

A COMPARATIVE ANATOMICAL INVESTIGATION OF THREE TAXA OF *BRASSICA* L. FROM BANGLADESH

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Key words: Brassica L.; Root anatomy; Stem anatomy; Stomata; Trichome.

Abstract

In the present study, micromorphology and comparative anatomy of young root, stem and leaf of three economically important taxa of *Brassica* L. viz., *B. rapa* L. subsp. *campestris* (L.) Clapham, *B. juncea* (L.) Czern. and *B. napus* L. are investigated in detail for the first time in Bangladesh by using light microscopy (LM). In anatomical studies, cross sections of young root, stem and leaf have been examined and biometric measurement of cell and tissues are presented. The micromorphological studies are related to the epidermal surface. In addition, the stomatal index and stomatal index ratio of these taxa have been calculated and presence of simple, non-glandular, unicellular trichome on stems and leaves also been observed except on the stem of *B. rapa* L. subsp. *campestris* (L.) Clapham. The anatomical study reveals that the investigated taxa have primary growth in roots and stems as well as amphistomatic and bifacial leaves with anisocytic stomata have been noticed. Finally, presence of tetrarch or single strand exarch xylem in vascular bundle of root, the size, shape and presence or absence of trichome over stem, shape of midrib of leaf have been considered to provide reliable features for identification of the *Brassica* species.

Introduction

The family Brassicaceae consists of 338 genera and 3709 species and dispersed all through the world, basically in temperate regions of the Northern Hemisphere (Warwick *et al.*, 2006; Kasem *et al.*, 2011). Among the genera, the genus *Brassica* L. includes around 100 economically important species with great hereditary and morphological assorted diversity (Jahan *et al.*, 2013). The principal distribution centers of the family are the Mediterranean, Irano-Turanian and Saharo-Sindian regions (Hedge, 1976). In Bangladesh, the Brassicaceae family is represented by 12 genera and 24 species (Ahmed *et al.*, 2008).

The genus is noteworthy for containing more important agricultural and horticultural crops like oilseed, vegetables, medicine and condiments than any other genus (Saha *et al.*, 2008). Oleiferous *Brassica* species viz. *B. rapa*, *B. juncea* and *B. napus* constitute the world's third most vital source of consumable oils (Gupta and Pratap, 2007), and are considered as one of the leading oilseed crops in Bangladesh (Razzaque and Karim, 2007).

Metcalf and Chalk (1957) studied the anatomy of Cruciferae and destined the diagnostic anatomical characteristics including epidermal cell type, stomata type and the array of the sclerenchymatic cells around the vascular bundles of the leaves. The quantity of morphological and taxonomic studies in Cruciferae has expanded in recent years (Khatun *et al.*, 2011; Kaya and Dirmenci, 2012; Bayirli *et al.*, 2014; Selvi *et al.*, 2014; Hayta *et al.*, 2014; Satil *et al.*, 2015).

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No micromorphological and anatomical study on the available and economically important species of *Brassica* L. from Bangladesh has been conducted so far. Therefore, the purpose of this paper is to investigate the anatomical and micromorphological properties of three important taxa of the genus *Brassica*.

Materials and Methods

Plant materials

Three species of *Brassica* L. namely, *B. rapa* L. subsp. *campestris* (L.) Clapham, *B. juncea* (L.) Czern. and *B. napus* L. were chosen for this study because of their availability and economic importance (Fig. 1). Among the three *Brassica* L. species, the seeds of *B. rapa* L. subsp. *campestris* (L.) Clapham were collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. Seeds of *B. juncea* (L.) Czern. and *B. napus* L. were collected from the Oilseeds Research Center (ORC) of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh. These three species were maintained in the Botanical garden, Department of Botany, Jagannath University, Dhaka.

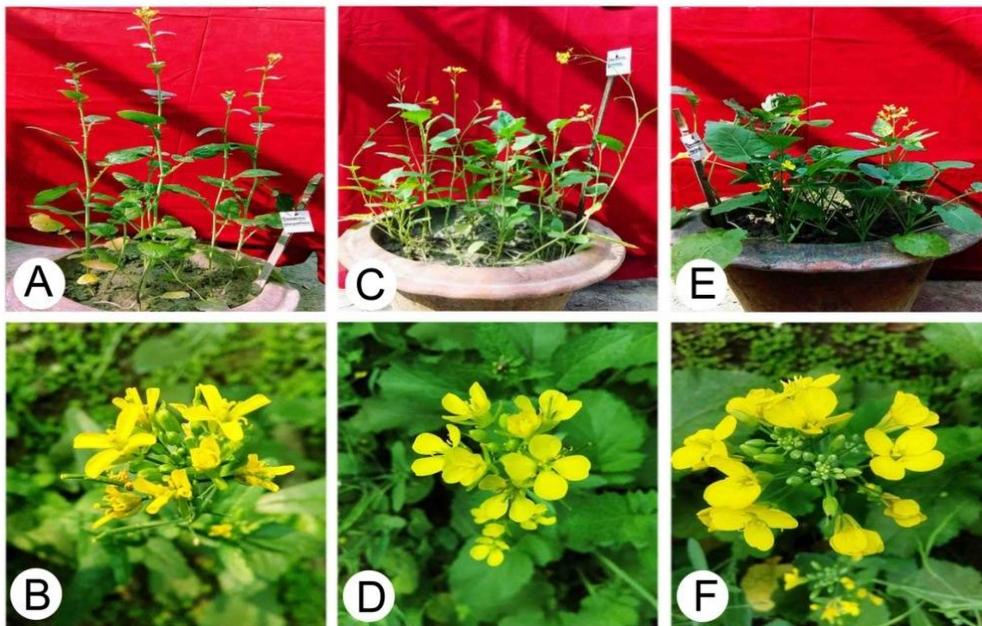


Fig. 1. Habit of three species of *Brassica* L. A–B. *B. rapa* subsp. *campestris*; C–D. *B. juncea*; E–F. *B. napus*; A, C, E: Plant morphology; B, D, F: Flowers.

Anatomical investigation

Fresh specimens were collected for the study of the internal structures of the studied species of *Brassica*. Transverse section of the vegetative organs, viz. young root, stem and leaf were chosen as they might provide discrete anatomical features of taxonomic importance. Free hand sectioning of young root, stem and leaf was made with the help of a razor blade. The sections were stained with safranin (0.1%) for 15 min. After that the well stained section were mounted in 20% glycerin (Shethi *et al.*, 2017) and selected sections were prepared for permanent slides.

Stomatal density on abaxial and adaxial surfaces of the leaf was counted under a light microscope. Stomatal index was calculated according to the method of Meidner and Mansfield (1968). Stomatal terminology and the leaf epidermal terminology were based on the classification provided by Dilcher (1974) and Wilkinson (1979), respectively. The trichomes were investigated on the both surface of leaves of all studied species and examined by light microscopy. Trichome description and classification followed Theobald *et al.* (1979). The permanent slides were studied under a compound light microscope Optica. Micrographs were captured from various regions of the sections using different magnifications through Euromex camera. Measurements of sections were made by using Image Focus 4 English software. Biometric measurements of anatomical characters were made by taking three times measurement of different cells and tissue of each species.

Results and Discussion

The comparative anatomy and micromorphology of *Brassica* species growing in Bangladesh are thoroughly investigated. The biometric measurements of the anatomical characteristics of *B. rapa* subsp. *campestris*, *B. juncea* and *B. napus* based on young root, stem and leaf are presented in Table 1. Comparative anatomical characters of young root, stem and leaf of the investigated species are given in Tables 2, 3 and 4, respectively. Leaf epidermal surface characters of the studied species of *Brassica*, examined micromorphologically, are shown in Table 5.

Root anatomy

In the cross-section of young root, the root exhibits a primary structure. There is epidermis which makes up the outermost layer of the root and consists of single layered, compactly packed, elongated barrel shaped cells, having no cuticle and stomata. The epidermal layer width is the lowest in *B. rapa* subsp. *campestris* (51.37 μm), while it is highest in *B. napus* (85.73 μm) (Table 1). On epidermal layer, root hairs are found to be present in *B. rapa* subsp. *campestris* and *B. juncea* but absent in *B. napus*. Immediately beneath the epidermis, there is cortex that consists of 4–9 thin-walled parenchyma cell layers, sometimes having intercellular spaces among them and cells are usually elliptical, circular and oval in shape. Below the cortex layer, a distinct endodermis layer is found, consisting of elliptical cells. The pericycle consists of thin walled parenchyma cells, found to be present as uniseriate which makes the outer boundary of the primary vascular bundle of the roots of examined species. The xylem forms four discrete strands- the tetrarch, alternating with the phloem in *B. rapa* subsp. *campestris* and *B. napus* while *B. juncea* exhibited with the xylem which is occupied the center. The xylem present in the vascular bundle of root in all the studied species are exarch. The phloem is located under the pericycle and is composed of 4–6 layered overlapping sequence of cells. The parenchymatous conjunctive tissues occur in between xylem and phloem strands. No cambium is observed in the examined species of *Brassica* L. Except in *B. juncea* the central part of the stele is occupied by well-developed pith which is found sclerenchymatous, while the pith is found inconspicuous or absent in *B. juncea* (Fig. 2 and Table 2).

Stem anatomy

In the transverse section of stem, a few variations reveal among the three different examined species of *Brassica*. The stems of studied species also exhibit primary structure. The contour of stem is round in *B. juncea* and ovoid in *B. napus* (Figs. 3C and 3E), while polygonal in *B. rapa* subsp. *campestris* (Fig. 3A). Margin of all the species have ridges and furrows. Except in *B. rapa* subsp. *campestris*, the other two species, viz. *B. juncea* and *B. napus* have sparsely situated trichomes on the outer surface of stem. *B. juncea* contains very few, small, non-glandular,

Table 1. Biometric measurement of anatomical characters of three species of *Brassica* L.

Parameters	<i>Brassica rapa</i> subsp. <i>campestris</i>				<i>Brassica juncea</i>				<i>Brassica napus</i>			
	Length (μm)		Width (μm)		Length (μm)		Width (μm)		Length (μm)		Width (μm)	
	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD	Mean	STD
Root												
Epidermal cells	76.49	8.97	51.37	6.65	87.86	0.51	78.68	9.87	112	15.57	85.73	9.16
Root hair	55.81	1.00	-	-	415.33	38.06	-	-	-	-	-	-
Cortex layers	-	-	68.92	10.99	-	-	347.09	33.88	-	-	443.45	7.91
Endodermal cells	21.60	2.87	11.30	2.72	60.14	16.17	50.24	13.58	73.82	6.77	62.21	8.29
Pericycle cells	62.30	11.37	48.04	5.71	64.92	11.90	53.93	6.90	54.63	16.17	49.11	3.92
Phloem layers	-	-	17.53	3.42	-	-	47.94	7.47	-	-	40.58	14.31
Tracheid cells	7.94	1.02	6.43	0.60	40.47	4.25	36.77	1.92	38.46	3.97	31.77	10.38
Stem												
Cuticle	-	-	7.01	0.75	-	-	7.77	4.49	-	-	5.36	1.02
Epidermal cells	36.22	2.77	29.45	1.64	32.10	5.36	23.76	7.95	23.75	10.37	16.15	3.77
Trichomes	-	-	-	-	75.25	3.67	-	-	342.82	77.97	-	-
Cortex layers	-	-	158.96	77.54	-	-	194.70	45.29	-	-	198.52	94.66
Endodermal cells	29.16	6.17	19.37	2.66	72.84	15.16	51.58	9.42	32.84	8.85	27.13	3.52
Pericycle layer	-	-	54.86	21.74	-	-	40.34	21.62	-	-	88.92	30.88
Phloem layers	-	-	19.38	12.62	-	-	28.89	10.00	-	-	47.55	13.66
Cambium layer	-	-	-	-	-	-	65.08	12.00	-	-	69.08	4.21
Tracheid cells	27.03	4.74	23.79	4.58	12.68	1.49	11.56	1.70	24.40	2.47	20.67	1.19
Pith region	-	-	963.89	18.12	-	-	694.15	16.46	-	-	1147.93	35.23
Pith cells	77.42	47.90	73.88	49.16	85.12	28.45	65.08	12.00	67.10	36.09	48.47	35.16
Leaf												
Cuticle	-	-	3.38	1.38	-	-	3.06	1.16	-	-	3.40	0.65
Trichome	81.75	99.30	-	-	510.32	34.97	-	-	371.78	41.35	-	-
Upper epidermis cells	31.70	8.77	14.42	1.87	39.82	20.16	23.48	9.56	46.47	16.53	19.41	5.95
Lower epidermis cells	20.90	5.15	12.60	1.41	31.90	15.30	18.25	3.69	29.96	6.69	11.16	3.73
Mesophyl layers	-	-	169.41	20.84	-	-	171.55	16.12	-	-	195.40	4.67
Palisade parenchyma	28.44	9.92	22.60	2.80	42.44	20.89	29.67	4.43	40.00	15.75	28.21	15.36
Spongy parenchyma	39.50	16.76	18.98	2.00	32.33	9.15	20.72	3.47	27.31	17.07	18.16	6.53

unicellular, conical-shaped, blunt tipped trichomes which are present sparsely on the outer surface (Fig. 3C). In contrast, long, non-glandular, unicellular, less number of sparsely arranged trichomes with a very pointed tip are found in *B. napus* (Fig. 3E). The epidermis is single layered with thick cuticle on the outside in all the three investigated species. The cuticle layer ranges from 5.36 μm in *B. napus* to 7.77 μm in *B. juncea* (Table 1). The epidermis is composed of compactly arranged square, rectangular, elliptical or circular cells. The epidermal layer (length) is found highest in *B.*

rapa subsp. *campestris* (36.22 μm) whereas it is lowest in *B. napus* (23.75 μm) (Table 1). The epidermis contains anisocytic stomata. The epidermal layer is followed by a single layer of hypodermis, which is made up of collenchyma tissues except in *B. juncea* (Fig. 3D). The cortex layer consists of cortex parenchyma and endodermis. The cortex parenchyma is chlorenchymatic

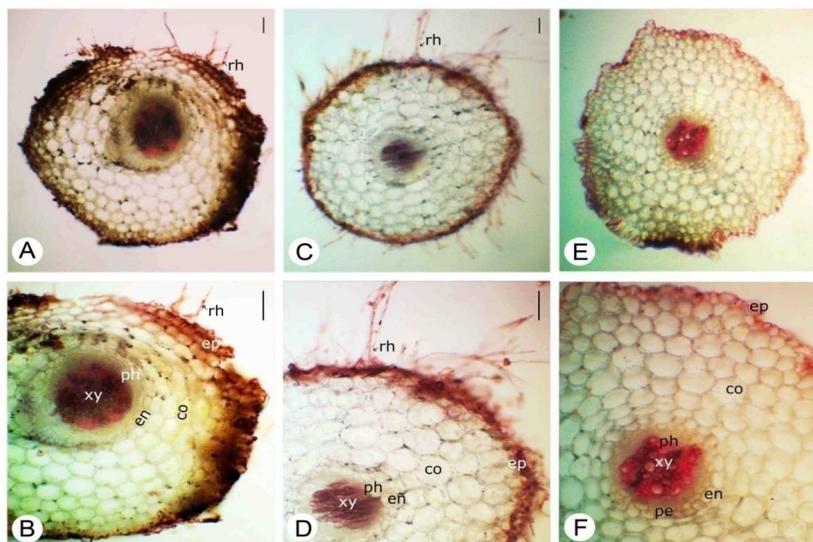


Fig. 2. Transverse section of root of three species of *Brassica* L. A–B. *B. rapa* subsp. *campestris*; C–D. *B. juncea*; E–F. *B. napus*; A, C, E: Full contour of root (4X); B, D, F: Detailed anatomical characters of root (10X). ep: epidermal cell, rh: root hair, co: cortex, en: endodermis, pe: pericycle, xy: xylem, ph: phloem. Bar = 100 μm .

Table 2. Young root anatomical characters of three species of *Brassica* L.

Taxa	Root hairs	Cortex		Pericycle	Xylem Strand	Phloem	Pith
		Parenchyma	Endodermis				
<i>B. rapa</i> subsp. <i>campestris</i>	Present	5-7 layered	1 layered	1 layered	Tertarch	4-6 layered	Present
<i>B. juncea</i>	Present	4-5 layered	1 layered	1 layered	Single strand	4-5 layered	Inconspicuous
<i>B. napus</i>	Absent	7-9 layered	1 layered	1 layered	Tertarch	4-5 layered	present

and is made up of 2–9 layers, thin-walled, with regular oval or circular cells. Cortex parenchyma also contains starch granules. The parenchymatous cells of *B. rapa* subsp. *campestris* and *B. juncea* contain more intense starch than *B. napus* (Fig. 3). Underneath the cortex parenchyma, there is a single layered rectangular or barrel shaped endodermis surrounding the vascular bundle of the stem. Pericycle is usually sclerenchymatic, 1–3 layered and surrounding the phloem layers of vascular bundles. The number of vascular bundles arranged in a ring varies from 7 to 25 in the studied species. 9 to 13 vascular bundles are found in *B. rapa* subsp. *campestris* whereas 7 to 12 and 17 to 25 vascular bundles are observed in *B. juncea* and *B. napus*, respectively (Table 3). Inter-fascicular region is located in between vascular bundles of *B. rapa* subsp. *campestris* and this region comprises sclerenchymatic cells while a continuous wavy band of multi-layered rectangular, sclerenchymatous conjunctive tissue is observed in *B. juncea* and *B. napus*. The type

of vascular bundle is conjoint, collateral and open in *B. juncea* and *B. napus* (Figs. 3D & 3F). The phloem is 2–7 layered and consists of irregular and squashed cells. The cambium is distinct and is located between phloem and xylem elements. The cells of cambium tissue are typically rectangular shaped and are arranged in 2–4 layers. Xylems of the vascular bundles are highly lignified. In *B. rapa* subsp. *campestris*, vascular bundles are found to be conjoint, collateral and closed (Figs. 3A–B and Table 3). The pith region located in the center of the stem is composed of large orbicular or polygonal parenchymatous cells and occupies much of the volume of stem in all the three studied species of *Brassica* (Figs. 3A, 3C & 3E). A comparative account of stem anatomical characters among the three *Brassica* species has been depicted in Table 3. The highest numbers of vascular bundles are found in *B. napus* followed by *B. rapa* subsp. *campestris*, while the lowest number is noticed in *B. juncea*. It can easily be distinguished from other species by its round shaped stem, small, non-glandular, sparsely located unicellular, blunt-tipped trichomes. *B. napus* can be identified by its sparsely located unicellular, non-glandular, long trichomes with pointed tips (Table 3).

Table 3. Stem anatomical characters of three species of *Brassica* L.

Taxa	Trichome	Cortex		Pericycle	Vascular bundle Type	No. of vascular Bundles	Phloem
		Parenchyma	Endodermis				
<i>B. rapa</i> subsp. <i>Campestris</i>	Absent	3-5 layered	1 layered	1 layered	Closed, Collateral	9-13	3-5 layered
<i>B. juncea</i>	Present	2-4 layered	1 layered	1 layered	Open, Collateral	7-12	2-3 layered
<i>B. napus</i>	Present	6-9 layered	1 layered	2-3 layered	Open, Collateral	17-25	4-7 layered

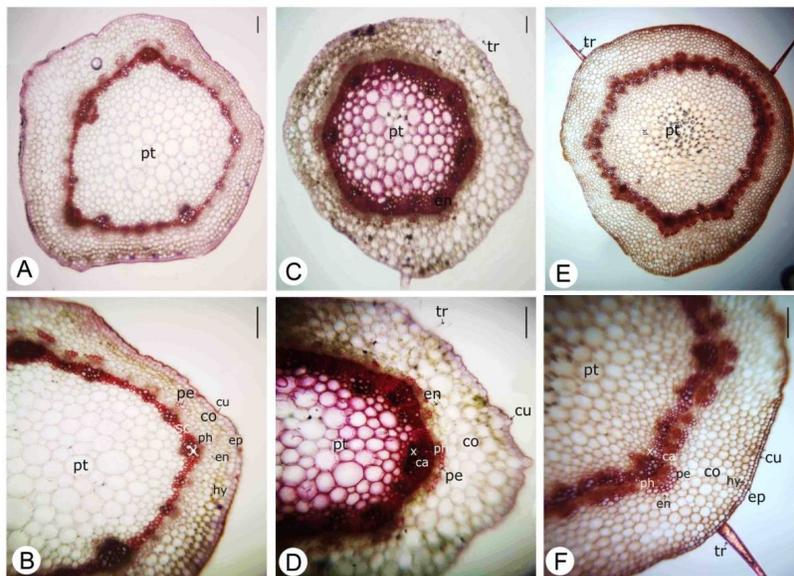


Fig. 3. Transverse section of stem of three species of *Brassica* L. A–B. *B. rapa* subsp. *campestris*; C–D. *B. juncea*; E–F. *B. napus*; A, C, E: Full contour of stem (4X); B, D, F: Detailed anatomical characters of stem (10X). cu: cuticle, ep: epidermis, co: cortex, hy: hypodermis, en: endodermis, pe: pericycle, sc: sclerenchyma, ph: phloem, ca: cambium, x: xylem, pt: pith, tr: trichome. Bar = 100 μ m.

Leaf anatomy

Transverse section of the dorsiventral leaves of the three species of *Brassica* provides diverse anatomical features in both leaf blade and midrib region. In the studied species, there is a single layer of epidermis observed on the adaxial and abaxial surface of the leaf. A thin layer of cuticle present on the upper and lower epidermis ranging from 3.06 μm in *B. juncea* to 3.40 μm in *B. napus* (Table 1). Epidermal cells of the both surfaces of lamina are rectangular to cubic in shape whereas the epidermal cells of midrib are ellipsoidal to oval in shape in all studied species. The laminar epidermal cells are much bigger than epidermal cells of midrib (Fig. 4). From the micromorphological observations of leaf epidermal surface, the epidermal cell walls are found irregular in shape and anticlinal cell wall are sinuate and undulating (Figs. 5A–5F, Table 5). The

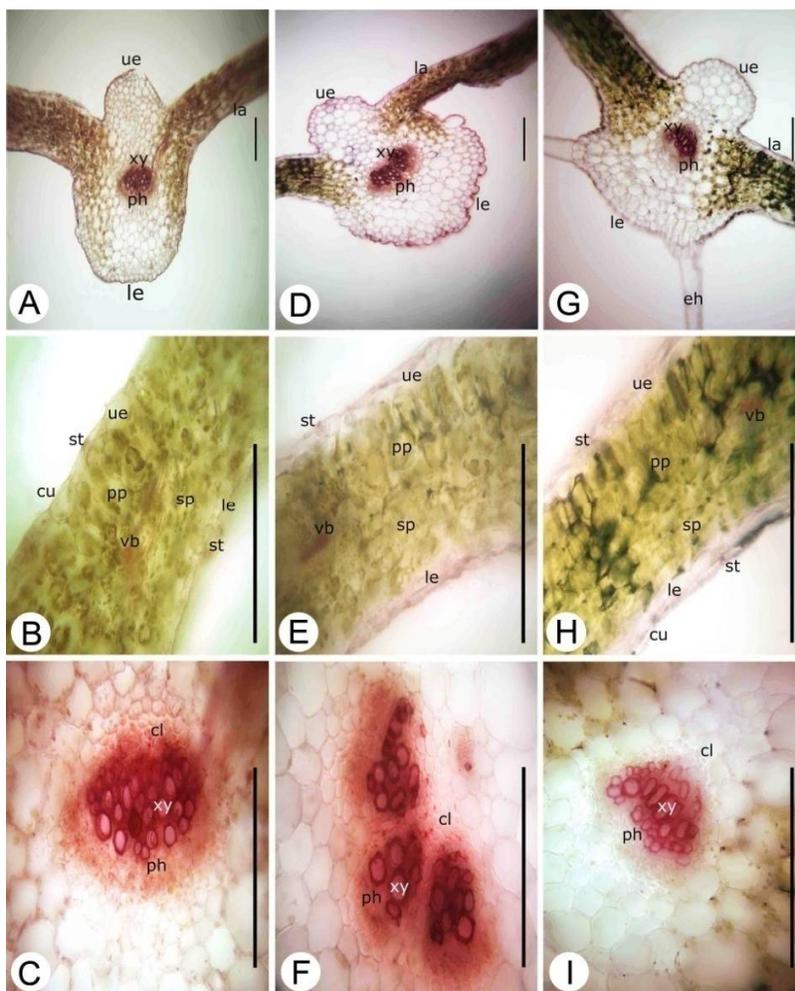


Fig. 4. Transverse section of leaf of *Brassica* L. species. A–C. *B. rapa* subsp. *campestris*; D–F. *B. juncea*; G–I. *B. napus*; A, D, G: Leaf section with lamina and midrib (10X); B, E, H: Detailed anatomical characters of lamina (40X); C, F, I: Detailed anatomical characters of midrib (40X); cu: cuticle, ue: upper epidermis, le: lower epidermis, eh: eglandular hair, vb: vascular bundle, st: stomata, cl: collenchymas, la: lamina, pp: palisade parenchyma, sp: spongy parenchyma, xy: xylem, ph: phloem. Bar = 100 μm .

abaxial leaf surface of all the three species are covered with simple, non glandular, unicellular trichomes which are straight or curved, stalked, flattened in the lower part and tapering above, long on the midrib and veins, otherwise shorter with straight or hooked tips. Trichomes are densely present in *B. rapa* subsp. *campestris* and *B. juncea*, but sparsely found in *B. napus* (Figs. 5G–5I and Table 4). Amphistomatic types of stomata are observed on epidermal layers of all the studied species. Stomata are found to be elliptic in all species. Stomata are bigger in *B. juncea* (24.47 μm), while they are smaller in *B. rapa* subsp. *campestris* (19.34 μm) on both surfaces of leaves (Table 5). They may be either mesomorphic or higromorphic. The density of stomata is higher in abaxial surface (112.00–184.40) than the adaxial surface (69.28–105.20) in all the studied species (Table 5). Based on the arrangement of subsidiary cells that enclosing the guard cells, the stomata of the three studied species are anisocytic type (Figs. 5A–5F). The stomatal

Table 4. Comparative leaf anatomical characters of three species of *Brassica* L.

Taxa	Trichomes	Palisade parenchyma	Spongy parenchyma	Midrib shape	Middle vascular bundle	
					Collenchyma on xylem	Collenchyma under phloem
<i>B. rapa</i> subsp. <i>Campestris</i>	Densly present	2-3 layered	3-5 layered	Biconvex, adaxial less angular, abaxial square shaped with no ridges and furrows	1-2 rows	3 rows
<i>B. juncea</i>	Densly present	2-3 layered	4-5 layered	Biconvex, adaxial more angular, semi-circular abaxial with prominent ridges and furrows	4-5 rows	1-2 rows
<i>B. napus</i>	Sparsely present	3-5 layered	4-5 layered	Biconvex, adaxial angular, abaxial semi-circular, somewhat flattened with no ridges and furrows	3-5 rows	3-4 rows

Table 5. Micro-morphological leaf epidermal surface characteristics of three species of *Brassica* L.

Characters	<i>Brassica rapa</i> subsp. <i>campestris</i>		<i>Brassica juncea</i>		<i>Brassica napus</i>	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Anticlinal cell wall	Sinuate	Sinuate	Sinuate	Sinuate	Undulate	Undulate
Shape of epidermis cells	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular
Stomata type	Anisocytic	Anisocytic	Anisocytic	Anisocytic	Anisocytic	Anisocytic
Stomatal shape	Elliptical	Elliptical	Elliptical	Elliptical	Elliptical	Elliptical
Stomata length (μm)	19.91 \pm 1.06	19.34 \pm 1.90	24.47 \pm 3.22	24.47 \pm 0.51	22.76 \pm 0.25	24.12 \pm 2.20
Stomata width (μm)	15.47 \pm 0.77	15.62 \pm 0.44	18.74 \pm 2.69	20.02 \pm 1.68	16.37 \pm 1.37	19.74 \pm 0.45
Number of stomata (1mm ²)	99.60 \pm 20.00	146.00 \pm 14.03	105.20 \pm 17.47	184.40 \pm 9.50	69.28 \pm 12.70	112.00 \pm 14.89
Number of epidermis cells	349.00 \pm 22.19	585.60 \pm 30.00	315.60 \pm 24.50	691.20 \pm 15.60	349.00 \pm 21.00	448.73 \pm 18.90
Stomata index	22.20	19.96	25.00	21.05	16.55	19.97
Stomata index ratio	1.11	0.89	1.19	0.84	0.82	1.21

index of the studied species ranges from 16.55–25.00 in the adaxial surface and 19.96–21.05 in the abaxial surface, while the stomatal index ratio for adaxial surface is between 0.82 (*B. napus*) and 1.19 (*B. juncea*), and for abaxial surface it is between 0.84 (*B. juncea*) and 1.21 (*B. napus*) (Table 5). In lamina, the mesophyll is bifacial (dorsiventral) in all species (Figs. 4B, 4E & 4H). The mesophyll layer consists of palisade and spongy parenchyma cells, where in the palisade parenchyma cells are 2–5 layered and cylindrical to rectangular in shape. The longest palisade parenchymatous cell has been found in *B. juncea* (42.44 μm) followed by *B. napus* (40.00 μm), while the smallest palisade parenchyma is noted in *B. rapa* subsp. *campestris* (28.44 μm) (Table 1). Spongy parenchyma cells are 3–5 layered, ellipsoidal or oval with no intercellular space. Vascular bundles are embedded in mesophyll and of the closed, collateral type, and are surrounded with a single layer of parenchymatous bundle sheath. Although the midrib is biconvex in all the examined species, each of them has some variations on either surface (Table 4, Figs. 4A, 4D & 4G). In *B. rapa* subsp. *campestris*, adaxial surface is found to be less angular, whereas the abaxial surface is square with no ridges and furrows. *B. juncea* exhibits with more angular adaxial surface and semi-circular abaxial surface with prominent ridges and furrows. In *B. napus*, adaxial surface is angular whereas abaxial surface is semi-circled, somewhat flattened, with no ridges and furrows. Single, closed, collateral vascular bundle has been observed in the midrib of *B. rapa* subsp. *campestris* and *B. napus*. On the contrary, three discrete closed, collateral vascular bundles are present in the midrib of *B. juncea*. The xylems that present in the middle vascular bundles are more lignified. Subsequent collenchyma layers are found to present on the xylem and below the phloem tissues in the middle vascular bundles. No sclerenchymatic tissues are present on phloem layers of vascular bundle (Figs. 4C, 4F & 4I).

The present study is probably the first as no micromorphology and anatomical investigation of *Brassica* in Bangladesh has been made earlier. Previously, several anatomical studies of various genera of the family Brassicaceae has been carried out to identify the members of Brassicaceae (Ančev and Goranova, 2006; Selvi and Paksoy, 2013; Tekin and Martin, 2017). The present study has revealed that stems of three studied *Brassica* species provide the general anatomical characteristics of the Cruciferae family (Fig. 3) which is in congruent with previous studies where the pith region has been found as parenchymatous and occupies a large proportion of the total diameter of the stem in the Cruciferae (Metcalf and Chalk, 1957; Tekin and Martin, 2017). In a comparison of the results stem anatomy of *Physoptychis haussknechtii* Bornm., distributed mainly in Eurasia (Rešetnik *et al.*, 2013), a member of Brassicaceae family (Tekin and Martin, 2017) and our results on examined *Brassica* species, showed the primary structure of stem which depicted with compactly arranged single layered epidermis covered with thick cuticle, followed by parenchymatous cortex layer, endodermis and sclerenchymatous pericycle. Beneath the pericycle, a continuous ring of sclerenchymatous cambium formed in between xylem and phloem elements of *B. juncea* and *B. napus*. The pith is formed from elliptical or circular parenchymatous cells in the center of stem. The results of our study shows that all the studied species of *Brassica* L. have similar anatomical properties as *P. haussknechtii* Bornm., such as having the stem of the primary structure, however the presence of cambium in *B. juncea* and *B. napus* have been found correspond with *P. haussknechtii* Bornm.

Metcalf and Chalk (1957) stated that the stomata of Cruciferae family are of the cruciferous (anisocytic) type. The stomata of *Brassica* species were surrounded by three subsidiary cells of which one is usually much smaller than the other two, the so-called Cruciferous (Anisocytic) type (Figs. 5A–5F). However, occurrence of such type of stomata is reported to be common in other genera, namely *Ricotia* L. and *Chrysochamela* (Fenzl) Boiss. of the same family by many workers (Selvi and Paksoy, 2013; Çakilcioğlu *et al.*, 2017). Due to the presence of same type of stomata in

different genera of the same family, this feature may be helpful in delimitation of ranking in taxonomic studies (Santos *et al.*, 2018).

Ančev and Goranova (2006) studied trichome morphology of eleven genera of tribe *Alyseae* of Brassicaceae and recognized four types, viz. simple, stalked 2 to 5 armed, stellate and dendritic trichomes. In the present study, trichomes found in leaf in all the studied species are simple, straight, stalked, flattened in the lower part, tapering above, long on the midrib and veins, otherwise shorter with straight or hooked tips (Figs. 5G–5I). According to Ančev and Goranova (2006), simple trichomes cover the leaves of *Lunaria rediviva* L. and mixed with stalked 2 to 3-armed ones, compose the leaf indumentums of *Camelina sativa*. Simple trichomes on the silicules of *Clypeola jonthlaspi* subsp. *microcarpa* are columnar with transverse furrows. The stem trichomes of *Brassica juncea* are simple, short, unicellular, conical-shaped and blunt at the tip, while it has been found long and tapering above in the stem of *B. napus* and no trichome been found in *B. rapa* subsp. *campestris*. Trichomes basically serve as defense tissue and provide worthy anatomical features to characterize species (Shethi *et al.*, 2019). Therefore, it may be considered that the size and shape and presence or absence of trichomes may provide information to recognize the species of *Brassica* genus.

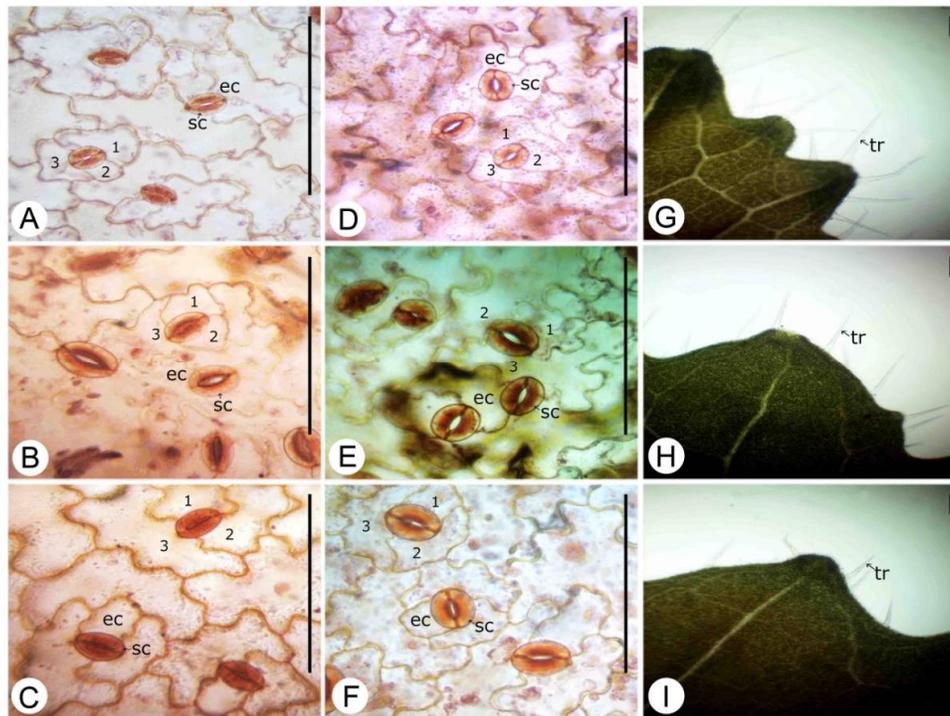


Fig. 5. Stomata of adaxial surface (A, B, C) (40X) and abaxial surface (D, E, F) (40X) and trichomes (G, H, I) (4X) of *Brassica* L. species. A, D, G. *B. rapa* subsp. *campestris*; B, E, H. *B. juncea*; C, F, I. *B. napus*. sc: stoma cell, ec: epidermal cell, tr: trichome. Bar = 100 μ m.

Results revealed from the present investigation on three *Brassica* species are discussed with previous studies on some other genera, i.e. *Ricotia* L. (Selvi and Paksoy, 2013) and *Chrysochamela* (Fenzl) Boiss. (Çakılcioğlu *et al.*, 2017) of the same family. In the leaf anatomy of our present studied species showed quite similarities with the species of *Ricotia* L. and

Chrysochamela (Fenzl) Boiss., viz. the epidermis of midrib and lamina is uniseriate and covered with thin cuticle, mesophyll is bifacial, spongy parenchyma is well developed with oval to circular cells with no intercellular space, and palisade parenchyma composed of cylindrical cells. Stomata are comparatively tiny and amphistomatic. In *Chrysochamela* (Fenzl) Boiss., the lower and upper epidermal cells of the middle vascular bundle are bigger than epidermal cells in the mesophyll layer (Çakilcioğlu *et al.*, 2017). However, in the studied *Brassica* species, the laminar epidermal cells are much bigger than the upper and lower epidermal cells of midrib. In the midrib, collateral type of vascular bundle (closed collateral) has been found in all the examined species of *Brassica* L.

According to Pandey (1978), number of xylem strands in the vascular bundle of root, is specific to species. As the studied species are found with tetrarch xylem (except, *B. juncea* which found with single strand xylem in the center) in root, it may be considered as an important character for distinguishing *Brassica* species. In our study, primary root structure has been found in three *Brassica* species. However, secondary root structure in some other genera of Brassicaceae has been noticed in previous studies (Orcan and Binzet, 2003; Tekin and Martin, 2017).

The present study on anatomical profiles of three *Brassica* species from Bangladesh is the first of its nature. Besides, other anatomical traits, viz. primary structure and tetrarch or single strand exarch xylem in root, size, shape and presence or absence of trichomes over stem, midrib shape of leaf are considered as distinctive characters for delimitation of the *Brassica* species investigated. Inclusion of additional species employing additional tools would throw more light on better understanding of species delimitation and interspecific relationships of the genus *Brassica*.

Acknowledgment

The authors are thankful to Bangladesh Agricultural Research Institute (BARI), Bangladesh Institute of Nuclear Agriculture (BINA) for providing the seeds of three species of *Brassica* used in this study.

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(Manuscript received on 20 October 2019; revised on 21 May 2020)