulna | 3110±0.48h | 0.67±0.02hijk | 0.16±0.02abc | 1980±0.09j | 2170±0.10t | 1580±0.41t | 1.07±0.11ij | 1600±0.52p |
| 15. | Jessore | 2290±0.33v | 0.57±0.05k | 0.14±0.04abc | 1970±0.36k | 2160±0.42u | 1750±0.41n | 1.05±0.09 | 1880±0.24h |
| 16. | Jhenaidah | 2950±0.19j | 0.99±0.01cdef | 0.07±0.03c | 1140±0.52v | 3050±0.05e | 2260±0.14g | 2.28±0.05d | 1770±0.17k |
| 17. | Faridpur | 3200±0.47f | 0.78±0.06ghi | 0.17±0.02abc | 1940±0.35m | 2320±0.16s | 1680±0.40q | 0.91±0.07j | 1550±0.38r |
| 18. | Shariatpur | 2750±0.34n | 0.66±0.04ijk | 0.13±0.03abc | 1970±0.39k | 2890±0.25h | 1650±0.29r | 1.33±0.03efgh | 1770±0.49k |
| 19. | Barguna | 1150±0.35x | 0.99±0.06cdef | 0.14±0.02abc | 1707±0.24q | 2640±0.46i | 830±0.29v | 0.27±0.05k | 260±0.41t |
| 20. | Khagrachari | 1790±0.32w | 1.02±0.01cde | 0.13±0.03abc | 2100±0.22h | 2970±0.10f | 2050±0.36k | 1.34±0.17efgh | 2210±0.41d |
| 21. | Bandarban | 2450±0.36t | 0.66±0.07ijk | 0.12±0.04abc | 2020±0.12i | 2460±0.25q | 1820±0.44m | 1.17±0.06hi | 1620±0.35o |
| 22. | Rangamati | 3120±0.36g | 0.77±0.02ghij | 0.19±0.03abc | 1840±0.45p | 2950±0.23g | 1740±0.40o | 1.20±0.06ghi | 1630±0.27n |
| 23. | Cox's Bazar | 3240±0.24e | 2.48±0.06a | 0.21±0.01ab | 3920±0.40c | 1800±0.14w | 1710±0.14p | 0.89±0.06j | 240±0.41u |
| 24. | St. Martin's Island | 3400±0.49d | 1.93±0.04b | 0.20±0.04ab | 6390±0.14a | 2070±0.17v | 1430±0.40u | 1.25±0.05fghi | 630±0.47s |

*Mean values followed by same lower-case letters (a, b etc.) are not significantly different (p<0.005) among different areas of cultivars as determined by Duncan's Multiple Range Test (DMRT, by SPSS 25).*

Endodermis thickness of the root was highest (6390 µm) in accession of Saint Martin's Island, 2nd highest value (4243 µm) in accession of Rajshahi, 3rd highest value (3920 µm) in accession of Cox's Bazar and lowest (1140 µm) in accession of Jhenaidah. Sclerenchyma thickness of root was highest (0.24 cm) in accession of Rajshahi and also high (0.21, 0.20 and 0.14 cm) values were found in accessions of Cox's Bazar, Saint Martin's Island and Barguna, respectively and lowest (0.07 cm) in accession of Jhenaidah. Intensive sclerification in the outer cortex, vascular region and endodermis layer was observed in the roots of Barguna, Cox's Bazar and Saint Martin's Island. In the roots of other accessions, low sclerification was noticed. Statistical analysis (Table 4) reveals that, except for sclerenchyma thickness, all the root anatomical characters showed significant differences at 0.001% level among the accessions of *C. dactylon* collected from different habitats of Bangladesh.

**Table 4:** Analysis of variance (ANOVA) subjected to variation of different anatomical attributes of root of *Cynodon dactylon* on the basis of 24 habitats of Bangladesh.

| Data source | Source of variation | DF | Mean sum of square | F-value |
|---|---|---|---|---|
| Epidermis thickness | Habitat | 23 | 1219255.908 | 3.439*** |
| | Error | 48 | 0.355 | |
| Cortex thickness | Habitat | 23 | 0.593 | 70.328*** |
| | Error | 48 | 0.008 | |
| Sclerenchyma thickness | Habitat | 23 | 0.005 | 1.229NS |
| | Error | 48 | 0.004 | |
| Endodermis thickness | Habitat | 23 | 3677782.348 | 1.031*** |
| | Error | 48 | 0.357 | |
| Metaxylem area | Habitat | 23 | 1359288.000 | 4.960*** |
| | Error | 48 | 0.274 | |
| Phloem area | Habitat | 23 | 1003406.522 | 3.021*** |
| | Error | 48 | 0.332 | |
| Pith thickness | Habitat | 23 | 1.140 | 91.943*** |
| | Error | 48 | 0.012 | |
| Pith cell area | Habitat | 23 | 1487178.516 | 3.751*** |
| | Error | 48 | 0.396 | |

***= significant at p≤ 0.001, NS= non-significant.

Fig. 4a-c showed the highest value (5148 μm$^2$) of metaxylem area of root was found in the accession collected from Rajshahi, and the lowest value (1800 μm$^2$) was in the accession of Cox's Bazar. The highest value (3350 μm$^2$) for the phloem area of the root was also found in an accession collected from Rajshahi, and the lowest value (830 μm$^2$) was found in Barguna. Pith thickness of root was highest (2.74 cm) in accession of Mymensingh and lowest (0.27 cm) in case of Barguna. Pith cell area of root was highest (3300 μm$^2$) in accession of Rajshahi and lowest (240 μm$^2$) in accession of Cox's Bazar.

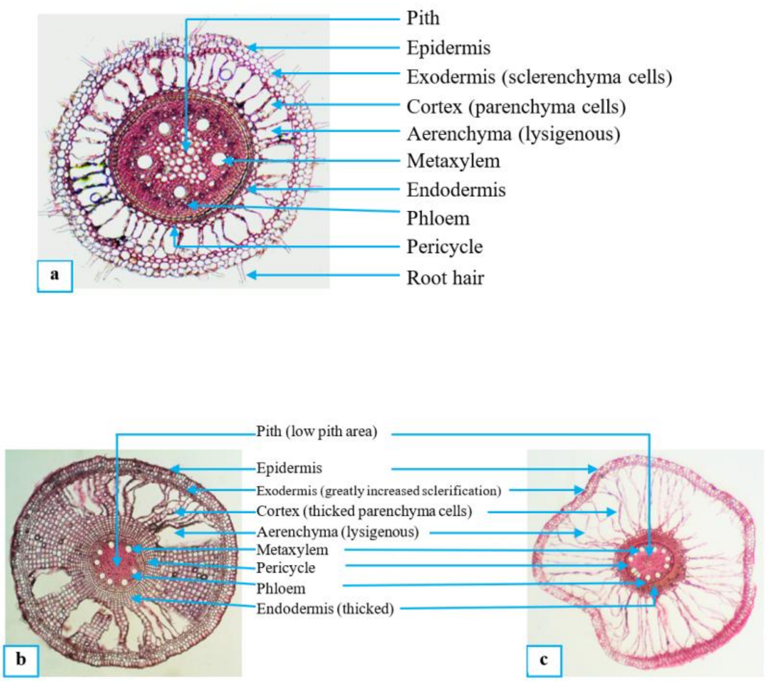



**Fig. 4(a-c):** Root anatomy of *C. dactylon* accessions collected from different habitats; a) Gazipur (non-saline habitat), b) Saint Martin's Island (saline habitat), and c) Barguna (saline habitat).

## Discussion

Anatomical characteristics are important features, which can be implicated in taxonomical diagnosis, as well as in explaining ecological conditions. It is now very evident that *C. dactylon* grows well on a wide variety of soils, from heavy clays to deep sands, provided fertility is not limiting. It tolerates both acid and alkaline soil conditions and is highly tolerant to saline conditions (Nitu et al. 2019b). This grass species survives some flooding but does best on well-drained sites. They are also drought-tolerant. Despite many types of unfavourable conditions, *C. dactylon* can survive by changing its anatomical and physiological characteristics. Considering this viewpoint, the anatomical features of the stem and root of 24 accessions of *C. dactylon* collected from different habitats were studied in the present investigation.

Usually, moderate soil temperature and acidic clay soil do not have drastic effect on their morphological, anatomical and physiological properties. But natural population of *C. dactylon* can have considerable genetic variation for tolerance to soil temperatures, salinity and drought (Speranza, 1995). Few accessions of this grass species in the present study have been found to tolerate salinity successfully. However, growth of this grass species is stimulated by moderate salinities and it can tolerate relatively high salinities (Mass and Hoffman 1977). The accessions collected from Barguna, Cox's Bazar and Saint Martin's Island studied in the present investigation revealed somewhat different anatomical features both in case of shoot and root. In case of hypodermis these three accessions showed greatly increased sclerification outside the vascular bundles. In other accessions, sclerification was low, and this is why sclerenchyma thickness values were high in all the accessions except those of Cox's Bazar and Saint Martin's Island. Similarly, epidermis thickness values in all the accessions were high compared to that of Cox's Bazar. Munns (2002) stated that this type of plant material can be directly used for re-vegetation of such affected areas, and the introduction of new salt-tolerant genes to increase the tolerance of crop cultivars also. In spite of different sorts of anatomical and morphological modification in plant bodies this grass species was found to be capable of minimizing detrimental effects of salts. Salt tolerant plant species shows a wide range of anatomical adaptive features like increased succulence both in case of root and stem, thick cuticle and deposition of wax, salt secretory trichome and glands, thick and many layered epidermis and well developed water storing tissues in the cortex, widening of casparian band and enhanced developments of root endodermis (Akram et al. 2002, Wahid 2003). In the present study, the cortex was parenchymatous, having thin-walled cells, but these cells surrounding the vascular bundle gradually sclerified into thick-walled cells. Cortex thickness values were significantly higher in all the accessions of bermudagrass except that of Saint Martin's Island and cortex, where the values were remarkably low.

In case of stem of bermudagrass in the present study cambium was found to be absent as the whole procambium was consumed in the formation of vascular tissues. The vascular bundles were, therefore, conjoint, collateral but closed. Number of vascular bundles per stem was highest in salt tolerant accession collected from Cox's Bazar and then 2$^{nd}$ highest value was found in case of Barguna, and lowest in that of Gaibandha. But Awasthi and Pathak (1999) reported increased vascular bundle area in relatively less salinity tolerant plants. The salt range population, however, showed slight decrease in this character along with increased in its number and that may be better adaptation for efficient water uptake as reported by Yujing et al. (2000). Xylem vessels are generally reduced under salt stress was reported by Gadallah and Ramadan (1997). This might be due to nature and type of plant species which varies from mesophyte to xerophyte. In salt tolerant population of *C. dactylon* from the salt range the stem was increased under saline regime. This increased succulence in stem may aid to store additional water and this is why this grass species shows better survival under unfavourable conditions. Hameed et al. (2010) reported that salt range population of *C. dactylon* showed specific root and stem anatomical adaptations for its better survival under harsh saline environment. Increased exodermis and sclerenchyma, endodermis, cortex, and pith parenchyma in roots were critical for reducing water loss and enhancing water storage capacity. In stem, increased stem area, increased epidermis and sclerenchyma thickness, increased cortex thickness and increased number and area of vascular tissue seemed to be crucial for its better survival under harsh saline environment.

Xylem of the stem in the present study was found to be made up of vessels, tracheids, xylem parenchyma and a few xylem fibres. The phloem was found to make up of sieve elements and phloem parenchyma. Younis et al. (2014) stated that salinity elevation in soil has contrary effects on xylem and phloem area of the stem and root as their area tended to decrease under salt stress condition. It was due to the reduction of water uptake by the plants under high saline conditions, and this reduction in water intake by the cells created an osmotic condition. However, plants growing under saline conditions undergo different anatomical and cytological changes (Winter 1988, Huang and Van Steveninck 1990). The modifications are different in organ to organ at different levels of organization (Mills 1989). Salt tolerance of natural populations depends on the existence of available genetic variability and evolutionary history of the habitats (Ashraf 2004). Exploration of genetic variation in natural populations, identification of genetically based markers and finally the incorporation of stress tolerance traits into glycophytic plants have been emphasized recently (Munns 2011).

Roots of a few plant species have been extensively studied, and surprisingly few details have been reported about the adaptive anatomy of wetland grass rhizomes or stolons. In the present study, the transverse section of bermudagrass roots showed that the epidermis was composed of a single thin-layered wall, tangentially elongated to irregularly shaped cells, and the highest value for epidermis thickness was higher in the accessions of the non-saline area compared to that of the saline area. On the other hand, cortex thickness was found to be higher in case of the accessions collected from saline belt. Yang et al. (2011) reported that in *C. dactylon*, the endodermis had lignified casparian bands and almost complete suberin lamellae with 5mm of the root tip; a few passage cells were present. The endodermis was heavily suberized with thick outer tangential walls and with lignified secondary cell walls in old roots. Adjacent cell layers of the inner cortex developed thick walls.

In the present study, lysigenous aerenchyma was observed in roots of *C. dactylon* in all the accessions, but comparatively, the aerenchyma area in accessions of the saline range was higher. Arber (1920), Justin and Armstrong (1987), Jackson and Armstrong (1999), and Evans (2003) stated that lysigenous aerenchyma is formed by the creation of gas spaces as a result of cell death and subsequent lysis, causing the collapse of cell files, e.g., root cortical cells. The stems of *C. dactylon* do not have selective barrier layers of endodermis and exodermis, but do have a thick, cuticular epidermis, while adventitious roots have extensive lysigenous aerenchyma (Yang et al. 2011). Air space tissues take two general forms, cavities (especially in pith but sometimes also in cortex) and aerenchyma (organized tissues, structured during their development) as per the suggestion of Jung et al. (2008) and Seago et al. (2005). Jung et al. (2008) also suggested that in grass roots, lysigenous aerenchyma is widespread. Takahashi et al. (2014) stated that the formation of aerenchyma tissue is an anatomical adaptation to excess water stress. Aerenchyma consists of longitudinally interconnected gas spaces that enable the rapid transport of gases between, and within shoots and roots (Evans 2003). In many wetland and aquatic plants, aerenchyma is developed in the shoots as well as the roots. In roots, primary parenchyma forms in the primary cortex and can be broadly classified into types: schizogenous aerenchyma and lysigenous aerenchyma (Armstrong and Armstrong 1994, Seago et al. 2005, Jung et al. 2008). In the present study, the identified aerenchyma might be lysigenous, since it develops based on the excess water stress during flooding and waterlogging. Soil waterlogging (or flooding of the soil) occurs when soil is saturated with water. Owing to the diffusion of gases through soil water, gas exchange between the soil and the atmosphere is strongly hindered (Colmer 2003). Soil waterlogging reduces plant growth as $O_2$ availability in the root zones decreases (Armstrong 1979, Jackson and Drew 1984). To cope with waterlogging, plants usually develop new roots with aerenchyma (Laan et al. 1989, Visser et al. 1996, Huber et al. 2009). Aerenchyma refers to tissue with air spaces that provide an internal pathway for oxygen diffusion in organs under water logged/submerged conditions (Armstrong 1979). Apart from this, plants may display other adaptive strategies that might act together to improve root aeration and oxygen consumption within the root (Cardoso et al. 2013).

In the present study, sclerenchyma thickness of the root of *C. dactylon* was found to vary from 0.07 cm to 0.24 cm, and intensive sclerification in the outer cortex, vascular region and endodermis layer was observed in roots of the accessions collected from Barguna, Cox's Bazar and Saint Martin's Island. In non-saline areas, sclerification in roots was found to be low. This might be due to the differential tolerance of the three ecotypes of bermudagrass to salt range, which relates well to the soil physicochemical properties of the habitats. Maintenance of ion balance in plants subjected to saline conditions is vital for sustaining growth and productivity under such environmental adversities (Munns and Tester 2008). Anatomical modifications to overcome high salinities are very specific not only in the grass species but also in the ecotypes (Hameed et al. 2011).

Vascular bundles in the roots of *C. dactylon* in the present study were found to be arranged in a ring of differential radials. The different characters of vascular bundles, like metaxylem area, phloem area, pith area and pith thickness, were found to differentiate among the accessions of non-saline and saline habitats in terms of their highest and lowest values. Ashraf (1994) and Hameed and Ashraf (2008) stated that plants growing on naturally salt-affected soils must have evolved a multitude of morpho-anatomical and physiological adaptive characteristics in view of the considerable length of time they have been exposed to the high selection pressure of the habitat, such as high salinity and aridity. This view point might be the region of differential response of the same plant species like *C. dactylon* in the present study. Moreover, Hameed et al. (2011) stated that specific anatomical and physiological modifications in plants exposed to stressful environments may enable them to thrive well on such environment. Although plants use a variety of physiological phenomena to counteract salt stress, regulation of ion homeostasis is one of the premier physiological processes operating in plants exposed to salt stress (Zhu 2003). This includes selective ion uptake (Flowers and Colmer 2008), accumulation of toxic ions, partially in terms of increased succulence (Hammed et al. 2009), and excretion of such unwanted toxic ions (Ramadan and Flowers 2004, Naz et al. 2009).

Twenty-four accessions of *C. dactylon* were studied in this investigation and a few of them were collected from that type of habitat which belongs to somewhat drought area as well as to different altitudes. Thus, the anatomical features of all these accessions were different to some extent. Altitudinal stress had a very little impact on stem anatomy. Epidermal cells were radially broader than tangential direction and no such differences were found in the length of epidermal cells of all the internodes. Hypodermis was found to be made up of thick walled lignified sclerenchyma cells. Down to hypodermis vascular bundle were found without cambium to show some pattern of arrangement. Ahmad et al. (2016) reported that epidermal cell was decreased significantly along the elevation in ecotypes from foot hill up to top hill sites. In contrast, sclerification increased significantly along with increase of elevation and the maximum increase in sclerification was recorded in top hill ecotype. Cortical cell area, vascular bundle area and numbers generally decreased along with increase in altitude. They also reported that root anatomical characteristics like epidermal cell area and cortical region thickness showed a consistent decrease from foot hill to top hill with increase in the elevation. Cortical and endodermal cell area significantly increased similarly but further increase in altitude showed significantly decreased cortical cell area in top hill ecotype. Sclerenchymatous thickness increased invariably in all ecotypes with increase in the altitude. However, in the present study, a very small variations in root and stem anatomy might be due to lack of significant differences in altitudinal variation from sea levels to the northern part of Bangladesh. Nevertheless, a few remarkable anatomical features were noticed in those accessions, collected from the Chittagong hill tracts and the saline areas.

On the contrary, root and shoot anatomical characteristics were found to show little variation among the accessions in the present study, and that might be due to seasonal drought conditions. Angiosperms that occupy continuously wet habitats have constitutive aerenchyma, but roots of many other angiosperms can respond to anaerobiosis by developing lysigenous or schizogenous cortical aerenchyma (Konings and Lambers 1991). In dry land plants, this response is mediated by increased ethylene levels, which are the result of reduced diffusion out of the roots under conditions of poor aeration or waterlogging as well as of increased ethylene production under low oxygen concentrations (Kawase 1981, Drew 1990, Konings and Lambers 1991). The most striking feature of the grass species *C. dactylon* under study was the positive response of root aerenchyma in both dryland and saline habitat. This is one of the adaptive responses of this grass species. This grass species had a well-differentiated hypodermis, including exodermis with a few isodiametric cell layers. However, it has been reported that a compact exodermis may play a role in preventing the collapse of the cortex. Simultaneously, a thickend exodermis may act as a barrier excluding toxic reduced ions and also reduce the outward radial fusion of oxygen (Moog and Janiesch 1990). The cell wall thickenings in exo-and endodermis under drought may function in protecting the stele and cortex from desiccation (Stasovski and Peterson 1991). Baruch and Merida (1995) stated that the effect of drought on root anatomy is less understood. Roots are exposed to drying soil may exhibit a pronounced suberization of the exodermis and endodermis, which is believed to protect the death of cortical cells.

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