

Morphological and Anatomical Studies on Some Members of Araceae of North Bengal

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Abstract

The present study aimed to characterize eight edible members of the family Araceae on the basis of their morphological and anatomical characteristics. The petiole length, leaf size, and corm size varied widely across the studied members. Significant variations in the stomatal index, stomatal length, and width were observed, although all were of paracytic type with elliptical to circular shapes. Leaf epidermal cells were found to be polygonal or puzzle shaped. Mesophyll cells were non-distinguishable as palisade or spongy parenchyma. The root vascular bundles were found to be exarch, either arranged circularly or remaining scattered in the ground tissue. Pith was also observed, either large, centrally located, or scattered. Petiole anatomy showed scattered vascular bundles with collateral xylem and phloem in almost all the species, except *Ol Kochu*, *Panchmukhi Kochu*, and *Ghot Kochu*. Presence of tannins was observed in the petioles of all the species, whereas calcium oxalate crystals in the form of raphides were found to be present in some members. Water vessels, either large or small were found in the petioles of all the species. The number of parenchyma cells varied from 2 to 6 depending on the species. All the morphological and anatomical characteristics would help identify the eight members of Araceae and provide information for future studies with them.



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Introduction

The family Araceae comprises many perennials, evergreen, herbaceous members with underground modified stems viz. rhizomes, tubers, and corms or climbing habits (Noltie, 1994). Most aroids usually prefer moist, well-drained, and nutrient-rich soil with frequent fertilization and warm temperature (nearly 16 °C.). Most varieties are propagated through vegetative propagation, like leaf cuttings, stem cuttings, layering, or division. However, seed propagation can sometimes be capricious (Al-Eisawi, 1998; Feinbrun- Dothan, 1986). Aroids are rhizome-bearing perennial plants. Most of these

plants have originated from the Mediterranean region but some are also found in Europe, Western to Central Asia, and Northern Africa. Corm size ranges from 8 inches to nearly 2 feet in height (20.5-60.5 cm). Leaves are alternate or apparently basal, usually petiolated with sheathing leaf bases. Various leaf blade patterns like- linear, simple, pinnate, radiate, pedate with cordate to sagittate types are also observed. Inflorescences are of spadix type often subtended by the membranous cataphyllus spathe, containing bisexual or unisexual flowers, depending on species. The leafy spathe may be violet, white, yellow, or brown coloured and may even be sweet or sharply scented. Flowers are usually red to orange in colour. Bisexual flowers show tepals (0, 4, or 6), stamens (4 – 6), bilocular anthers, and 3 loculed ovary. In the case of unisexual flowers, male flowers

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are represented by single stamen of synandrous type (fused stamens, subsessile); female flowers consist of single ovaries (sometimes associated with a sterile staminode), commonly unilocular (sometimes with 3 or 4 locules), ovules 1-many per locule, positioned parietal, basal or apical. Spadix is sometimes sterile, merely a terminal appendage in nature (Noltie, 1994).

The Araceae family comprises 3500 species worldwide. In India, there are 25 genera and 187 taxa distributed mainly in North-Eastern India, Eastern Himalayas, and Western Ghats (Botanical Survey of India, 2019). Some of the commonly growing members of Araceae that have been consumed by the people of North Bengal include *Colocasia* sp., *Alocasia* sp., *Xanthosoma* sp., *Ariopsis* sp., *Amorphophallus* sp. Out of these species, some are cultivated and some are gathered from the wild. *Colocasia* sp. is an important crop growing in humid areas, and used as a vegetable (Al-Obeidi et al., 2022). It is considered an ancient crop based on geographical distribution and genetic origin (Matthews, 2014). They are perennial tropical plants with roots (height about 3 m), with green leaves having upwardly directed reticulate veins. Flowers are not distinctly visible due to their tiny size and are arranged compactly with a succulent spathe with a height of approximately 20 cm (al-Obeidi et al., 2022). *Alocasia macrorrhizos* also known as giant taro from the same family, is mainly used as an ornamental crop, but in a few countries (Western Samoa, Tonga, Wallis, etc.), it has been used as a food crop. Taro grows its corm above the ground up to 1 meter to 2 meters in length and 20 cm in diameter (Garcia et al., 2008). *Amorphophallus* is economically important as a good source of starch, and as traditional medicine and grows wild as well as in cultivated conditions in India, Sri Lanka, China, Japan, and Indonesia (Gholave et al. 2019). *Xanthosoma* is widely cultivated in Asia, Africa, and Oceania with about 124 species and valued for edible purpose. The plant is nutritionally rich with energy, protein, starch, macro- and micronutrients and vitamins (Boakye et al. 2018). However, the aroid members are very difficult to identify due to the great complexity in their morphological and anatomical characters.

In this context, our study aims to investigate and understand some Araceae members' morphological and anatomical variations found in the North Bengal region. Characterization of the Araceae members will help in the proper identification and documentation that will benefit future studies.

Materials and methods

Collection of plant samples

Local underutilized varieties of eight different aroid plant samples under study were collected freshly from three districts of North Bengal (Table 1). Ol, Mukhi, and Shola (black, white) Kochu were collected near Matigara, Darjeeling; Ghot Kochu was collected from Balurghat, Dakshin Dinajpur; Maan and Dudh Kochu were collected from Bhote patty, Jalpaiguri; and Panchmukhi Kochu collected from Dhupguri, Jalpaiguri region. All the collected varieties were submitted to the North Bengal University Herbarium (NBU) for proper identification. After this, all the samples were declared to be the family of Araceae.

Morphological parameters

All the samples were propagated in the experimental garden, and the morphological observations on their habit and corm were performed. The length of the petiole, corm size, and leaf size of the samples were measured. Five petioles of each sample were measured using a scale, and the average values were recorded.

Anatomical parameters

Leaf anatomy

Epidermal peels from the median portion of leaf lamina were obtained following the standard method (Metcalf (1960); Arogundade and Adedeji (2016). The peels were stained with safranin, later mounted in dilute glycerine, and observed under microscope (MD-52A). The epidermal cell's shape, size, and number of stomata were measured. The stomatal index of the leaf surfaces was calculated using the formula:

$$\text{Stomatal Index} = \frac{S}{S+E} \times 100$$

Where S = Number of stomata and E = Number of ordinary epidermal cells plus the subsidiary cells in the same unit area.

Root anatomy

The transverse section of the roots was obtained with the help of a sharp blade and stained with safranin and cotton blue. The sections of roots were then passed through different concentrations of ethanol gradation (50, 60, 70, 80, 90, and 100%). The section was later mounted on 25% glycerine and observed

under light microscope (Leica DM LS2). Photomicrographs of the different root sections were taken under the 40X. The Length and diameter of the sections were measured with the aid of an ocular and stage micrometre.

cm), whereas the smallest petiole was observed in Mukhi Kochu (Length- 10 to 30 cm, and diameter- 3 to 7cm). The petiole sizes indicated the growth pattern, corm size, and storage ability (calcium, raphides, tannins, acicular crystals), and meant for

Table 1. List of the aroid species (*Kochu*) and their collection site

Local/common name	Scientific name	Collection area
<i>Ol Kochu</i>	<i>Amorphophallus paeoniifolius</i> (Dennst.) Nicolson.	Shivmandir, Matigara, Darjeeling
<i>Ghot Kochu</i>	<i>Xanthosoma</i> sp.	Balurghat, Dakshin Dinajpur
<i>Mukhi Kochu</i>	<i>Colocasia esculenta</i> (L.) Schott.	Shivmandir, Matigara, Darjeeling
<i>Shola Kochu (White)</i>	<i>Colocasia esculenta</i> (L.) Schott.	Matigara, Darjeeling
<i>Shola Kochu (Black)</i>	<i>Colocasia esculenta</i> (L.) Schott.	Matigara, Darjeeling
<i>Panchmukhi Kochu</i>	<i>Colocasia esculenta</i> (L.) Schott.	Dhupguri, Jalpaiguri
<i>Maan Kochu</i>	<i>Alocasia macrorrhizos</i> (L.) G. Don.	Bhote Patty, Maynaguri, Jalpaiguri
<i>Dudh Kochu</i>	<i>Alocasia</i> sp.	Bhote Patty, Maynaguri, Jalpaiguri

Petiole anatomy

The transverse section of the petioles was done by the same method as described in the above section.

Results and discussion

Morphological parameters

Length of the petiole

Petiole length of the varieties was found to be distantly different from each other (Figure 1). The length of the petiole ranged from 10 to 110 cm and the diameter of the petiole measured from 3 to 20 cm. The petiole size was found to be highest in Dudh

Kochu (Length- 30 to 110 cm and diameter- 12 to 15 cm), whereas the smallest petiole was observed in Mukhi Kochu (Length- 10 to 30 cm, and diameter- 3 to 7cm). The petiole sizes indicated the growth pattern, corm size, and storage ability (calcium, raphides, tannins, acicular crystals), and meant for

Corm size

Corm size of all the varieties ranged from 7 to 100 cm in length and 5 to 8 cm in diameter (Figure 1). The highest corm size was observed in Maan Kochu (Length- 50 to 100cm and diameter- 15 to 20cm), and the smallest corm size was observed in Mukhi Kochu (Length- 7 to 15cm, and diameter- 5 to 8cm). Corm size basically depends on the soil nutrition as well as the sizes of the petiole, leaves, and age of the plants (Ravi et al., 2011).

















	Plant habit	Corm	Corm size	Petiole size	Leaf size
Panchmukhi			L- 14 to 20 cm, B- 11 to 15 cm	L- 55 to 70 cm, D- 4 to 5 cm	L- 22 to 30 cm, B- 18- 24 cm
Shola (White)			L- 50 to 60 cm, B- 9 to 13 cm	L- 60 to 80 cm, D- 7 to 10 cm	L- 25 to 30 cm, B- 15 to 22 cm
Shola (Black)			L- 20 to 30 cm, B- 6 to 10 cm	L- 30 to 40 cm, D- 6 to 8 cm	L- 15 to 20 cm, B- 10 to 15 cm
Ghot			L- 15 to 20 cm, B- 10 to 13 cm	L- 50 to 60 cm, D- 5 to 7 cm	L- 20 to 28 cm, B- 15 to 20 cm
Dudh			L- 12 to 70 cm, B- 10 to 15 cm	L- 30 to 110 cm, D- 12 to 15 cm	L- 15 to 35 cm, B- 7 to 25 cm
Maan			L- 50 to 100 cm, B- 15 to 20 cm	L- 40 to 90 cm, D- 15 to 20 cm	L- 30 to 60 cm, B- 25 to 50 cm
Mukhi			L- 7 to 15 cm, B- 5 to 8 cm	L- 10 to 30 cm, D- 3 to 7 cm	L- 5 to 18 cm, B- 5 to 12 cm
Ol			D- 7 to 20 cm	L- 30 to 70 cm, D- 10 to 15 cm	L- 10 to 15 cm, B- 5 to 7 cm

Figure 1. Morphological observations on eight local varieties of *Kochu* collected and propagated in the experimental garden

Leaf size

The leaf size of all the varieties ranged from 5 to 60 cm in length and 5 to 7 cm in breadth (Figure 1). Out of eight varieties, the largest leaf size was observed in Maan Kochu (Length- 30 to 60cm and breadth- 25 to 50cm), and the smallest corm size was observed in Ol Kochu (Length- 10 to 15cm, and breadth- 5 to 7cm). Size of the leaves varied according to the age of the plants, size of the corms, and also in the nutritional condition of the soil (Ravi et al., 2011).

Anatomical parameters

Leaf anatomy

Both the upper and lower epidermis were found to be composed of parenchymatous cells arranged in a single row of uniseriate polygonal and puzzled cells (outer and inner tangential cells, stomatal cells, crystal cells) (al-Obeidi et al., 2022). The epidermal cells' outer and inner tangential walls were thick and straight. The stomatal complexes were found to be paracytic type, where the kidney-shaped guard cells were arranged parallelly with the stomatal aperture.

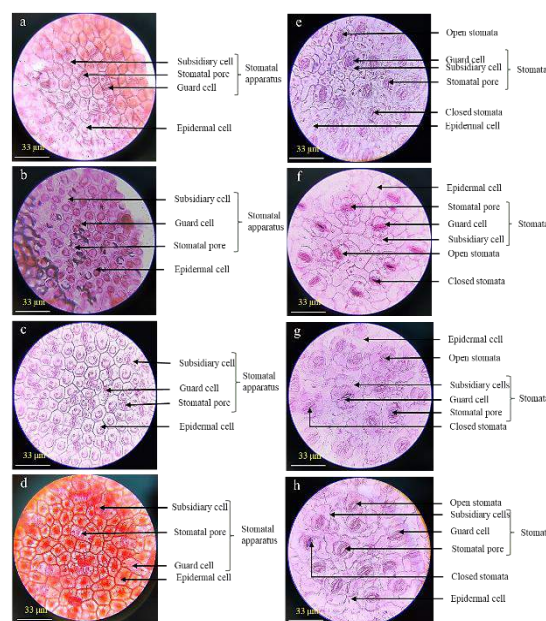


Figure 2. Leaf anatomy of eight varieties of Kochu under 40X – a) *Panchmukhi Kochu*, b) *Shola Kochu* (White), c) *Shola Kochu* (Black), d) *Ghot Kochu*, e) *Dudh Kochu*, f) *Maan Kochu*, g) *Mukhi Kochu*, h) *Ol Kochu*

Table 2. Quantitative attributes of the leaf anatomy of aroid varieties

Variety	Leaf anatomical parameters					
	Epidermal cell	Stomatal shape	Stomata type	Stomatal Index	Stomatal Length	Stomatal Width
Panchmukhi Kochu	Polygonal	Elliptical, Circular	Paracytic	27.8	13.55	8.32
Shola Kochu (white)	Polygonal	Elliptical, Circular	Paracytic	25.53	10.35	9.22
Shola Kochu (black)	Polygonal	Elliptical, Circular	Paracytic	20.65	14.65	7.26
Ghot Kochu	Polygonal	Elliptical, Circular	Paracytic	18.75	13.92	11.65
Dudh Kochu	Puzzle	Elliptical, Circular	Paracytic	28.94	18.63	14.12
Maan Kochu	Polygonal	Elliptical, Circular	Paracytic	17.14	17.32	11.83
Mukhi Kochu	Puzzle	Elliptical, Circular	Paracytic	26.38	18.85	15.25
Ol Kochu	Puzzle	Elliptical, Circular	Paracytic	31.03	20.65	14.21

The dimensions of the stomata also varied among the species as shown in Table 2. According to Suratman et al., (2016); and al-Obeidi et al., (2022), such variation of stomata depends on the surface area (both upper and lower) of the leaf. Raphides were found to be abundant, a characteristic feature of the family Araceae (Genua and Hillson, 1985). The mesophyll cells were not easily distinguishable as palisade and spongy parenchyma, as previously observed by Oluwabunmi et al. The xylem was composed of vessels arranged in rows of about 2-3 rows separated by parenchymatous cells.

Figure 2 and Table 2 showed that the stomatal index of Ol Kochu was the highest (31.03) and Maan Kochu's was the lowest (17.14) among all the studied varieties. However, the highest length of the stomata was found in Ol Kochu (20.65 μm) and lowest (10.35 μm) in Shola Kochu (white). On the contrary, Mukhi Kochu showed maximum stomatal width (15.25 μm), and the minimum stomatal width was observed in Shola Kochu (black).

Root anatomy

Transverse sections of the roots showed multilayered endodermis and pericycle composed of compactly arranged parenchymatous cells (Figure 3 and Table 3). The vascular bundle was found to be of exarch type, either arranged in a circular pattern (in Shola Kochu [white & black], Ghot Kochu, Dudh Kochu, Maan Kochu, and Mukhi Kochu) or scattered in the ground tissue (Panchmukhi and Ol Kochu). Pith was found to be conspicuous, made up of loosely arranged parenchymatous cells only in the).

varieties having vascular bundles arranged in a circular pattern. The cortex region consisted of many polygonal cell layers containing acicular calcium oxalate crystals (raphides). In some members like-Shola Kochu [white and black], chains of rectangular parenchymatous cells were found to be present that contain rosette crystals. Large air vacuoles were also found to be present in the cortical region of some members like- Shola Kochu (white and black

Table 3. Quantitative attributes of the root anatomy of aroid varieties

Variety	Root anatomical parameters					
	Vascular Bundle	Pericycle layer	Parenchymatous cell	Aerenchymatos cells	Pith	Endodermis layer
Panchmukhi Kochu	Exarch type, Scattered	2 to 3	Absent	Absent	Scattered	Multilayer
Shola Kochu (white)	Exarch type, Circular	2 to 4	Present	Present	Central, Small	4 to 6
Shola Kochu (black)	Exarch type, Circular	2 to 3	Present	Present	Central, Small	3 to 5
Ghot Kochu	Exarch type, Circular	2 to 3	Absent	Absent	Central, Large	Multilayer
Dudh Kochu	Exarch type, Circular	3 to 4	Absent	Absent	Central, Large	Multilayer
Maan Kochu	Exarch type, Circular	2 to 3	Absent	Absent	Central, Large	Multilayer
Mukhi Kochu	Exarch type, Circular	3 to 5	Absent	Absent	Central, Small	Multilayer
Ol Kochu	Exarch type, Scattered	3 to 4	Absent	Absent	Scattered	Multilayer

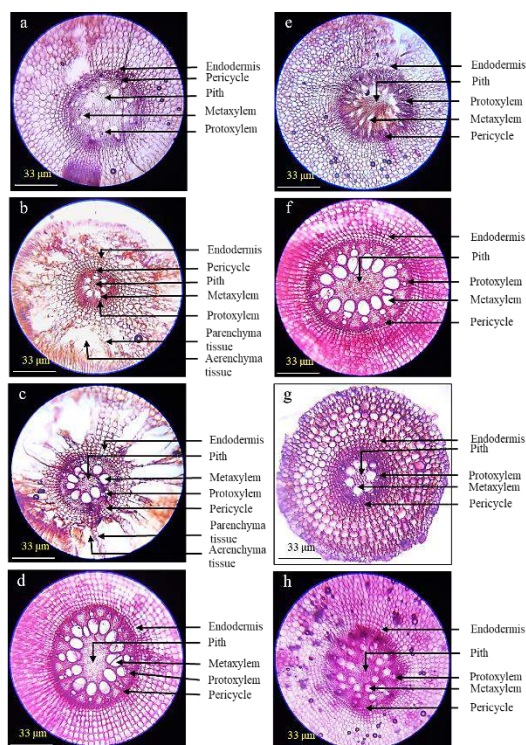


Fig. 3 Root anatomy of eight varieties of *Kochu* under 40X – a) Panchmukhi *Kochu*, b) Shola *Kochu* (White), c) Shola *Kochu* (Black), d) Ghot *Kochu*, e) Dudh *Kochu*, f) Maan *Kochu*, g) Mukhi *Kochu*, h) Ol *Kochu*

Petiole anatomy

All the anatomical data related to the petiole are included in Figure 4 and Table 4. The transverse section of the petiole is composed of layers from the outside to the inside of the epidermis of the layer of cells enveloping the petiole interspersed with parenchymatous cells, smooth layer of cuticle, scattered type of vascular bundle surrounded by a bundle sheath. Distinct protoxylem and metaxylem cavities were observed, which could be correlated with the previous observations by Suleiman (2003). Smaller water vessels were also found in Panchmukhi, Shola (black), and Mukhi *Kochu* varieties, whereas the larger vessels were observed in Shola (white), Ghot, Dudh, Maan, and Ol *Kochu*. Out of the eight varieties acicular crystals were absent in Ghot *Kochu* and raphides were absent in Shola *Kochu* (white), Mukhi *Kochu*, and Ol *Kochu*. Tannins were present in all the samples. Collateral vascular bundles with scattered distribution pattern were present in Shola *Kochu* [white & black], Dudh

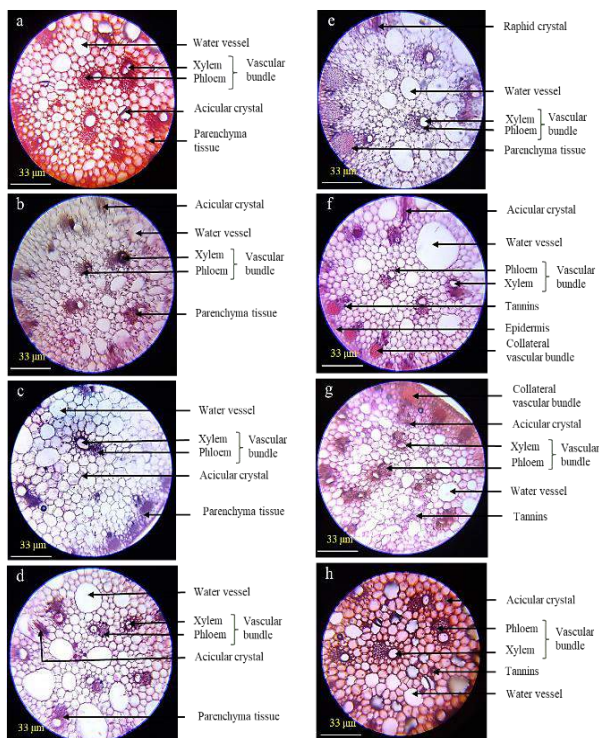


Fig. 4 Petiole anatomy of eight varieties of *Kochu* under 40X – a) Panchmukhi *Kochu*, b) Shola *Kochu* (White), c) Shola *Kochu* (Black), d) Ghot *Kochu*, e) Dudh *Kochu*, f) Maan *Kochu*, g) Mukhi *Kochu*, h) Ol *Kochu*

Kochu, Maan *Kochu*, and Mukhi *Kochu*), and Collateral vascular bundles with scattered distribution were absent in the varieties of Panchmukhi *Kochu*, Ghot *Kochu*, and Ol *Kochu*. Number of parenchymatous cells were observed to be in ascending order in the varieties of Mukhi and Dudh (3 – 6 cells), Maan (3 – 5 cells), Ol (3 – 4 cells), Panchmukhi and Shola *Kochu* [black] (2 – 4 cells), shola *Kochu* (1 - 4 cells), and Ghot *Kochu* (1 – 2 cells).

Conclusion

From the above results, it can be concluded that the eight members of Araceae vary significantly in terms of their morphological and anatomical characteristics. These data represent an essential basis for the differentiation of these species of the Araceae family. Further research is required to evaluate the physicochemical, biochemical, and other important properties that will help to characterize these locally available aroids for their nutritional benefits and commercial utilization.

Table 4. Quantitative attributes of the petiole anatomy of aroid varieties

Variety	Petiole anatomical parameters						Collateral Vascular bundle
	Vascular Bundle	Parenchymatous cells	Water Vessel	Acicular crystal	Raphides	Tannins	
Panchmukhi Kochu	Scattered type	2 to 4	Present, small	Present	Present	Present	Absent
Shola Kochu (white)	Scattered type	1 to 4	Present, Large	Present	Absent	Present	Present, Scattered
Shola Kochu (black)	Scattered type	2 to 4	Present, small	Present	Present	Present	Present, Scattered
Ghot Kochu	Scattered type	1 to 2	Present, Large	Absent	Present	Present	Absent
Dudh Kochu	Scattered type	3 to 6	Present, Large	Present	Present	Present	Present, Scattered
Maan Kochu	Scattered type	3 to 5	Present, Large	Present	Present	Present	Present, Scattered
Mukhi Kochu	Scattered type	3 to 6	Present, small	Present	Absent	Present	Present, Scattered
Ol Kochu	Scattered type	3 to 4	Present, Large	Present	Absent	Present	Absent

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