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Microscopic Study of the Major Plant Part of a Euphorbia, *Croton bonplandianum* Baill

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Abstract: *Croton bonplandianum* Baill. is an herbaceous medicinal plant, and was taken for anatomical study in the present investigation. Prime parts, i.e., stem, leaf, and root, were studied anatomically in free-hand sections after being stained with 1% safranin under a light microscope. The micrographs were taken with an accessory digital camera, and the diameter was measured from the micrographs. The stem was angular in cross-cut view and consisted of an epidermal layer including a layer of spindle-shaped cells (22.3mm). The cortex had seven to eight layers of elliptical, thick-walled compact parenchyma cells (43.5mm). The secondary xylem in the vascular bundle was endarch and thick-walled. Secondary phloem grew in a continuous cylinder around the xylem. Pith was wide, composed of parenchymatous cells, and 85.7 mm in diameter. The transverse section of the leaf showed the isobilateral arrangement. The epidermal layer was made up of a single layer of compressed to elongated parenchymatous cells. The horizontal palisade layer was present in the adaxial sub epidermal part of the midrib. The midrib was represented by a vascular bundle which was made up of phloem (outer) and xylem (inner). Calcium oxalate crystals were seen in phloem parenchyma. The crystal layer was one or two cells thick. A sclerid layer was seen around the vascular bundle. The surface preparation showed the paracytic type of stomata. Number of stomata was 0.227/mm^2 and the index was 21.5. The transverse section of the root was composed of the outer epidermis, which was made up of a few layers of irregular and compressed cork cells. Sclerenchymatous cells were also found to be scattered throughout the cortex and were lignified in nature. The vascular bundle was collateral in nature, with phloem arranged outwards and the xylem (lignified, 28.3mm in diameter) situated inwards, occupying the whole area of the section. The secondary phloem was well preserved. It included parenchyma cells and sieve elements associated with the companion cells. The central pith was found to be absent.

Keywords: *Croton bonplandianum*, Flowering Plant, Leaf, Plant Anatomy, Root, Stem

1. Introduction

Croton is a genus comprising around 1300 species, widespread in tropical regions, and *Croton bonplandianum* Baill. is one of them. It belongs to the family Euphorbiaceae. It is a flowering plant. *C. bonplandianum* is a much-branched woody herb, 20–50 cm tall, with branches that are moderately stellate-hairy to subglabrous. Traditionally, this plant is used to treat liver and skin diseases, including ring worm infection, and also to cure the swelling of the body [4]. This plant's leaves are used to treat cuts and wounds, venereal sores, and cholera [22]. The pharmacological properties of leaf extracts have been evaluated for antioxidant and wound healing properties. The effect of the leaf residue of *C. bonplandianum* on the growth and metabolism of parthenium was studied [5, 20]. The part that has medicinal value is seed and seed oil. The seeds are used for the treatment of jaundice, acute constipation, abdominal dropsy, and internal abscesses [25]. The fresh juice of the plant is used for headaches by ethnic groups. Latex from plants has a healing effect on wounds and cuts [8, 6]. The latex of the plant (1:5 v/v in 50% acetone) showed anti-fungal activities by causing absolute inhibition against two ring worm fungi, *Microsporium gypsum* and *Trichophyron mentagrophyt* [3]. People use *Croton bonplandianum* Baill as both fuel and detergent. Firstly, the stems and branches are used as fuel, and afterwards the ash is collected and kept in a bottle for 5–6 days. Then the ash is put in warm water and used as a detergent for cleaning cotton garments.

Anatomical work in higher plants has been done by several authors [7, 2, 27, 23, 11, 10, 28, 17, 14, 16]. The anatomy of

C. bonplandianum Baill. has received little attention. The report on leaf anatomy was published by Prasanna and Karpagam [21]. They discovered that *C. bonplandianum* Baill. shared characteristics such as calcium oxalate crystals distributed throughout the leaf and well-represented vascular bundles in the petiole, leaf, stem, and root. Their distinct characteristics, such as vasculature in the petiole, sclerenchyma, and lactifers, were observed. The leaf lamina revealed loosely arranged spongy parenchyma with plenty of air space. The root showed an oxidative hermidin blue layer with crushed cortex. Although anatomical characters of stem, leaf, and root were carried out once for *C. bonplandianum*, it was a very limited study. From the above context, it is clear that more anatomical research is needed to elucidate the anatomical features of *C. bonplandianum*. In the present study, the internal features of the stem, leaf, and root of *C. bonplandianum* were studied.

2. Materials and Methods

Croton bonplandianum Baill. is naturally grown in different places at Rajshahi University campus, Rajshahi, Bangladesh (Figure 1A). The plants were collected from the Rajshahi University campus. Healthy whole plants were collected and the stems, leaves, and roots of the studied plant were separated and washed by tap water to remove dust or soil for section preparation.

Free-hand cross-cut thin sections were prepared from stem and root slices without using supporting materials, but the leaf sections were cut with the support of potato blocks. To cut sections, Platinum Coated Hi-Stainless Steel Razor Blades (Japan) were used to cut sections. Epidermal peels of the lower surface were made by placing the leaf blade taken from a standard median portion of the leaves on a clean glass slab, with the surfaces to be studied facing down. The specimens were irrigated with water holding it downwards from one end and then the epidermis above the desired surface was scraped off carefully with a sharp razor blade. Thin and uniformed sections as well as peels were separated and stained with 1% safranin and mounted on a temporary slide for microscopic observation. The cells in photographs of all sections were under taken to measure diameter.

3. Results

3.1. Stem Anatomy of Croton bonplandianum Baill

The major criteria selected for this study involve the internal structures of *Croton bonplandianum* Baill. The stem was angular in cross section. It consisted of an epidermal layer, a cortical zone, secondary xylem, phloem, and a wide pith. The epidermal layer included a layer of spindle-shaped, thick-walled cells (22.3 mm) covered with cuticle (Figure 1B, 2A). The cells were compactly arranged without any intercellular spaces. It was represented by the cells with slightly thicker walls.

The cortex had seven or eight layers of elliptical, thick-walled compact parenchyma cells (43.5 mm, Figure 2A). Located in the inner cortical portion were small clusters of lignified sclerenchyma cells (Figure 1B). Beneath the cortical layer was the vascular bundle. The vascular bundle was endarch, collateral, and conjoint. The secondary xylem was thick-walled. It consisted of radially aligned lines of vessels. The vessels were circular to elliptical, thick-walled, and were solitary or in radial multiples. The vessels were wide. Xylem fibers were squarish in sectional view; they were in regular, compact radial lines. The cells were thick-walled and lignified (Figure 1B). Secondary phloem formed in a continuous cylinder that surrounded the xylem. The phloem elements were in short radial lines. Medullary rays running throughout the whole length of the xylem layer were of the uniseriate type. The central portion of the stem was made of wide pith with a thick parenchymatous layer that was round to oval in shape and lacked intercellular spaces. Pith was wide and 85.7mm in diameter (Figure 1B, 2A).

3.2. Leaf Anatomy of Croton bonplandianum Baill

The leaf is the primary photosynthetic organ of the plant. The leaf consisted of a thick midrib and lateral vein (Figure 1C). Histologically, the transverse section of the leaf showed the isobilateral arrangement. The midrib of the leaf was planoconvex in sectional view, with a flat adaxial side and a semicircular wide abaxial part (Figure 1C). The epidermal layer of the midrib was thin and the cells were distinct, squarish in shape, and thick-walled. A thick cuticle was present in the epidermal layer. The epidermal layer was made up of a single layer of compressed to elongated parenchymatous cells. The ground tissue consisted of thick-walled and compacted parenchymatous cells. The palisade layer was present in the adaxial sub epidermal part of the midrib.

The central portion of the midrib was represented by the U-shaped collateral vascular bundle, which was made up of phloem (outer) and xylem (inner). The vascular strand was single. The arrangement of xylem (26.7mm) was normally endarch and lignified. Pholem occurred along the lower part, circular discrete masses with narrow gaps in between (Figure 1C, 2B). Cambium was present between xylem and phloem. Calcium oxalate crystals were seen regularly shaped within the phloem parenchyma. The crystal layer was one or two cells thick. A sclerid layer was seen around the vascular bundle (Figure 1C). The surface preparation showed the paracytic type of stomata; the number of stomata was $0.227/mm^2$ and the index was 21.5 (Figure 1D).

3.3. Root Anatomy of Croton bonplandianum Baill

The transverse section of the root was composed of the outer epidermis, which was made up of a few layers of irregular and compressed cork cells. The root was rough with irregular fissures on the surface. The root exhibited slightly eccentric growth with a greater amount of secondary growth on one side than the other (Figure 1E).

The cortical zone was narrow and the cortical cells indicted in certain regions and collapsed into dark portions in other

regions. The cortical region, comprising of randomly arranged parenchymatous cells, lies below the epidermal. Sclerenchymatous cells were also found to be scattered throughout the cortex and were lignified in nature (Figure 1E).

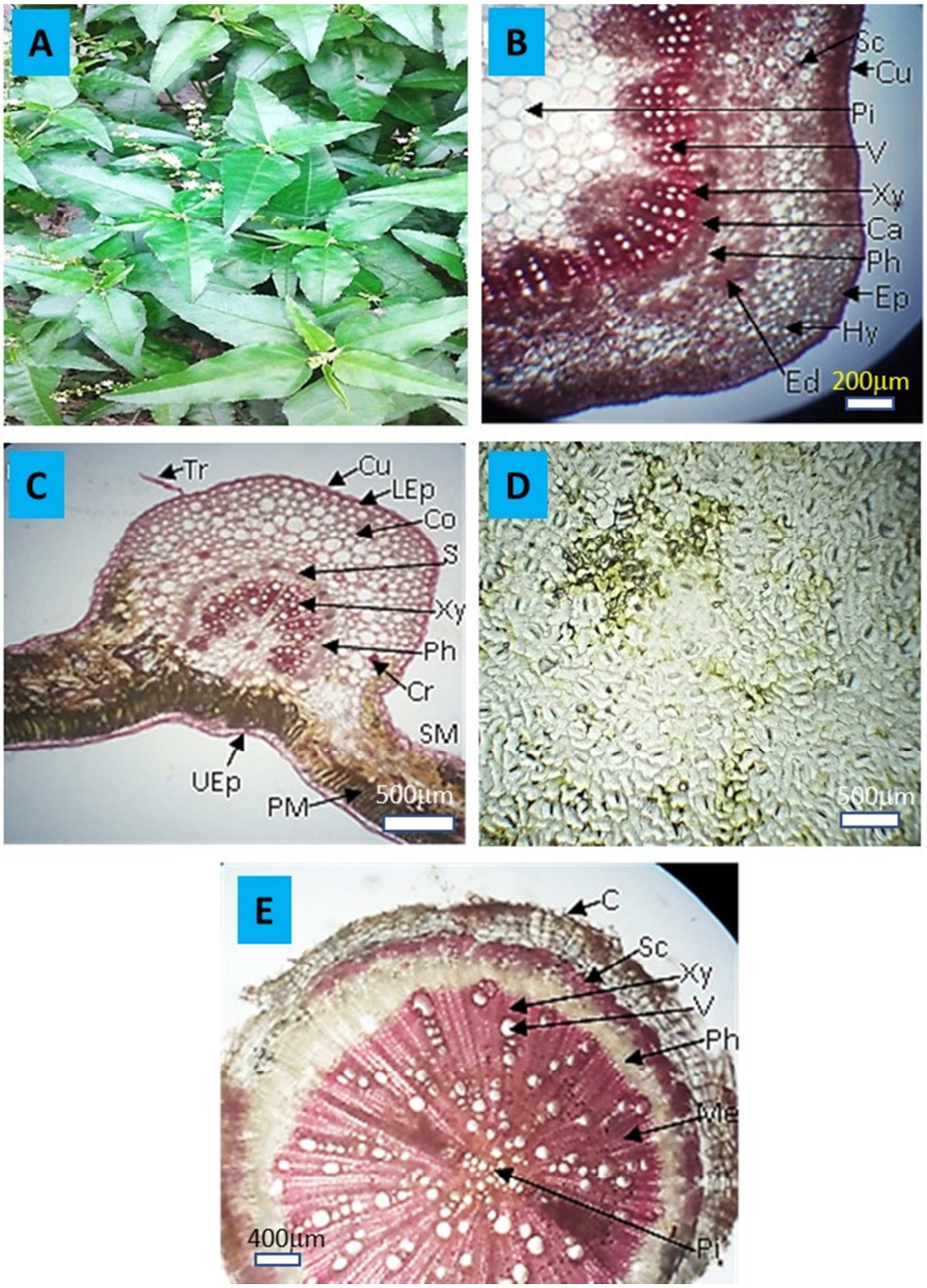

Figure 1. *Anatomy of Croton bonplandianum* Baill. *A) Plants with flowers, B) TS of stem, C) TS of leaf, D) Stomatal features on lower surface of leaf, E) TS of Root. Ep: Epidermis, Hy: Hypodermis, Co: Cortex, Ph: Phloem, Ca: Cambium, V: Vessel, Ed: Endodermis, Pi: Pith, LEp: Lower Epidermis, UEp: Upper Epidermis, SM: Spongy Mesophyll, PM: Palisade Mesophyll, Tr: Trichome, S: Sclerid, Cr. Crystal, Sc: Sclerenchyma, Me: Medullary ray, C: Cork.*

The vascular bundle was collateral in nature, with phloem arranged outwards and the xylem (lignified, 28.3 mm in diameter) situated inwards, occupying the whole area of the section. The secondary phloem was well preserved. It included parenchyma cells and sieve elements associated with the companion cells (Figure 1E, 2C). The phloem elements were wide and thick-walled. Secondary xylem consists of diffusely distributed vessels in the ground tissue of xylem fibers.

The vessels in the central part of the root were narrow and those towards the periphery were wider (Figure 1E). The vessels were solitary, circular, and thick-walled. The xylem fibers were thick-walled and lignified. The fiber lumen was narrow. The cells were compact and dense. Medullary rays (uniseriate) were found to be penetrating throughout the whole length of the xylem. The central pith was found to be absent (Figure 1E).

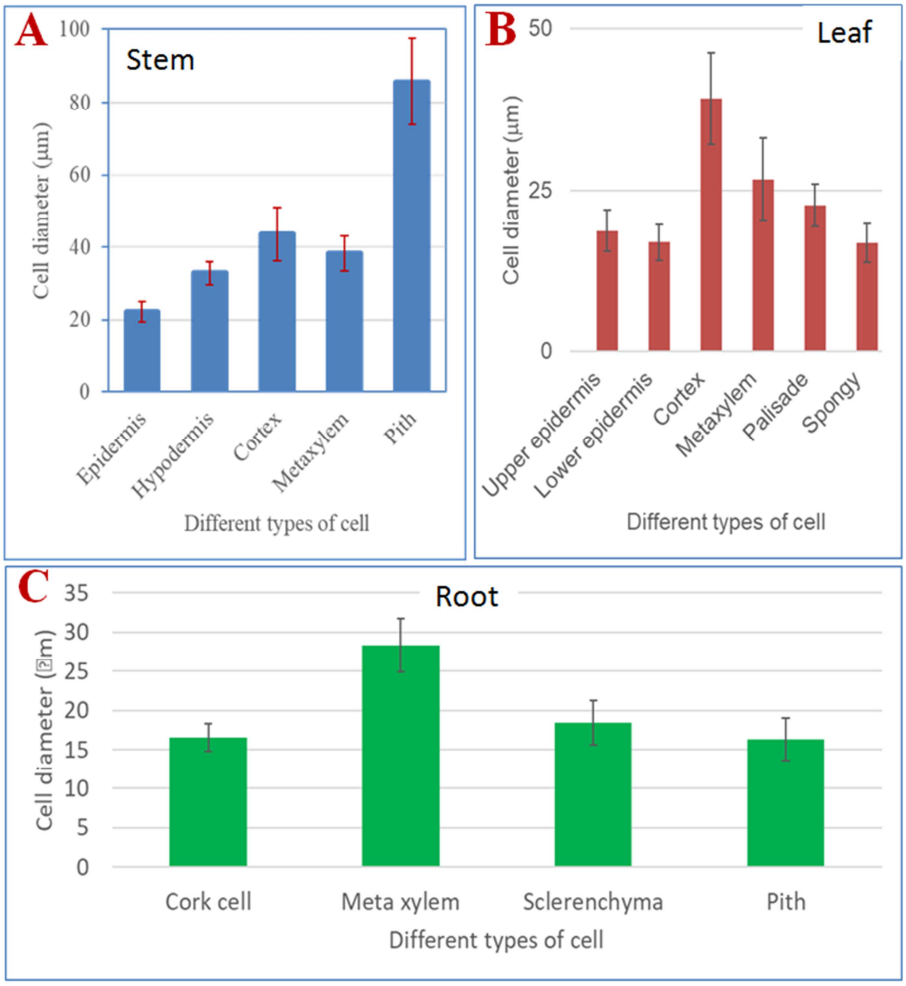

Figure 2. *Diameter of different cells of Croton bonplandianum* Baill*. A) Assorted stem cells diameters, B) Diameter of various cells in leaf, C) Diameter of different cells of root.*

4. Discussion

The investigation showed that the stem varied in the outline shape; it was mostly angular in *Croton bonplandianum* Baill. The results are in dissonance with the findings of Prasanna and Karpagam [21], who found that the transverse section of the stem showed a circular outline. The stem anatomy of examined samples showed general features such as a single-celled epidermal layer, parenchymatous cortical cells, a discontinuous layer of lignified sclerenchymatous cells, and a bicollateral vascular bundle. The findings also agree with the results of Prasanna and Karpagam [21]. The extra feature was not observed in the cortical area of the studied plant, whereas *C. cordiifolius* has laticifers, crystals, druses, and starch in the stem cortex [15, 26]. The central portion of the stem was made of wide pith with a thick parenchymatous layer that was round to oval in shape and lacked intercellular spaces. The findings in the studied samples report are similar to the report of Narendra et al. [19]. In other species of Croton, *Croton cordiifolius* was reported with stem anatomical traits by Iasmine et al. [15]. The findings are similar to more or less the present studied sample. In the pith of the stem were found lignified cells, stone cells, crystals, druses, and starch that were not observed in the studied plants.

The leaf is a variable organ. In Euphorbiaceae, leaves are mostly alternate but may be opposite or whorled, and they are simple or compound or sometimes highly reduced. The leaf anatomy of Euphorbiaceae is unique in the sense that it is latex-yielding. The phenomenon is not found in the studied sample. In the studied plant samples, the common anatomical characteristics reported in midrib and leaf blade are those illustrated earlier in *C. bonplandianum* [21], *C. sparsiflorus* [9], *C. cordiifolius* [15] and *C. lanjouwensis* [1].

The laticifers are distributed in several genera of Euphorbiaceae [18]. They can be found in all vegetative organs, as in the case of species of Euphorbia [12]. According to Rudall [25], in Croton, the laticifers are non-articulated and their abundance depends on the environment. The laticiferous phenomenon is not found in the studied plant (*Croton bonplandianum* Baill.). In leaf section, calcium oxalate crystals were seen in regular phloem parenchyma. The crystal layer was one or two ells thick. The vascular bundle was surrounded by a sclerid layer. Elumalai et al. [9] discovered star-shaped cystolith (calcium oxalate and

calcium carbonate) as a storage product in leaf sections of *C. sparsiflorus*. The lower region of mesophyll cells has a black-coloured substance known as phlafofeen (an oxidized product of tannin).

According to Haiad [13], the common characters in the leaf blades of Croton species: amphistomatic leaves, paracytic stomata, dorsiventral mesophyll, and biconvex midrib, with collateral vascular bundles. However, in *C. cordiifolius*, the leaves are hypostomatic, the mesophyll is isobilateral, and the midrib has a concave-convex contour. But, some of these characteristics of *C. cordiifolius* have already been described in other species of Croton, such as hypostomatic leaves in *C. lanjouwensis* [1] and isobilateral mesophyll in *C. rhamnifolioides* [24]. *Croton rhamnifolius* presents a midrib with a plan-convex contour [24]. The lower surface preparation shows the paracytic stomata in the studied plant.

The common features of epidermal, ground, and vascular tissue systems in the root were observed and reported earlier [21]. The central pith was found to be absent. A similar observation in the root anatomy of *C. bonplandianum* Baill. was reported by Prasanna and Karpagam [21]. In other species of *Croton*, *Croton cordiifolius* was reported with root anatomical traits by Iasmine et al. [15]. They reported that the root showed a cylindrical contour. Randau et al. [24] did not mention the presence of laticifers in the roots of *C. rhamnifolius* and *C. rhamnifolioides*, as it was not identified in this study.

5. Conclusion

The present study attempted to identify anatomical and histochemical features of *Croton bonplandianum*. The identified chemical in the histochemical study was found in leaf sections but not in stem and root sections. The present investigation was useful for the study of higher studies.

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