STEM ANATOMY OF THREE SPECIES OF PINANGA FROM ASSAM, INDIA

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STEM ANATOMY OF THREE SPECIES OF PINANGA FROM ASSAM, INDIA. The stem anatomy of the genus *Pinanga* Blume distributed to Assam of northeast India remains unattended. An anatomical study was conducted to address this research gap, where the plant samples to *Pinanga* were collected from different parts of Assam and identified as *P. gracilis*, *P. griffithii*, and *P. sylvestris*. The findings recorded anatomical variation among the species. Freehand sections of the stems were conducted, double-stained, quality specimens mounted, and suitable slides were observed under a light microscope. The types of epidermal cells and metaxylem vessels were variable among the studied species. In this study, vascular bundles with more than one metaxylem vessel were also recorded, which can be used for identification.

Keywords: *Pinanga*, stem, anatomy, Assam, India

ANATOMI BATANG TIGA SPESIES PINANGA DARI ASSAM, INDIA. Anatomi batang dari genus Pinanga *Blume yang tersebar di Assam di timur laut India masih belum diketahui. Sebuah studi anatomi dilakukan untuk mengatasi kesenjangan penelitian ini, di mana sampel tanaman* Pinanga *dikumpulkan dari berbagai daerah di Assam dan diidentifikasi sebagai* P. gracilis*,* P. griffithii*, dan* P. sylvestris*. Temuan ini mencatat variasi anatomi di antara spesies tersebut. Bagian batang yang dipotong secara bebas, diwarnai dua kali, spesimen berkualitas dipasang, dan slide yang sesuai diamati di bawah mikroskop cahaya. Jenis sel epidermis dan pembuluh metaxylem bervariasi di antara spesies yang diteliti. Dalam penelitian ini, bundel pembuluh vaskular dengan lebih dari satu pembuluh metaxylem juga dicatat, yang dapat digunakan untuk identifikasi.*

Kata kunci: Pinanga*, batang, anatomi, Assam, India*

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I. INTRODUCTION

The subtribe Arecinea of the tribe Areceae includes three palm genera viz. *Areca* L., *Nenga* H. Wendl. & Drude and *Pinanga* Blume (Baker & Dransfield, 2016). The genus *Pinanga* shows size variation in different ecologies (Dransfield, 1991) and is represented by around 131 species (Randi, Hikmat & Heatubun, 2019) which are distributed in India, China, Bhutan, Bangladesh, Nepal, Myanmar, Thailand, Laos, Cambodia, Malaysia, Indonesia, Vietnam, and Papua New Guinea (Renuka & Sreekumar, 2012). Among these countries, Indonesia occupied the highest position in terms of species richness under the genus (Witono, 2003). Compared to Indonesia, only five species were reported from India, viz. *P. dicksonii* (Roxb.) Blume, *P. gracilis* Blume, *P. griffithii* Becc., *P. manii* Becc., and *P. sylvestris* (Lour.) Hodel (Renuka & Sreekumar, 2012). Interestingly, all these five species were not reported from Indonesia (Henderson, 2009). Three out of the five species were reported from the state Assam of north-eastern India, viz. *Pinanga gracilis*, *P. griffithii* and *P. sylvestris* (Mehmud & Roy, 2021b; Renuka & Sreekumar, 2012). The distribution of these species also extended up to neighbouring countries viz. *P. griffithii* in Myanmar, *P. gracilis* in China, Nepal, Bhutan and Bangladesh, and *P. sylvestris* in China, Myanmar, Thailand, Cambodia, and Laos (Henderson, 2009).

Significant research work was conducted to study the anatomy of stem, leaf, lamina axis, root and nature of vascular bundles in a few species of *Pinanga* (Tomlinson, 1961) but excluded *P. gracilis*, *P. griffithii* and *P. sylvestris*. The study also highlighted the importance of anatomical features in taxonomy. Morphological features were considered in a phenetic study on clustered *Pinanga* of Java and Bali, where ecological parameters especially light intensity and temperature were found to link with wide morphological variations (Witono, 2003). Thomas and Boura (2015) investigated the stem anatomical features, primarily the diameter of vessels and raised a key question to investigate the causes of palm diversity i.e., whether the existing diversity is a consequence of phylogenetic or the influence of climatic factors. Except for *P. gracilis*, leaf morphologically of both *P. griffithii* and *P. sylvestris* are quite similar to each other. The importance of stem anatomical studies and their uses for ascertaining the identity of different palm species in India were conducted (Bhat, Nasser & Thulasidas, 1993; Mathew & Bhat, 1997; Mehmud & Roy, 2020a, 2020b; Renuka, Bhat & Pandalai, 2010). This paper studies three species of *Pinanga* and observes the anatomical attributes of the stem that are used for their taxonomic determination.

II. MATERIAL AND METHOD

A. Study Site

In the present study, the location of the samples of *Pinanga gracilis* and *P. griffithii* was recorded as 24°26'N to 92°49'E in Cachar district of Barak Valley, and for *P. sylvestris* as 25°50'N, 92°14'E in West Karbi Anglong district of Assam. The state shows tropical monsoon rainfall with high humidity (Borborah, Borthakur & Tanti, 2016). But within the state, a subtropical monsoon climate was reported in the hilly district of Karbi Anglong (Barooah & Ahmed, 2014) and a tropical monsoon climate in Barak Valley (Borah, Rabha & Athokpam, 2016).

B. Methods

The field survey was conducted in various districts of Assam for material collection. Furthermore, the materials were processed for herbarium preparation and also stored in formalin for anatomical study. The collected specimens were to be identified based on the works of Henderson (2009) and Renuka and Sreekumar (2012). Further, the identification was confirmed by visiting herbaria houses of the Botanical Survey of India viz., Eastern Regional Centre, Shillong (ASSAM) and Central National Herbarium (CAL). Perpared vouchers were deposited in ASSAM and at the Herbarium of the Department of Botany, Cotton University. Two samples for each species were selected for the study. The samples

were internodes from the middle portion of the stem. With the help of a sharp blade, free hand transverse sections were done. Sections were stained by using safranin and light green and also passed through different alcoholic grades as mentioned by Chamberlain (1924) except the use of D.P.X. for mounting.

C. Data Analysis

The prepared slides of the sections were observed under a light microscope (Lawrence Mayo, N300M). The nature and measurements of different cells were recorded and photographs (Figure 1-3) were taken. The observations made in the present study were compared with relevant studies (Thomas & Boura, 2015; Tomlinson, 1961).

III. RESULTS AND DISCUSSION

In all species, the outline of the stem was round and the diameters of the stems were around 1.5–2.3 cm, nodes were prominent, and the internodes were 5–9 cm long. Cork cells were observed in the section of all the species, followed by the epidermis, hypodermis, ground parenchyma, scattered fibrovascular bundles and fibres. The anatomical features recorded for each species are included below.

A. *Pinanga gracilis* Blume

The epidermis is a single layer; cells are round to rectangular, ca. 11–12 × 8–10 µm and covered by cuticle (Figure 1A). Hypodermis is two to three layers, cells are irregular, round to ovate and thin-walled; few fibre bundles are present in this layer. The peripheral fibrovascular bundles are congested, variable in size, ca. 220 × 168 µm, with one or two metaxylem vessels and one phloem strand. The ground parenchyma cells are thin-walled, variable in size, round to ellipsoid. The central fibrovascular bundles are scattered (Figure 1B), ovate to round, irregular in size, ca. 110–147 × 80–98 µm; metaxylem vessel 1–4, ovate to round (Figure 1B-C), ca. 28–34 µm in diameter; phloem strand single, ovate or kidney-shaped, ca. 25–56 µm long, with many sieve elements; protoxylems one to eight, thick-walled, round, ca. 26–29 µm in diameter. Few tannin cells and fibres bundle are present in the ground tissue.

B. *Pinanga griffithii* Becc.

The epidermis is a single layer; cells are drop-shaped or ovate, ca. 12–14 × 13–16 µm, cuticle present, epidermal cells ovate or drop-shaped (Figure 2A). Hypodermis are two or three layers, cells are round to hexagonal with few fibre bundles. Peripheral fibrovascular bundles

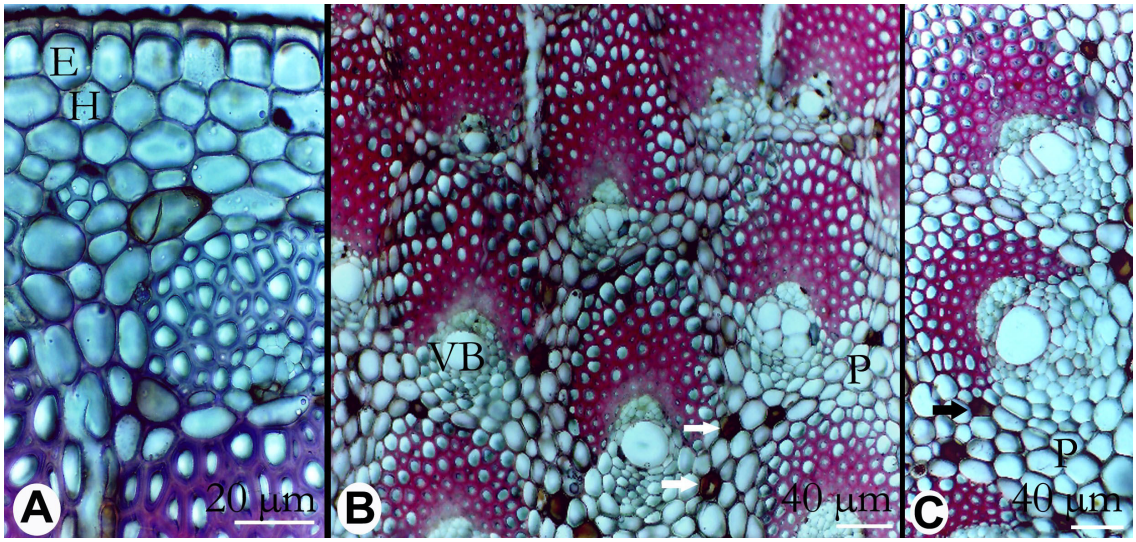

Figure 1. *Pinanga gracilis* Blume, **A**. Epidermis (E) and hypodermis (H) **B-C**. Fibrovascular bundles (VB) and ground parenchymatous tissue (P) (arrow showing tannin cells)

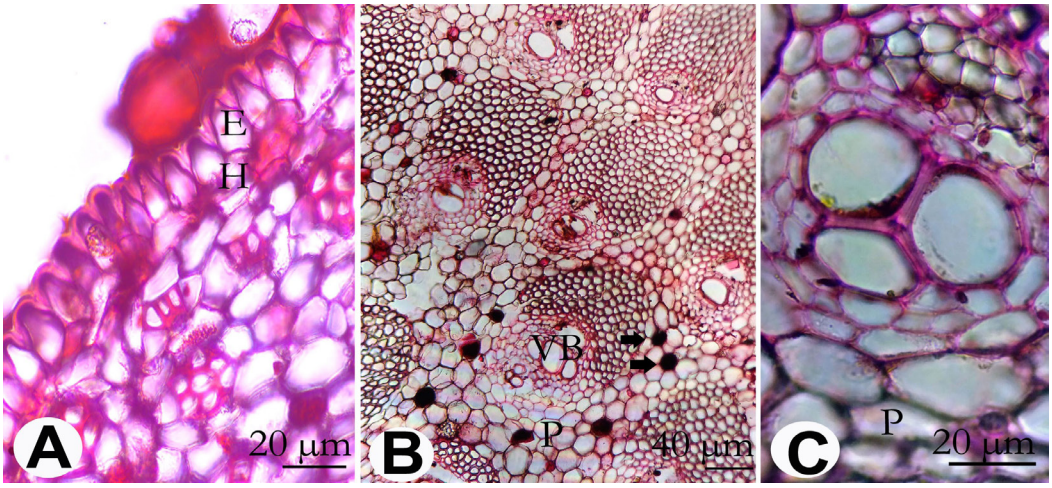

Figure 2. *Pinanga griffithii* Becc, **A**. Epidermis (E) and hypodermis (H) **B-C**. Fibrovascular bundles (VB) and ground parenchymatous tissue (P) (arrow showing tannin cells)

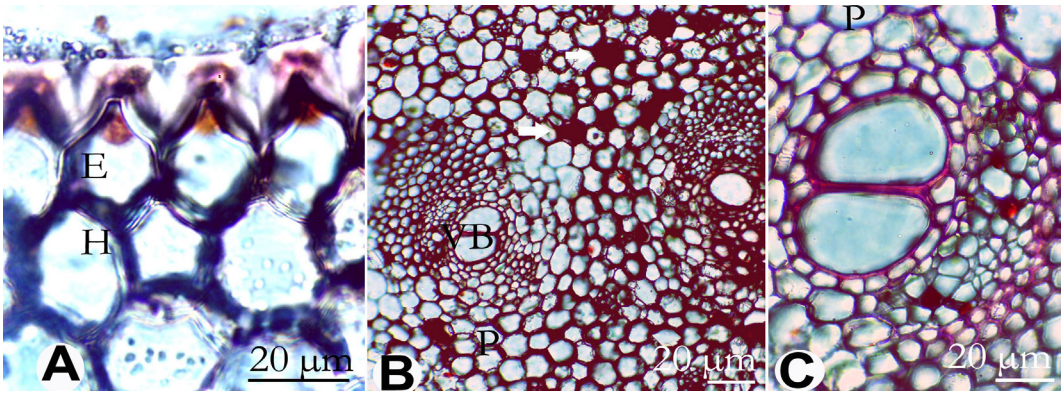

Figure 3. *Pinanga sylvestris* (Lour.) Hodel, **A**. Epidermis (E) and hypodermis (H) **B-C**. Fibrovascular bundles (VB) and ground parenchymatous tissue (P) (arrow showing tannin cells)

are round or elongated, variable in size, ca. 192 × 137 µm, with a single metaxylem vessel and a phloem strand. Ground tissues are variable in size, cells are round or ellipsoid, thin-walled, ca. 18–22 × 36–52 µm. Central fibrovascular bundles (Figure 2B-C) are irregular in size, ovate to round or ellipsoid, ca. 120–135 × 79–82 µm; metaxylem vessels one to five, ca. 22–35 µm in diameter; single phloem strand ca. 31–35 µm long with many sieve elements; protoxylem absent, or one to seven, thick-walled, round, ca. 8–22 µm in diameter. Few fibres bundles are scattered in the ground tissue.

C. ***Pinanga sylvestris* (Lour.) Hodel**

The epidermis is a single layer; cells are drop-shaped or ovate, ca. 13–16 × 15–17 µm, with cuticle present (Figure 3A). The hypodermis are three to four layers, thin-walled cells, round to angular, with few scattered fibre bundles. Peripheral fibrovascular bundles are congested and irregular in size, ca. 156 × 113 µm with one metaxylem vessel and a phloem strand. Ground cells are irregular, thin-walled, round to angular, ca. 12–32 µm wide. Central fibrovascular bundles are round or ellipsoid (Figure 3B-C), ca. 104–145 × 82–107 µm; metaxylem vessels one to three, round, ca. 38–55 µm in diameter;

phloem strand single, ovate or kidney-shaped, ca. 32–43 μm long with many sieve elements; protoxylem absent or one to four, thick-walled, round, ca. 19–21 μm in diameter. Few fibres bundles are present in the ground tissue.

In this study, epidermal cells were observed in drop-shaped or ovate in *Pinanga griffithii* and *P. sylvestris* but epidermal cells of *P. gracilis* are round or rectangular. The number of vessels is generally one in all the three studied species but the number of metaxylem vessels in some vascular bundles was found up to four in *P. gracilis*, up to three in *P. sylvestris* and up to five in *P. griffithii*. Whereas, Tomlinson, (1961) reported that the metaxylem is composed of one vessel in *Pinanga*. The presence of fibre strands noted in all three species, was also reported by Tomlinson, (1961) in other species of *Pinanga*. A key is prepared based on the stem anatomy of these three species.

1. Epidermal cells
   a. Round to rectangular…*Pinanga gracilis*
   b. Drop shaped or ovate……2
2. Metaxylem and protoxylem
   a. Metaxylem one to five, protoxylem up to seven……*Pinanga griffithii*
   b. Metaxylem one to three, protoxylem up to three……*Pinanga sylvestris*

In Arecoideae, Thomas & Boura (2015) reported the occurrences of single vessels in the genera *Areca*, *Nenga*, and *Pinanga*. The study also recorded one-two vessel in *Aiphanes* Willd., *Bactris* Jacq. ex Scop., *Cocos* L., *Gaussia* H. Wendl., *Leopoldinia* Mart., *Manicaria* Gaertn., and *Sygrus* Mart. Except for *Gaussia* and *Syagrus* which belong to the dry period and mixed-genus biome respectively, the remaining of all other genera are belongs to the tropical rainforest biome (Thomas & Boura, 2015). Thomas & Boura (2015) also considered that the number of vessels and their diameter observed in a different member of the Arecoideae were adapted to the climatic condition. In the present study, *Pinanga gracilis* and *P. griffithii* were collected from the region exhibiting tropical monsoon climate and in both species, the diameter of metaxylem vessels was found almost similar. Whereas, *Pinanga sylvestris* was collected from the subtropical monsoon climate region of the state and the diameter of metaxylem vessels were found comparatively larger than the other two species. The numbers of metaxylem vessels were found more than one in all the studied species. Interestingly the metaxylem vessels were also found more than one in *Calamus meghalayensis* Henderson in Assam (Mehmud & Roy, 2021a). Generally in India, the other members of this genus were represented by a single vessel (Mathew & Bhat, 1997; Renuka et al., 2010). Environmental factors influence plant vascular system (Baas, Werker & Fahn, 1983; Lindorf, 1994; Qaderi, Martel & Dixon, 2019), whether the variation in the number of vessels recorded in the present study is an influence of climatic conditions or the nature of some species needs to be studied in future.

IV. CONCLUSION

The result of the present anatomical study, especially the epidermal cells and numbers of vessels, the hypodermal cells, fibrovascular bundles, and numbers of protoxylems, were found useful anatomical features to separate the three studied *Pinanga* species. Variation of the size of the metaxylem vessels observed in the present study in all three studied species collected from Assam. Hence, the present study is not only helpful in the identification of these species based on anatomy but also provides additional information to future researchers to understand the relationship that exists among *Pinanga* species globally.

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