RESEARCH ARTICLE

Micro-morphological and Morphometric Attributes Analysis of Brachysclereids (Stone Cells) on Fruit Mesocarp Cells of Six Selected Taxa of *Diospyros* L. in the Family Ebenaceae Based on Analytical Microscopy: A Systematic Approach

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Abstract

Micro-attributes are an important systematics tool for plant identification and classification nowadays. Brachysclereid (Stone Cell) was studied in six species belonging to the genus *Diospyros* (*D. ebenum, D. sylvatica, D. melanoxylon, D. kaki, D. blancoi, D. peregrina*) under the family Ebenaceae. Light Microscope and Scanning Electron Microscope were used for the anatomy and ultrastructural study of brachysclereid. Phenetic relationships of *Diospyros* species were studied by cluster analysis through UPGMA (Unweighted Pair Groups Method with Arithmetic Average). The species are grouped in two groups, 'A' and 'B,' and a cluster labeled 'X' on the phylogenetic tree. The important characteristics of the brachysclereid include some quantitative characters (Shape, Sclereid cell layers, Sclereid cell cluster number, Cluster crystal, Porosity of the stone cells, lumen shape, Sclereids arrangement) and some quantitative characters (Sclereid cell length, Sclereid cell width, Sclereid index and Sclereid cell lumen diameter). Within a species, almost all of the traits under study showed little variation. Principal Component Analysis (PCA) was applied using all polymorphic characteristics. The PCA revealed the sclereid index, sclereid cell layer, sclereid cell width, and sclereid cell lumen diameter. The Sclereid index and Sclereid cell layer strongly influence principal component-1 (PC1). Principal component (PC-1) and principal component-2 (PC-2) jointly explain 77.41%.

Highlights

- Micromorphological and ultrastructural characterization of brachysclereid i.e. stone cells of six species of *Diospyros*.
- Phenetic relationships of *Diospyros* species were studied by cluster analysis through UPGMA (Unweighted Pair Groups Method with Arithmetic Average).
- Principal component analysis (PCA) was applied using all polymorphic characteristics.
- Brachysclereid, i.e., stone cells, remain stained with phloroglucinol-HCL and show a violet-red color.
- The degree of variation in brachysclereid (length, breadth, and lumen diameter) among the selected plant species was assessed using analysis of variance (ANOVA).

Keywords: Brachysclereid, Scanning electron microscope, Principal component analysis, Numerical analysis.

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Introduction

The 500 to 600 species that make up the genera *Diospyros* and *Euclea* are part of the pantropical Ebenaceae (ebony family). Very few of them reach temperate regions. Southeast Asia, Madagascar, Tropical Africa, and South America are the primary centers of variety (Wallnofer, 2001). This genus contains many species, including *D. celebica*, *D. pilosanthera, D. cauliflora*, and *D. ebenum,* which are recognized for producing expensive, high-quality wood, that is used for making crafts and musical instruments (Bennet, 2016). Since several species, such as *D. discolour*, *D. malabarica*, and *D. hasseltii*, are known to yield edible persimmons, the genus is important economically (Kinho, 2013; and Bakhuizen van Den Brink, 1937).

A number of the medicinal bioactivities of the *Diospyros* species that have been the focus of current research include anti-microbial (Nematollahi *et al*., 2011), anticancer (Pratiwi and Nurlaeli, 2020), antioxidant, anti-inflammatory, analgesic, and anti-diabetic properties. Medium-sized, dioecious *Diospyros* trees are rarely shrubs and typically have firm, black boles. There are simple, alternating leaves; in the inflorescence, there may be an axillary cyme or solitary flowers with pistil-late blossoms. There are a few to many green, white, or yellow flowers in the arrangement.

Numerous morpho-taxonomical studies have been conducted on *Diospyros*, such as those that analyze the phenetic

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relationships and morphology of widespread *Diospyros* spp. in the Mascarene Islands (Mauritius) (Venkatasamy *et al*., 2006), the macro and micromorphology of *Diospyros* spp. in China (Yi *et al*., 2016), the morphometric variation of pollen grains, fruit, and seeds of some *Diospyros* species in Ukraine (Grygorieva *et al*., 2017 and Grygorieva *et al*., 2013), and the leaf clearing pattern of some *Diospyros* species (Putri and Chikmawati, 2015).

Typically, sclereids are short cells with multiple simple pits, thick secondary walls, and strong lignification. Nonetheless, some sclereids have comparatively thin secondary walls, making them hard to differentiate from sclereid parenchyma cells. Sclereids can be found in seeds, fruits, leaves, and stems. Sclereids can be found in many parts of fruits. Brachysclereids, which are single or clustered stone cells, are dispersed throughout the fleshy portions of pears (*Pyrus*) and quinces (*Cydonia*). The distinct grainy feel of pears is attributed to the sclereid clusters.

Tschirch, 1881, the first researcher in this field, first proposed the four basic groups of sclereids: brachysclereids, osteosclereids, and astrosclereids, macrosclereid. A few researchers have recently revealed that distinct sclereid species can be found in many genera within a family or in different species within the same genus. In the family Marcgraviaceae, (Richter, 1920) classified sclereids into three categories: ophiurosclereids, librosclereids, and astrosclereids.

A substantial amount of lignin and cellulose that is found in the mesocarp layer in either aggregated or various forms known as sclereids are referred to as "stone cell," or brachysclereid (Qiao *et al*., 2005; Schroeder, 1982). Sclerenchyma cells give rise to sclereids. Some species have crystals implanted in their secondary walls (Kuo-Huang, 1990).

Stone cell "rosettes" with thicker, highly lignified cell walls developed since parenchyma cells, which had thin walls made of cellulose, encircled the stone cells. (Tian *et al*., 2011) explain that the stone cells serve as particular structures, or "strong points," that preserve the fruit's hardness and firmness, while lignin imparts resilience, rigor, and resistance. They are the same size and quantity as when the 'Bluecrop' fruit growing phase started.

This study has concentrated on the skeletal morphology, ultra-structure, and numerical analysis of the fruit mesocarp of six different *Diospyros* species. Numerous methods, including optical microscopy, scanning electron microscopy (SEM), PCA (Pincipal component Analysis), and UPGMA (Unweighted Pair Groups Method with Arithmetic Average), have been used for the identification and analysis.

Materials and Methods

Diospyros ebenum (Latitude 22° 27′07.93″ N; Longitude 86°52′54.4″ E) J. Koenig ex Retz., *D. sylvatica* (Latitude 22° 27′07.93″ N ; Longitude 86°52′54.47″ E) Roxb., *D. kaki* L. f., *D. melanoxylon* (Latitude 22° 37′55.46″ N; Longitude 86°45′51.34″ E) Roxb., *D. blancoi* (Latitude 22° 06′37.18″ N; Longitude 86°02′14.79″ E) A.DC., and *D. peregrina* (Latitude 22° 06′37.18″ N; Longitude 86°02′14.79″ E) (Gaertn.) are the six plants (Fruits). The greater part of Gurke resides within the Jhargram district of West Bengal, India's Chilkigarh. (Fig.1 and Fig.2)

Plant sample preparation for Light Microscopy (LM)

The collected fruits were preserved by fixing them in an FAA (5:5:90: Formalin: Glacial Acetic Acid) solution. The material was dehydrated (Tao et al., 2009) at 30°C using successive percentages of 70, 85, 95, and 100 ethanol for 30 minutes at a time. Throughout the transverse portion, the material was cut into tiny sections no thicker than 10 mm. The cutting sample was coated with one or two drops of 1% $C_6H_3(OH)_3$-ethanol solution and placed on a glass plate. Two to three minutes later, the same sample was treated with one drop of 35% HCl. Stone cell photos were captured while the sample was being examined under a light microscope with the model number LEICA DM 3000.

Fig. 1: Fruit of *Diospyros* Species. **A.** *D. ebenum* J. Koenig ex Retz. **B**. *D. Sylvaticum* Roxb., **C**. *D. melanoxylon* Roxb., **D**. *D. Kaki* L. f., **E**. *D. blancoi* A. DC. **F.** *D. peregrina* Gaertn.) Gurke

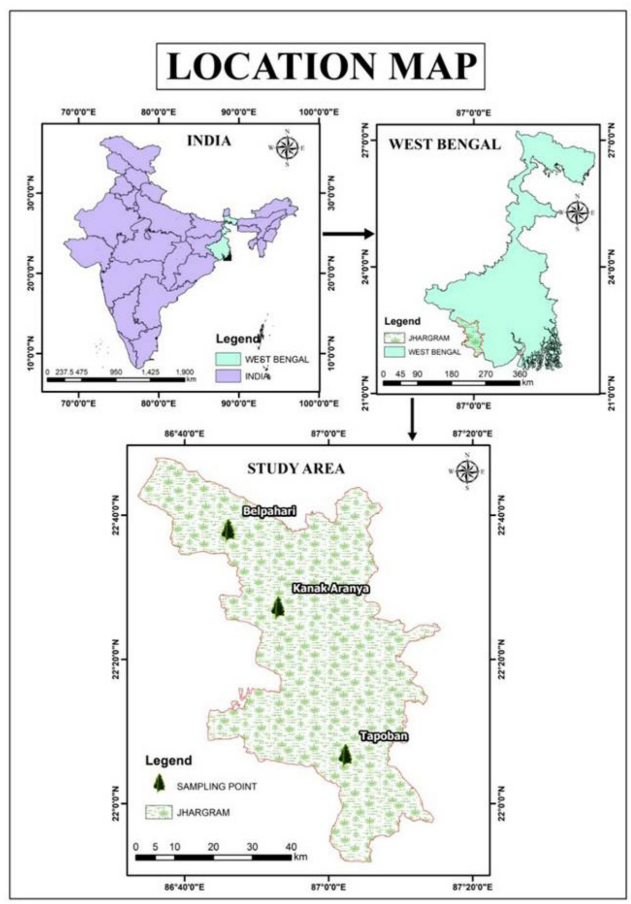

Fig. 2: Location map shows the study area of six *Diospyros* species

Plant Sample Preparation for SEM (Scanning Electron Microscopy)

Razor blades were used to slice the fruit tissue into longitudinal sections that were 200 m thick. The cutting sample was allowed to cure in the sunlight at room temperature before being sputter-coated with gold. To view and analyse the Ultra-structural image of the final sample, it was examined. It was removed using a ZEISS Supra-40 scanning electron microscope (SEM) at the Indian Institute of Technology in Kharagpur (Xu et al., 2006).

Phylogenetic Interpretation

Using the photos that were captured, polymorphic features were noticed and noted (Table 1). For cluster analysis, each anatomical trait of the sclereid shown in (Table 1) was coded. Using the information in (Table 1) as a starting point, a data matrix (Table 2) was created to calculate for the purpose of calculating the similarity between species. In order to narrow the dimension of the elements in this study, the 15 variables were analyzed using principal component analysis (PCA). Using the statistical program NTSYS-pc, version 2.2 (Rohlf, 1992), phenetic analysis was performed on the data matrix in (Table 2) data row. The coefficient of similarity was calculated using the average taxonomic distance. Using arithmetic averages, the unweighted pair group method (UPGMA) has been used to create a phenogram (Sokal, 1986). It was also calculated to find the cophenetic correlation coefficient (r). Using a basic matrix that was standardized, principal component analysis (PCA) was performed.

Table 1: Fruit sclereid features and character states used in the phylogenetic analysis

Character	*States*
Sclereid shape (SH)	0= Ellipsoid; 1=Ovoid
Sclereid type (ST)	0=non-branched; 1=Branched
Mesocarp cell layer (MCL)	0=1-3; 1=4-5; 2=6-7; 3=8-9; 4= 10-12
Sclereid cell layer (SCL)	0=1-2; 1=3-4; 2=5-6; 3=7-9; 4=10-14
Sclereid cell cluster No. (SCCN)	0=1-2; 1=3-5; 2=6-8; 3=9-10
Cluster crystal (CC)	0=Absent; 1= Present
Porosity of the stone cells (PC)	0=Finely porous; 1=Less Porous
Lumen shape (LS)	0=Shorter; 1= Narrow
Tannin deposits in lumen (TC)	0=Prominent; 1= Not Prominent
Sclereids arrangement (SA)	0=Irregular in rows; 1=Regular in rows
Sclereid lobed (SL)	0=Absent; 1= Present
Sclereid cell length(μm) (SCCL)	0=33.00-34.81; 1=34.82-35.96; 2=35.98-36.80; 3=36.85-39.45, 4=39.50-55.50
Sclereid cell width (μm) (SCW)	0=33.00-34.81; 1=34.82-35.96; 2=35.98-36.80; 3=36.85-39.45; 4= 39.50-55.50
Sclereid index (SI)	0=0.33-0.45; 1=0.45-0.49; 2=0.50-0.54; 3= 0.55-0.56; 4= 0.57-0.59; 5= 0.60-0.68
Sclereid cell lumen diameter (μm) (SCLW)	0=4.25-5.60; 1=4.30-5.85, 2=5.90-6.90; 3=6.95-8.10; 4=8.20-8.60

Results

Fruits from six genera of *Diospyros* were studied. Fifteen characters were examined (Table 1): eleven qualitative and four quantitative. In the study by Jaime Bonilla-Barbosa *et al*., (2000), those characteristics were assigned binary or multistate scores (Table.2).

Sclereid Micromorphological Description in Six Plant Taxa

In this study, it was found that *D. ebenum* has a non-branched, ellipsoidal-shaped brachysclereid. Sclereids are arranged irregularly in four layers rows, narrow shaped lumen, finely porous sclereids are found, sclereids lobed are present. Eight sclereid cell clusters are present (Fig. 3).

D. sylvatica has a non-branched, ellipsoidal-shaped brachysclereid. Sclereids are arranged regularly in six-layer rows, narrow shaped lumen, less porous sclereids are found. Eight number of sclereid cell clusters is present (Fig. 4).

D. kaki has a non-branched, ovoid-shaped brachysclereid. Sclereids are arranged irregularly in four-layer rows, narrow-shaped lumen, cluster crystal, and less porous sclereids are found. Ten number of sclereid cell clusters is present (Fig. 5).

D. melanoxylon has a non-branched, ellipsoidal-shaped brachysclereid. Sclereids are arranged irregularly in four layers of rows, with shorter lumen, finely porous sclereids are found, and lobed sclereids are absent. Two sclereid cell clusters are present (Fig. 6).

D. blancoi has non-branched, ellipsoidal-shaped brachysclereid. Sclereids are arranged regularly in thirteen layers

Table 2: Micro-morphological characters indicated by 0,1, 2, 3, 4, 5 for dendrogram analysis of six species under the family Ebenaceae

S.N.	*Character* / *Taxon*	*SS*	*ST*	*MCL*	*SCL*	*SCCN*	*CC*	*PC*	*LS*	*TC*	*SA*	*SL*	*SCCL*	*SCW*	*SI*	*SCLW*
1.	*D. ebenum*	0	0	3	1	2	0	0	1	1	0	1	3	2	0	1
2.	*D. sylvatica*	0	0	2	2	2	0	1	1	1	1	1	2	3	4	3
3.	*D. kaki*	1	0	1	0	3	1	1	1	1	0	0	0	2	1	2
4.	*D. melanoxylon*	0	0	4	1	0	0	0	0	1	0	0	5	4	2	4
5.	*D. blancoi*	0	0	1	4	2	0	0	1	1	1	0	4	0	5	0
6.	*D. peregrina*	1	0	0	3	1	0	0	0	0	0	1	1	1	3	1

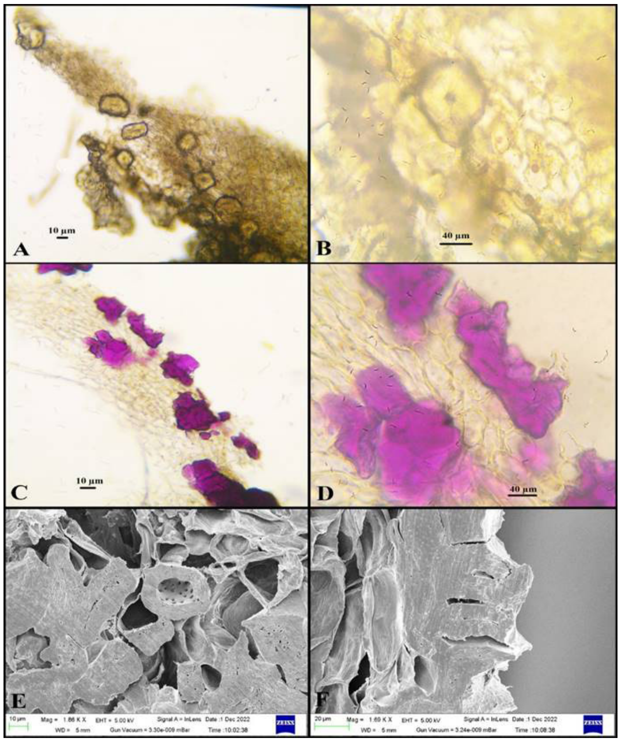

Fig. 3: *D. ebenum*: LM: A. Transverse section of fruit epicarp shows brachysclereid scattered in mesocarp (10x). B. Magnification (40x) of ellipsoidal shaped brachysclereid. C. Brachysclereid stained with phloroglucinol-HCl (10x). D. Magnified (40x) stained brachysclereid shows distinct narrow shaped lumen. SEM: E &F. Ultrastructure of brachysclereid shows thick lignified cell wall, narrow lumen and ramiform pit, Intercellular space, parenchyma cell.

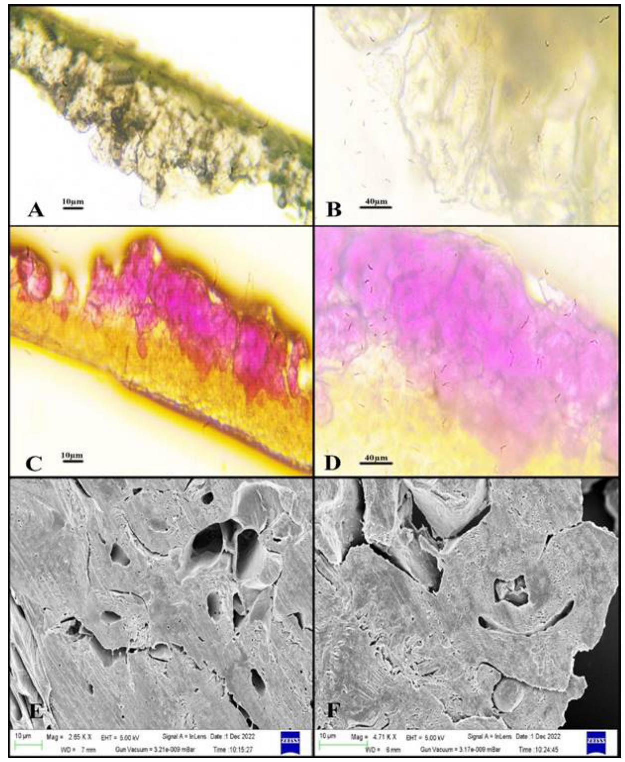

Fig. 4: *D. sylvatica*: LM: A. Transverse section of fruit epicarp shows brachysclereid arranged regularly in mesocarp (10x). B. Magnification (40x) of ellipsoidal-shaped brachysclereid. C. Brachysclereid stained with phloroglucinol-HCl (10x). D. Magnified (40x) stained brachysclereid shows distinct narrow-shaped lumen. SEM: E & F. Ultrastructure of brachysclereid shows thick lignified cell wall, narrow lumen and ramiform pit, Intercellular space, and parenchyma cel

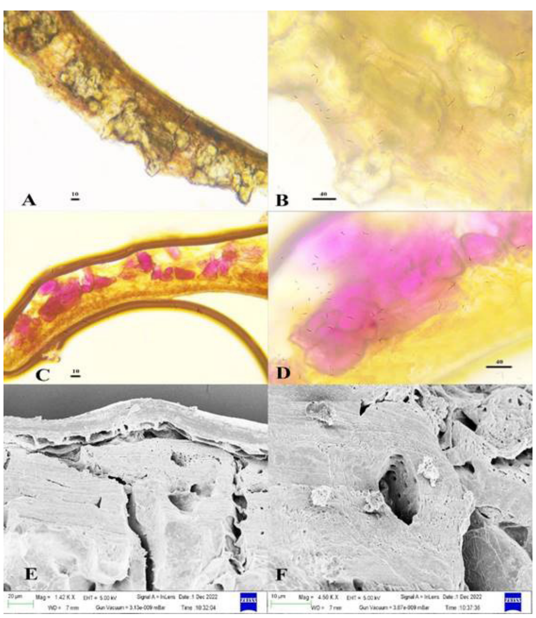

Fig. 5: *D. kaki*: LM. A. Transverse section of fruit epicarp shows brachysclereid arranged irregularly in mesocarp (10x). B. Magnification (40x) of ovoid-shaped brachysclereid. C. Brachysclereid stained with phloroglucinol-HCl (10x). D. Magnified (40x) stained brachysclereid shows a distinct narrow-shaped lumen. SEM: E & F. Ultrastructure of brachysclereid shows thick lignified cell wall, narrow lumen and ramiform pit, cluster crystal.

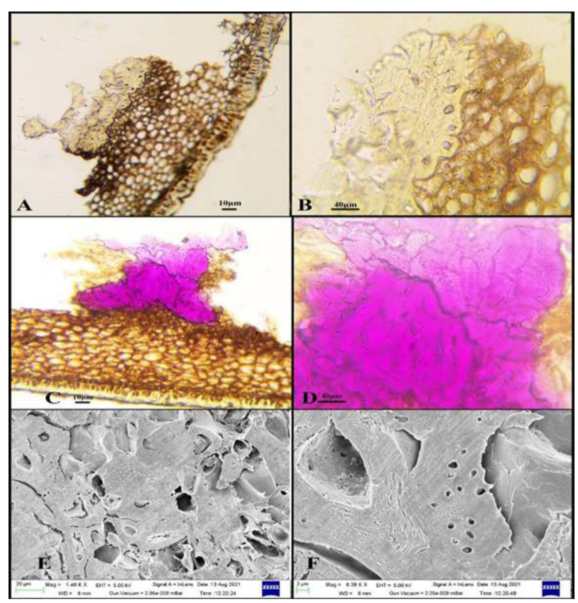

Fig. 6: *D. melanoxylon*: LM: A**.** Transverse section of fruit epicarp shows brachysclereid arranged irregularly in mesocarp (10x). B. Magnification (40x) of ellipsoid- shaped brachysclereid. C. Brachysclereid stained with phloroglucinol-HCl (10x). D. Magnified (40x) stained brachysclereid shows distinct shorter lumen. SEM: E & F. Ultrastructure of brachysclereid shows thick lignified cell wall, cavity, simple and ramiform pit.

rows, narrow lumen, cluster crystal, finely porous sclereids are found, sclereids lobed are absent. Eight number of sclereid cell clusters is present (Fig. 7).

D. peregrina has a non-branched, ovoid-shaped brachysclereid. Sclereids are arranged irregularly in eight-layer rows, with shorter lumen, finely porous sclereids are found, and lobed sclereids are present. Five number of sclereid cell clusters is present (Fig. 8).

Cluster Analysis

Based on fifteen different fruit sclereid characters (Table 1) we create a phylogenetic tree to find out the correlations among six species. In the phylogenetic tree, the species are arranged in two groups such as 'A' and 'B', and a cluster named 'X' (Fig. 9). In this phylogenetic tree, we found that the similarity coefficient of the species *D. peregrina* with the other five species is 0.265. The similarity coefficient of the species *D. kaki* with the other four species (*D. melanoxylon, D. blancoi, D. ebenum, D. sylvatica*) is 0.30. These five species create group 'A'. The coefficient of similarity of the species *D. melanoxylon* with the species *D. ebenum, D. sylvatica, and D. blancoi* is 0.38. These four species create group 'B'. And the three species such as *D. ebenum, D. sylvatica, and D. blancoi* are situated in cluster 'X' with the value of similarity coefficient 0.465 (Fig. 9).

Among these six species, three species such as *D. ebenum, D. sylvatica, D. blancoi* are strongly similar because they have maximum similar characteristics such as ellipsoid shaped sclereid, non-branched sclereid, sclereid cell cluster number are ranged between 6-8, cluster crystal absent, lumen shape narrow, prominently tannin are not deposited in the lumen, etc. as because their similarity coefficient (0.465) is much higher than other species. The species of *D. peregrina* is not strongly similar to the other five species because it does not share the same characteristics as the other species. Because its similarity coefficient is 0.265. The overall phylogenetic relationship of these six species based on fruit sclereid characters is represented in the dendrogram (Fig. 9).

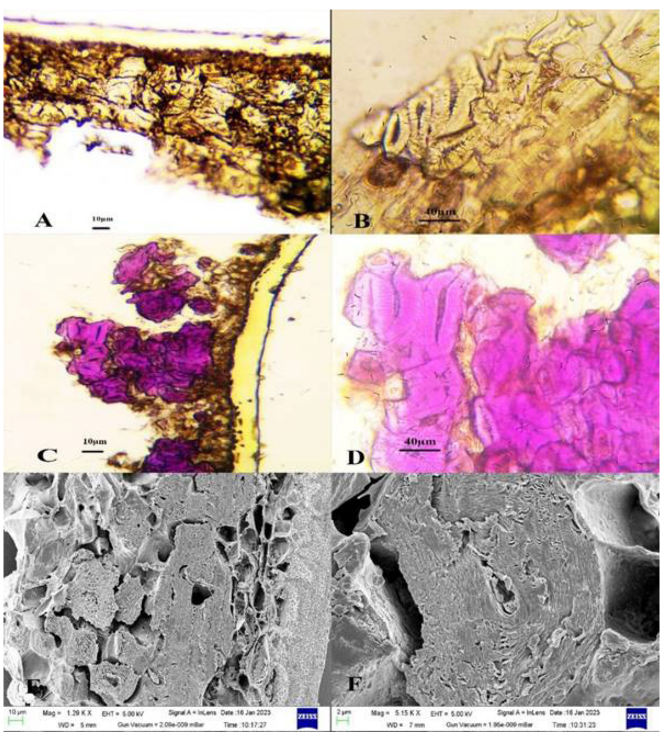

Fig. 7: *D. blancoi*: LM. A. Transverse section of fruit epicarp shows brachysclereid arranged regular in mesocarp (10x). B. Magnification (40x) of ellipsoid shaped brachysclereid. C. Brachysclereid stained with phloroglucinol-HCl (10x). D. Magnified (40x) stained brachysclereid shows distinct narrow lumen. SEM: E & F. Ultrastructure of brachysclereid shows thick lignified cell wall, lamellar structure, occluded cavity.

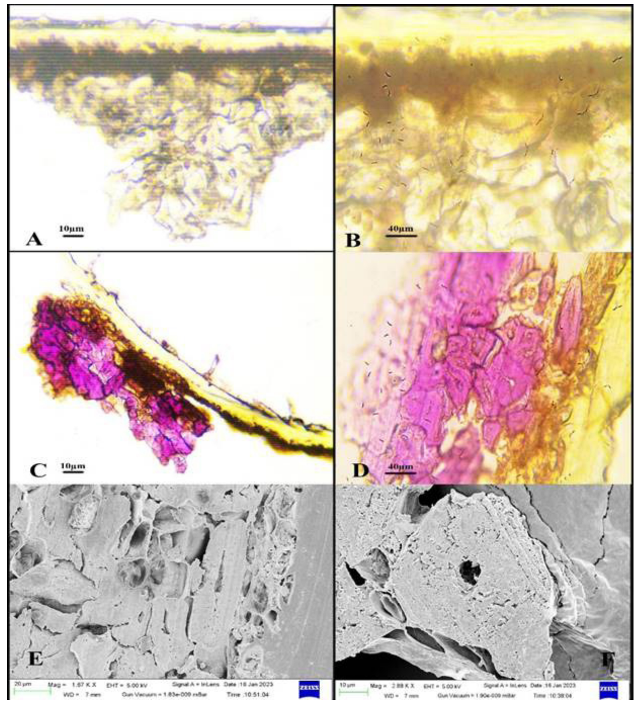

Fig. 8: *D. peregrina:* LM: A. Transverse section of fruit epicarp shows brachysclereid arranged irregular in mesocarp (10x). B. Magnification (40x) of ovoid shaped brachysclereid. C. Brachysclereid stained with phloroglucinol-HCl (10x). D. Magnified (40x) stained brachysclereid shows distinct shorter lumen. SEM: E & F. Ultrastructure of brachysclereid shows thick lignified cell wall, lamellar structure, occluded cavity.

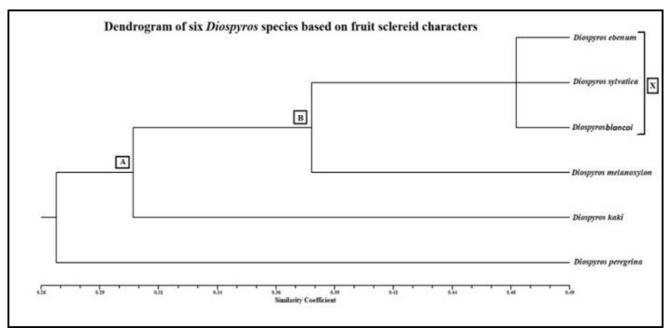

Fig: 9: A dendrogram showing the relationships between the six species in the Ebenaceae family based on the brachysclereid's micromorphological features.

Principal Components Analysis (PCA)

SI and SCL are positively correlated. Both SCL and SI are negatively correlated with SCW and SCLW. SI and SCL strongly influence PC 1 (Principal component 1). Both PC 1 and 2 jointly explain 77.41% of variation. PC 1, PC 2 and PC 3 altogether explain 90.53% of variants. (Abeysinghe, P.D. and Scharaschkin, T., 2019)

Characteristics of *D. ebenum*, *D. kaki and D. peregrina* are different from the characteristics of *D. blancoi*. *D. sylvatica,* and *D. melanoxylon* have share similar types of values. In overall all the species form cluster based on their character from the PCA (Fig. 10).

Statistical Analysis

The degree of variation in brachysclereid (length, breadth, and lumen diameter) among the selected plant species was assessed using analysis of variance (ANOVA). ANOVA was used to test the experimental hypothesis that there are significant differences in brachysclereid size among species belonging to the same morphological group. Before the ANOVA, descriptive statistics for the brachysclereid's length, width, and lumen diameter were calculated. These data included ranges, minimums, maximums, means, and standard deviations. There is statistical proof of a significant difference at the $p<0.05$ level in Table 3-5.

Mesocarp of fruits, and fruit parts of six selected species showed abundant brachysclereid (stone cells). The present study investigated different shapes of stone cells like ellipsoid, ovoid (Fig. 3-8). The average length, breadth and lumen diameter shows in Table.3-5. The length of brachy sclereid is 92.55 ± 4.84 µm in *D. melanoxylon*. The breadth of brachysclereid highest is 55.44 ± 5.61µm in *D. sylvatica*. The lumen diameter of brachysclereid highest is 8.53 ± 1.35µm in *D. melanoxylon.*

Table. 3: Length of brachysclereid in fruit of six selected plant taxa under the family Ebenaceae

Sl. No.	*Scientific Name*	*Mean ± Se*	*SD*	*SE*	*Mini*	*Maxi*
1.	*D. ebenum*	88.52 ± 5.59	4.93	2.85	84.57	94.06
2.	*D. sylvatica*	87.85 ± 6.76	5.98	3.45	84.02	94.75
3.	*D. melanoxylon*	92.55 ± 4.84	4.28	2.47	88.24	96.8
4.	*D. kaki*	82.82 ± 4.21	3.72	2.15	79.32	86.74
5.	*D. blancoi*	91.45 ± 2.10	1.85	1.07	89.72	93.43
6.	*D. peregrina*	83.47 ± 4.55	4.03	2.32	79.09	87.02

Table 4: Breadth of brachysclereid in fruit of six selected plant taxa under the family Ebenaceae

Sl. No.	*Scientific Name*	*Mean ± Se*	*SD*	*SE*	*Mini*	*Maxi*
1.	*D. ebenum*	36.73 ± 5.21	4.62	2.66	32.45	41.63
2.	*D. sylvatica*	55.44 ± 5.61	4.95	2.86	50.13	59.95
3.	*D. melanoxylon*	39.27 ± 3.90	3.46	1.99	36.46	43.14
4.	*D. kaki*	36.13 ± 4.47	3.95	2.28	32.14	40.04
5.	*D. blancoi*	34.81 ± 3.39	3.00	1.73	32.20	38.1
6.	*D. peregrina*	35.96 ± 1.84	1.62	0.94	34.28	37.53

Table 5: Lumen diameter of brachysclereid in fruit of six selected plant taxa under the family Ebenaceae

Sl.No.	*Scientific Name*	*Mean ± Se*	*SD*	*SE*	*Mini*	*Maxi*
1.	*D. ebenum*	5.84 ± 0.71	0.63	0.36	5.13	6.36
2.	*D. sylvatica*	8.06 ± 2.76	2.44	1.41	5.43	10.27
3.	D. melanoxylon	8.53 ± 1.35	1.20	0.69	7.42	9.82
4.	*D. kaki*	6.88 ± 0.61	0.55	0.31	6.36	7.46
5.	*D. blancoi*	5.56 ± 0.78	0.70	0.4	5.04	6.37
6.	*D. peregrina*	5.83 ± 0.37	0.32	0.19	5.46	6.07

Discussion

Liu and Qin, 2013, suggest that the fruit form of plant groups is a useful trait in taxonomy. The physiological functions of the trichomes, that are epidermal organs, are briefly explained. These include the balance of energy, protection against the sun, endurance to drought, gas exchange, reflectivity, and resistance to diseases and insects (Xiao *et al*., 2017). Different types, quantities, densities, and other features of vegetative trichomes are defined by their genetic composition and are encoded by particular genes, such as *Phlomoides* (Lamiaceae) (Seyedi and Salmaki, 2015), (Alliaceae) (Osman, 2012), Convolvulaceae (Ashfaq *et al*., 2019; Wang *et al*., 2019; Yang *et al*., 2018).

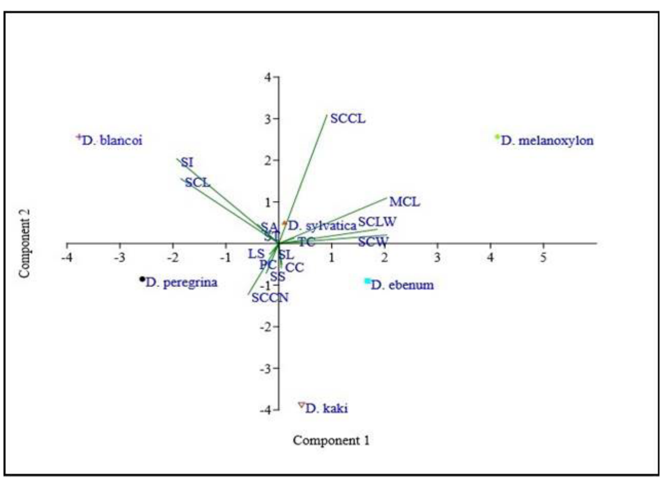

Fig. 10: Principal components (PC) of 15 informative qualitative and quantitative characters of *Diospyros* species.

Sclereids of the seed coat in *Nymphaea* can vary in placement (e.g., in longitudinal rows) and shape (e.g., lobulate), and they have thickened lignified cell walls. (Conard, 1905) described sclereids for *Nymphaea flava* seeds, and (Haines and Lye, 1975) illustrated sclereids in *N. caerulea* Sav. Additionally, they have been described for the subgenera *Victoria cruziana* and *Hydrocallis* (Wiersema, 1987; Valla and Martin, 1976). According to (Corner, 1976), sclereids can have stellate, subsquare, or rectangular shapes. Sclereids can have smooth, granulate, rugulate-reticulate, or verrucate surface topography, according to (Schneider and Ford, 1978; Wiersema 1987; Schneider and Ford, 1978; Conard, 1905). We found that sclereids of the subgenus *Nymphaea,* which includes *N. mexicana,* have a rugulate-reticulate surface, in contrast to (Capperino and Schneider's, 1985) observation that *N. mexicana* seeds had a smooth surface.

In this study we found the different light microscopy and ultrastructure (Fig. 3-8)) of brachysclereid and its eleven qualitative and quantitative (Table.1) character. The mature fruit mesocarp of six genera of *Diospyros* species consisted of brachysclereid, parenchyma tissue (Wang *et al.,* 2020). A few edible fruits, such as *Psidium guajava* (in the Myrtaceae family), *Malus sylvestris*, *Pyrus communis*, *Frageria vesca* (in the Rosaceae family), *Manilkara achras*, *Mimusops elengi*, and *Madhuca indica* (in the Sapotaceae family) contain sclereids, although some differentiation was also noted. The ultrastructure of sclereid of *Mimusops elengi* was well defined (Khatun *et al*., 2023). There are differences in the distribution and organization. In *Frageria vesca*, the big length of the sclereid is 1017.16µm. In *Pisum sativum*, sclereid has a narrow width of 16.3 µm. (Mondal and Khatun, 2011). Sclereid shape, Sclereid type, Mesocarp cell layer, Sclereid cell layer, Sclereid cell cluster no., Cluster crystal, Porosity of the stone cells, Lumen shape, Tanin deposition in lumen, Sclereids arrangement, are considered as a qualitative character. Sclereid cell length, sclereid cell width, Sclereid cell lumen diameters are considered as a quantitative character to construct the phylogenetic tree. Among six genera of *Diospyros,* 92.55 ± 4.8µm (Fig.11 Table.3) is the highest length of brachysclereid in *D. melanoxylon*. 55.44 ± 5.61µm (Fig. 12, Table 4) is the highest breadth in *D. sylvatica.* The highest lumen diameter is 8.53 ± 1.35µm. (Fig. 13, Table 5 in *D. melanoxylon*. The phylogenetic tree shows the species are arranged in two groups such as 'A' and 'B' and a cluster named 'X' (Fig. 9).

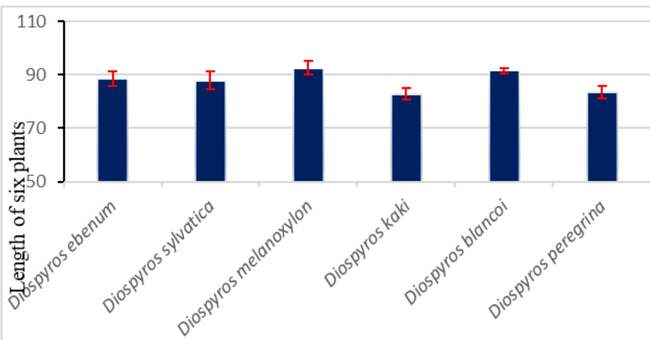

Fig. 11: Bar diagram shows length of brachysclereid in fruit of six selected plant taxa under the family Ebenaceae

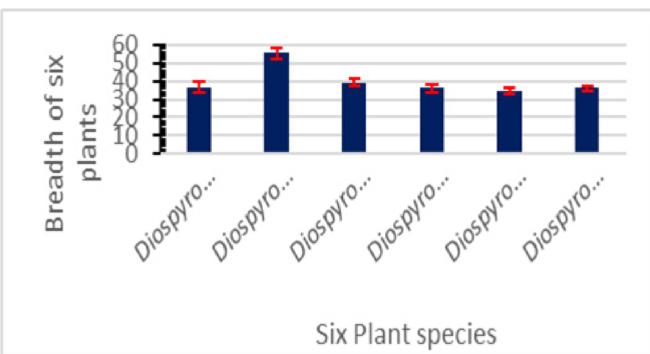

Fig. 12: Bar diagram shows breadth of brachysclereid in fruit of six selected plant taxa under the family Ebenaceae

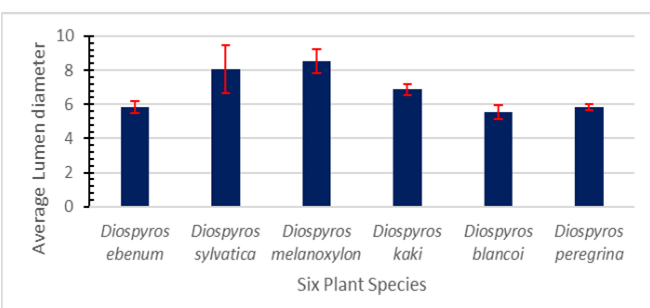

Fig. 13: The Bar diagram shows the lumen diameter of brachysclereid in the fruit of six selected plant taxa under the family Ebenaceae

Conclusion

Brachysclereid analysis of Jhargram district taxa has shown a great deal of variation in size and shape. The brachysclereid cells exhibit a high degree of variability and can be used as a recognizing and diagnostic tool. Important taxonomic features are the bachysclereids' size, shape, location, and arrangement. Phylogenetic tree shows the similarity coefficient of the species *D. peregrina* with the other five species is 0.265. The similarity coefficient of the species *D. kaki* with the other four species (*D. melanoxylon, D. blancoi, D. ebenum, D. sylvatica*) is 0.30. These five species create group 'A'. The coefficient of similarity of the species *D. melanoxylon* with the species *D. ebenum, D. sylvatica, and D. blancoi* is 0.38. These four species create group 'B'. The three species such as *D. ebenum, D. sylvatica, and D. blancoi* are situated in cluster 'X' with the value of similarity coefficient 0.465. PCA analysis shows SI and SCL are positively correlated. Both SCL and SI are negatively correlated with SCW and SCLW. SI and SCL strongly influence PC 1 (Principal component 1). Both PC 1 and 2 jointly explain 77.41% of the variation. PC 1, PC 2 and 3 altogether explain 90.53% of variants. The results of this study which focused on the micromorphological features of the sclereids of fully ripened fresh fruits, suggest that the morphological variety seen in *Diospyros* sclereids can be valuable in determining relationships and defining taxa.

Author contributions

Mamtaj Khatun and Amal Kumar Mondal have planned the research article. Mamtaj Khatun conducted the experiment, and characterized and analyzed the data. Amal Kumar Mondal edited the manuscript. All authors viewed and accomplished on the manuscript.

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