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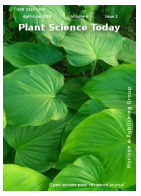

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Research Article

Seedling Morphology of some selected members of Commelinaceae and its bearing in taxonomic studies

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Abstract

Seedling morphology of eight species from four genera of the family Commelinaceae viz. *Commelina appendiculata* C.B. Clarke, *C. benghalensis* L., *C. caroliniana* Walter, *C. paludosa* Blume, *Cyanotis axillaris* (L.) D. Don ex Sweet, *C. cristata* (L.) D. Don, *Murdannia nudiflora* (L.) Brenan and *Tradescantia spathacea* Sw. are investigated using both light and scanning electron microscopy. The seedling morphological features explored include germination pattern, seed shape, surface and hilum, root system, cotyledon type, cotyledonary hyperphyll (apocole), cotyledonary hypophyll (cotyledonary sheath), hypocotyl, first leaf and subsequent leaves. All taxa studied had hypogeal and remote tubular cotyledons. However, differences in cotyledon structure (apocole, cotyledonary sheath), seed, hypocotyl, internodes, first leaf and subsequent leaves were observed. Variations of those characters were used to prepare an identification key for the investigated taxa. *Commelina* spp. and *Murdannia nudiflora* of the tribe Commelineae were found to differ from *Cyanotis* spp. and *Tradescantia spathacea* of tribe Tradescantieae in the petiolate first leaf with papillate margins on upper surface with 6-celled stomata and the glabrous epicotyl. The presence of an elongated cotyledonary sheath, long apocole and extended periblast region appear to be synapomorphies for *Commelina* spp. and *T. spathacea*. The affinity of the investigated taxa as revealed through multivariate analysis supports some of the relationships inferred by pollen morphology, floral morphology and DNA (rbc-L, 5S NTS, trnL-trnF) data stated by previous authors.

Keywords: Seedling morphology; Commelinaceae; Artificial key; Identification; Phylogeny.

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Introduction

The family Commelinaceae is small in comparison to other larger monocotyledonous families, like Orchidaceae and Poaceae (1). The family includes annual or perennial, erect, creeping or rarely climbing herbs and comprises 41 genera and 650 species distributed throughout the warmer parts of the world (2). The family has been extensively studied in the light of different botanical disciplines for a better understanding of the interrelationships and phylogeny of various taxa within the family. As such, Evans et al. (3,4) conducted a cladistic

Fig. 1. Diversity of seedling first leaves, **A** - *Commelina appendiculata* showing oblanceolate first leaf; **B** - *Commelina benghalensis* with elliptic first leaf; **C** - *Commelina caroliniana* showing narrowly elliptic first leaf; **D** – *Commelina paludosa* with elliptic-lanceolate first leaf; **E** - *Cyanotis axillaris* showing linear first leaf; **F** - *Cyanotis cristata* showing narrowly elliptic first leaf; **G** - *Murdannia nudiflora* with linear first leaf; **H** - *Tradescantia spathacea* showing oblanceolate first leaf.

analysis of the family based upon vegetative, reproductive and rbc-L (chloroplast DNA region) data sets but it does not include the seedling features. Even Woodson (5), Brenan (6) and Faden and Hunt (7) in their classification of the Commelinaceae did not consider the seedling characters. Seedling features are morphologically and genetically stable and may play a key role in distinguishing taxa (8). Readily available characters are the germination pattern, cotyledon type, shape and surface of the seed, cotyledonary hyperphyll, cotyledonary hypophyll, hypocotyl, first leaf as well as subsequent leaves. Tillich (9) and Paria (10) reported seedling structures as a key characteristic for systematic as well as phylogenetic studies.

The earlier work reported the Commelinaceae seedlings as hypogeal with the cotyledon consisting of a long sheathing base connected to the suctorial tip by a slender, thread-like stalk (11). The seed germination and development pattern of the seedlings of *Commelina virginica* reported few features of seedlings (12). Some of the Commelinaceae seedlings were treated as a rare type due to a long tubular cotyledonary sheath and the thread-like hyperphyll, *i.e.* apocole sharply bent down and elongated like the sheath (9,13). These reports revealed that the available data on the Commelinaceae seedlings are inadequate to get a clear idea on their complicated structure. As such further research in this line is necessary.

Materials and Methods

Seedling morphological characters from eight species belonging to four genera of Commelinaceae viz. *Commelina appendiculata* C.B. Clarke, *C. benghalensis* L., *C. caroliniana* Walter, *C. paludosa* Blume, *Cyanotis axillaris* (L.) D. Don ex Sweet, *C. cristata* (L.) D. Don, *Murdannia nudiflora* (L.) Brenan and *Tradescantia spathacea* Sw. occurring in natural habitats of Kolkata (India) were collected (Fig. 1) and studied in different developmental stages. These eight taxa have been considered due to availability of specimens with reference to their germination through seeds. Seeds of all these taxa were also collected from the identified adult plants and grown in the Experimental Botanic Garden of the Department of Botany, University of Calcutta. Voucher numbers, habitat and date of collection are provided in Table 1. The different developmental stages of each seedling taxon were considered. Seedlings raised in garden were compared to those collected from the field. No significant differences found between the garden raised and those from the wild collected. Ten to fifteen specimens of different developmental stages were studied for each taxon and taking from different habitats. Similar data on seedling morphology were noted in both the cases. The specimens were photographed, worked out, described and preserved in the form of herbarium sheets following the standard and modern herbarium techniques (14). The herbarium sheets are deposited in the Calcutta University Herbarium (CUH). The terminology proposed by Tillich (13) is used to describe the seedling morphological characters.

Table 1. Voucher table for the investigated species of Commelinaceae.

Taxa	Voucher Numbers	Locality (West Bengal, India)	Habitat	Date
Commelina appendiculata C. B. Clarke	*Bose 2126* (CUH)	Rajarhat, Kolkata	Pond side areas, Road side areas	24 Jun. 2013
	Bose 2232 (CUH)	Ballygunge, Kolkata	Natural vegetation in Ballygunge Science College	14 Jul. 2013
Commelina benghalensis L.	*Bose 2137* (CUH)	Rajarhat, Kolkata	Road side, pond side areas	24 Jun. 2013
	Bose 2226 (CUH)	Ballygunge, Kolkata	Rail line surroundings	14 Jul. 2013
	Bose 3379 (CUH)	Salkia, Howrah	River side areas	10 Apr. 2014
Commelina caroliniana Walter	*Bose 2115* (CUH)	Rajarhat, Kolkata	Small forest areas	24 Jun. 2013
	Bose 2224 (CUH)	Ballygunge, Kolkata	Rail line surroundings	14 Jul. 2013
	Bose 3376 (CUH)	Salkia, Howrah	River side areas	10 Apr. 2014
Commelina paludosa Blume	*Bose 2162* (CUH)	Sealdah, Kolkata	Rail line surroundings	02 Jul. 2013
	Bose 3817 (CUH)	Salt Lake, Kolkata	Natural vegetation in open land	02 Apr. 2015
Cyanotis axillaris (L.) D. Don ex Sweet	*Bose 2130* (CUH)	Rajarhat, Kolkata	Road side areas	24 Jun. 2013
	Bose 3821 (CUH)	Salt Lake, Kolkata	Natural vegetation in open land	02 Apr. 2015
Cyanotis cristata (L.) D. Don	*Bose 2237* (CUH)	Ballygunge, Kolkata	Natural vegetation in Ballygunge Science College	14 Jul. 2013
	Bose 4082 (CUH)	Dhakuria, Kolkata	Rail line surroundings	09 Jul. 2015
Murdannia nudiflora (L.) Brenan	*Bose 2123* (CUH)	Rajarhat, Kolkata	Road side, pond side areas	24 Jun. 2013
	Bose 3828 (CUH)	Salt Lake, Kolkata	Natural vegetation in open land	02 Apr. 2015
Tradescantia spathacea Sw.	*Bose 2234* (CUH)	Ballygunge, Kolkata	Natural vegetation in Ballygunge Science College	14 Jul. 2013
	Bose 3823 (CUH)	Salt Lake, Kolkata	Natural vegetation in open land	02 Apr. 2015

For SEM studies, matured first leaf of some selected taxa were dried following Barthlott (15), Barthlott et al. (16) with slight modifications. After complete drying the sample were mounted on aluminum stubs with double adhesive tape and coated with palladium gold. Selected materials were viewed under CARL ZEISS EVO 18 scanning electron microscope in the Centre for Research in Nanoscience and Nanotechnology, University of Calcutta.

Multivariate analysis was performed for 45 characters of seedling, seed and first leaf upper surface following average linkage method with correlation coefficient distance by using Minitab 17 software (17).

With such an idea the present study aims at screening seedling morphological characters in different stages of growth and development of eight species belonging to four genera of Commelinaceae with a view to understand their taxonomic affinity.

Results

***Germination and Cotyledon*:** The investigated seedlings reveal hypogeal and remote tubular germination pattern (Figs. 1A-H, 2A-X). According to Tillich (13) in remote germination a non-haustorial part of the cotyledonary hyperphyll (apocole) creates some distance between the seed and the sheath. In monocots the cotyledon is usually partly inside and partly outside the seed, these terms phanerocotylar and cryptocotylar in germination are hardly applicable to their seedlings. The term tubular is due to the structure of cotyledonary sheath or coleoptile, *i.e.* tubular. Tubular cotyledon is divided into two parts, cotyledonary hyperphyll and cotyledonary hypophyll. Cotyledonary hyperphyll is represented by apocole and haustorium. Apocole is the elongated white-coloured, thread-like, glabrous part of the cotyledon that buries the plumule in case of the hypogeal, remote germination. Haustorium is the suctorial organ of cotyledonary hyperphyll that remains inside the testa. Cotyledonary hypophyll is represented by cotyledonary sheath, *i.e.* the basal, always bifacial part of a cotyledon embracing the cotyledonary node and protecting the plumule.

Apocole is attached with the cotyledonary sheath and their attachment region is varied. Three different possible attachment of apocole with the apical, basal and middle region of the cotyledonary sheath is noticed among the taxa (Figs. 3A-H, 4). Size of apocole is identical for *C. appendiculata* and *C. benghalensis* as both have 5 – 15 mm length but apocole arises from middle region of the cotyledonary sheath in the earlier and apical region of the sheath in the later. In the other two lengths are *C. caroliniana* with 5 – 7 mm and *C. paludosa* with 5 – 8 mm but both of their apocole arises from middle region of the sheath. In *C. axillaris* apocole is very short, *i.e.* 1 – 2 mm, whereas in *C. cristata* it is 2 – 4 mm. *M. nudiflora* have identical length with *C. cristata*. These three taxa show basal region of apocole attachment with the sheath. In *T. spathacea* length is 8 – 20 mm and

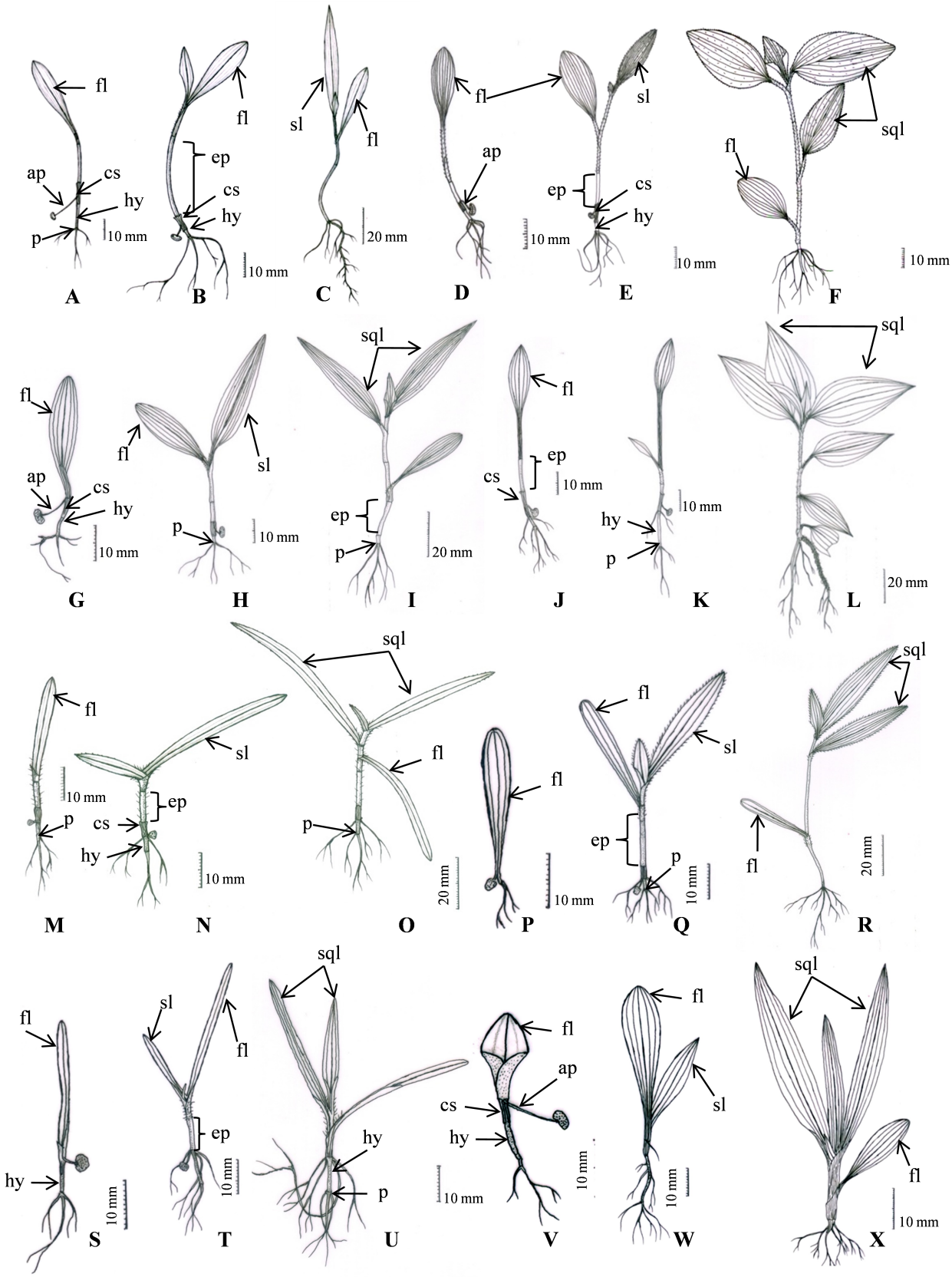

Fig. 2. Diversity of seedlings stages, **A-C**: *Commelina appendiculata* – **A**: first leaf stage, **B**: first leaf stage with initiation of second leaf, **C**: second leaf stage with initiation of third leaf; **D-F**: *Commelina benghalensis* – **D**: first leaf stage, **E**: second leaf stage, **F**: fifth leaf stage; **G-I**: *Commelina caroliniana* – **G**: first leaf stage, **H**: second leaf stage, **I**: third leaf stage with initiation of fourth leaf; **J-L**: *Commelina paludosa* – **J**: first leaf stage, **K**: first leaf stage with initiation of second leaf, **L**: sixth leaf stage; **M-O**: *Cyanotis axillaris* – **M**: first leaf stage, **N**: first leaf stage with initiation of second leaf, **O**: third leaf stage with initiation of fourth leaf; **P-R**: *Cyanotis cristata* – **P**: first leaf stage, **Q**: second leaf stage with initiation of third leaf, **R**: third leaf stage with initiation of fourth leaf; **S-U**: *Murdannia nudiflora* – **S**: first leaf stage, **T**: second leaf stage, **U**: third leaf stage; **V-X**: *Tradescantia spathacea* – **V**: first leaf stage, **W**: second leaf stage, **X**: fourth leaf stage. [ap-apocole, cs- cotyledonary sheath, ep-epicotyl, fl- first leaf, hy-hypocotyl, p- periblast, sl-second leaf, sql- subsequent leaves]

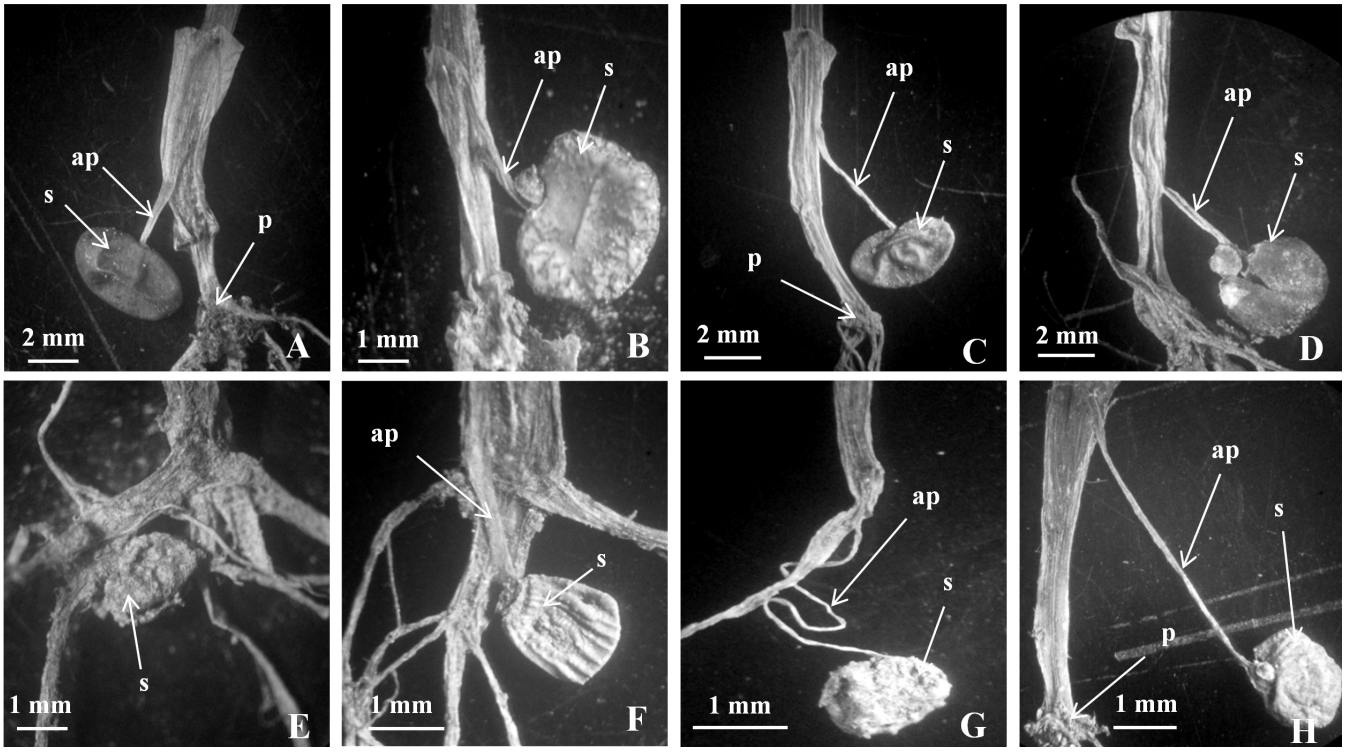

Fig. 3. Diversity of apocole attachment with cotyledonary sheath, **A** - middle region in *Commelina appendiculata*; **B** - apical region in *Commelina benghalensis*; **C** - middle region in *Commelina caroliniana*; **D** - middle region in *Commelina paludosa*; **E** - basal region in *Cyanotis axillaris*; **F** - basal region in *Cyanotis cristata*; **G** - basal region in *Murdannia nudiflora*; **H** - apical region in *Tradescantia spathacea*. [ap: apocole, s: seed, p: periblast]

apocole arises from apical region of the cotyledonary sheath. Cotyledonary sheath is tubular, membranous, long or short, with inconspicuous venation in the investigated taxa. Length of the sheath varies between 1 mm in *M. nudiflora* to 8.5 – 12 mm in *C. paludosa*. The respective length of sheath of the other taxa mentioned in Table 2. The apical and basal regions of the sheath are noticed with some differences in width of *C. appendiculata* and *T. spathacea*. Another significant observation is the presence of constriction at the apical region of the sheath, *i.e.* in *C. benghalensis*, *C. paludosa* and *T. spathacea.* The tubular cotyledon is followed by the manifestation of epicotyl (first internode), first leaf and subsequent leaves.

Root and Periblast: The development of root system is mostly dependent on growing conditions. The length of the roots may be characteristic, but observation is often difficult especially of the lateral ones. The position of collet may be distinct or uncertain. The primary or tap root is soft or hard, straight or flexuous. It directly develops from the radicle. Adventitious roots are borne by the collet as seen in most of the investigated Commelinaceae. In all the taxa, tap roots are short-lived, while persistent roots are adventitious. The taproot is mostly short and appearance of adventitious roots in the early stages of germination is prominent. Adventitious roots arise from the hypocotyl apex, periblast and from the lower nodes of seedling. Both short-living tap root and persistent adventitious roots are soft, fibrous, whitish, branched. Collet region is distinguishable in the investigated taxa. Considerable swelling of the collet region finally leading to a prominent disc-like structure is known as periblast (13). Periblast region is a circular disc-like structure so its width has been studied and mentioned in Table 2. Numerous adventitious roots normally cover the periblast region.

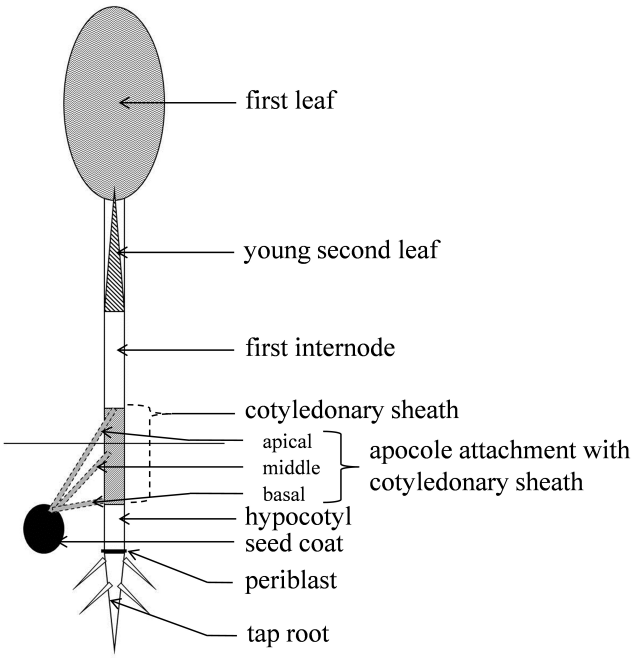

Fig. 4. Schematic diagram of Commelinaceae seedling.

***Seed*:** In the studied seedlings the seeds remain attached with the apocole up to second or third leaf stages, its features (shape, size, surface,

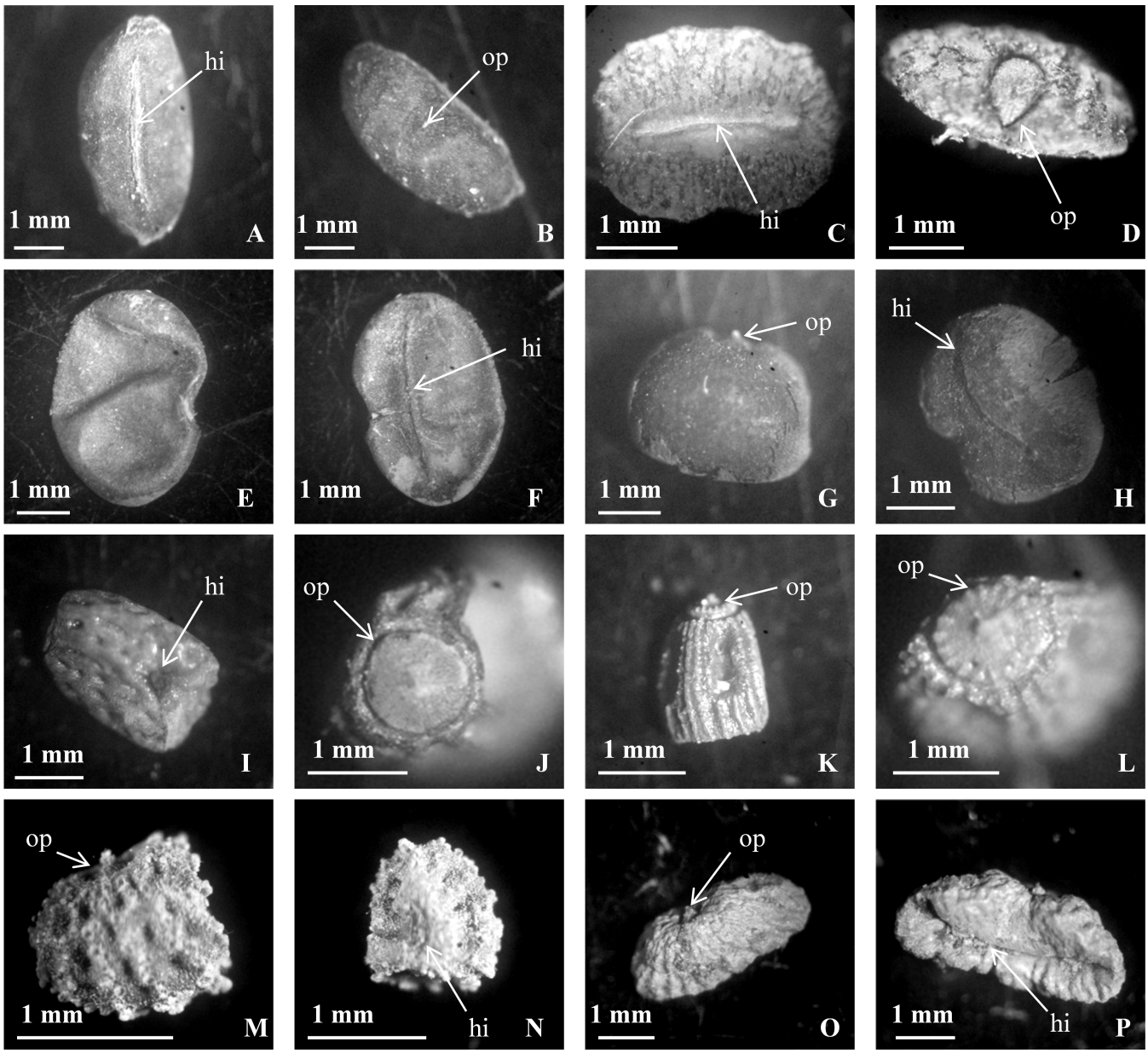

Fig. 5. Diversity of seeds, **A-B**: *Commelina appendiculata*; **C-D**: *Commelina benghalensis*; **E-F**: *Commelina caroliniana;* **G-H**: *Commelina paludosa;* **I-J**: *Cyanotis axillaris*; **K-L**: *Cyanotis cristata*; **M-N**: *Murdannia nudiflora*; **O-P**: *Tradescantia spathacea*. [hi: hilum, op: operculum]

operculum, hilum and colour) are considered under seedling characters (Fig. 5A-P; Table 3). Based on light microscopic (LM) studies, seeds of the investigated taxa reveal some promising identification characters. The four species of *Commelina* show four different types of seed shape. Oblong-cylindrical in *C. appendiculata,* oblong in *C. benghalensis,* ellipsoid in *C. caroliniana and* subglobose in *C. paludosa*. The surface pattern in *C. benghalensis* has ridges and furrows on the upper surface and a smooth lower surface, in contrast to the smooth upper and lower surfaces in the other three taxa. The two species of *Cyanotis* reveals oblong shaped seeds with upper and lower surfaces striate in between deep reticulations. *C. axillaris* with puncticulate surface is distinguishable from *C. cristata* having 1 – 4 circular pits. Seed of *M. nudiflora* is ovoid with upper and lower surface punctuate. *T. spathacea* have similar shape of seed with *C. appendiculata, i.e.* oblong-cylindrical, but their surface differs. Upper and lower surface of *T. spathacea* seed is irregularly striate. An interesting observation in the seeds of *C. appendiculata*, *C. caroliniana and M. nudiflora* is the presence of white farinose over their surface.

The seed size alone is of little taxonomic significance. There is random size distribution of seeds in the taxa studied. The size differences between the species of a genus vary within a narrow limit (Table 3). The largest seeds are noticed in *C. appendiculata* and *C. caroliniana* with size (length x breadth x width) 3.5 – 5 mm x 1.5 – 2 mm x 1.2 – 1.8 mm and 3 – 4.5 mm x 2 – 2.8 mm x 1.5 – 2 mm respectively. The smallest seeds are of *M. nudiflora* having size 1 – 1.2 mm x 0.9 – 1 mm x 0.5 – 0.6 mm.

Table 2. Seedling morphological data of the investigated species of Commelinaceae

Taxa Characters	***Commelina appendiculata* C.B.Clarke (*Figs. 1A, 2A-C, 3A, 4A-B*)**	***Commelina benghalensis* L. (*Figs. 1B, 2D-F, 3B, 4C-D*)**	***Commelina caroliniana* Walter (*Figs. 1C, 2G-I, 3C, 4E-F*)**	***Commelina paludosa* Blume (*Figs. 1D, 2J-L, 3D, 4G-H*)**	***Cyanotis axillaris* (L.) D.Don ex Sweet (*Figs. 1E, 2M-O, 3E, 4I-J*)**	***Cyanotis cristata* (L.) D. Don (*Figs. 1F, 2P-R, 3F, 4K-L*)**	***Murdannia nudiflora* (L.) Brenan (*Figs. 1G, 2S-U, 3G, 4M-N*)**	***Tradescantia spathacea* Sw. (*Figs. 1H, 2V-X, 3H, 4O-P*)**
Germination	hypogeal, remote	hypogeal, remote	hypogeal, remote	hypogeal, remote	hypogeal, remote	hypogeal, remote	hypogeal, remote	hypogeal, remote
Hypocotyl								
length (mm)	4 – 28	9 – 15	2 – 5	10 – 18	4 – 6	4 – 9	3 – 9	8 – 28
surface	glabrous	glabrous	glabrous	glabrous	glabrous	glabrous	glabrous	minutely hairy
Periblast (mm)	1 – 1.5	0.8 – 1.5	1 – 1.5	1.5 – 2	1.2 – 1.5	0.5 – 1	0.5 – 1.2	0.8 – 1.5
Cotyledon								
type	tubular	tubular	tubular	tubular	tubular	tubular	tubular	tubular
apocole	5 – 15 mm, from the middle region of the sheath	5 – 15 mm, from the apical region of the sheath	5 – 7 mm, from the middle region of sheath	5 – 8 mm, from the middle region of sheath	1 – 2 mm, from the basal region of the sheath	2 – 4 mm, from the basal region of the sheath	2.5 – 4 mm, from the basal region of the sheath	8 – 20 mm, from the apical region of the sheath
cotyledonary sheath	7 – 12 mm, apex slightly wider, no constrictions at apex	4 – 10 mm, apex and base equal, constrictions at apex	5 – 7 mm, apex and base equal, no constrictions at apex	8.5 – 12 mm, apex and base equal, constrictions at apex	2.5 – 3 mm, apex and base equal, no constrictions at apex	1.2 – 3 mm, apex and base equal, no constrictions at apex	1 mm, apex and base equal, no constrictions at apex	4 – 6 mm, apex slightly wider, constrictions at apex
Internodes	first – glabrous, subsequent - hairy	first – glabrous, subsequent - hairy	glabrous	glabrous	hairy	hairy	glabrous	hairy
First leaf petiolate/ sessile	petiolate	petiolate	petiolate	petiolate	sessile	sessile	petiolate	sessile
blade shape	oblanceolate	obovate / elliptic	narrowly elliptic	elliptic-lanceolate	linear	oblanceolate / narrowly elliptic	linear	oblanceolate / oblanceolate-ovate
size (mm)	30-40 x 7-10	13-33 x 7-16	24-38 x 6.5-11	25-31 x 7-9	40-50 x 4-5	23-42 x 2.5-4	26-50 x 2-3	33-51 x 12-15
apex	obtuse	obtuse / rounded	obtuse	obtuse / rounded	acute	obtuse / rounded	narrowly acute	obtuse / rounded
base	narrowly cuneate	cuneate	attenuate	cuneate	truncate	truncate	rounded	attenuate
surface	glabrous	glabrous	glabrous	glabrous	glabrous	glabrous	glabrous	glabrous
Subsequent leaves								
petiolate/sessile	petiolate	petiolate	petiolate	petiolate	sessile	sessile	sessile	sessile
blade shape	narrowly elliptic / lanceolate	ovate / lanceolate-ovate	oblong-lanceolate	lanceolate / elliptic lanceolate	linear	oblong / oblong lanceolate	linear	narrowly elliptic / lanceolate
size (mm)	50-65 x 8-15	20-55 x 7-23	46-82 x 7.5-15	40-90 x 9-25	50-70 x 5-6	44-90 x 5-16	35-65 x 3-5	94-105 x 12-16
apex	acute	acute	narrowly acute	acuminate	narrowly acute	acute	narrowly acute	narrowly acute
base	cuneate	obtuse	cuneate	cuneate	rounded	rounded	rounded	cuneate
surface	lightly hairy on the apical margin	Hairy	glabrous	glabrous	lightly hairy on the apical margin	hairy	glabrous	glabrous

Embryotega or operculum, a dehiscent cup of a seed that opens during germination is commonly found in the seeds of Commelinaceae. Shape of operculum is circular (ovate in *C. benghalensis*). Position of operculum is lateral in *Commelina* spp., *M. nudiflora* and *T. spathacea*, whereas in *Cyanotis* spp. position is terminal. Hilum is the attachment scar of the funicle. It refers only to the scar left on the seed by the stalk of the ovule. Hilum is linear and situated at the central region of the seed in *Commelina* spp., *M. nudiflora* and *T. spathacea* (in *C. paludosa*, hilum is slightly curved). Length of the hilum varies with the size of the seed. In *Cyanotis* spp. hilum is terminal, basal and punctiform. Characters of colour of seed have been utilised by different authors in describing different taxa (18,19). Colour of seed in the investigated taxa is normally a mixture of grey and brown colours.

***Hypocotyl*:** A very short tap root and adventitious roots longer than tap roots with short hypocotyl are present below the cotyledonary node. The hypocotyl is the basal portion of the ascending axis and consists of one internode only. It is situated between the cotyledonary node and the collet. Hypocotyl is a significant character as it is the primary organ of a seedling that faces the environmental consequences first. In species germinating in hypogeal manner the hypocotyl is much reduced in the embryo as well as in the seedling. Hypocotyl is mainly terete, erect (slightly curved in *C. appendiculata*), white (purple in *M. nudiflora*) in the investigated eight taxa. Length of hypocotyl varies between 2 – 5 mm in *C. caroliniana* to 8 – 28 mm in *T. spathacea*. Almost identical length of hypocotyl is noticed in *C. appendiculata, C. benghalensis and C. paludosa. Cyanotis* spp. and *M. nudiflora* shows shorter hypocotyl in comparison to others that varies between 3 – 9 mm. Surface of hypocotyl is glabrous in *Commelina* spp., *Cyanotis* spp. and *M. nudiflora* but in *T. spathacea* surface is minutely hairy.

***Internodes*:** Internodes are straight (sometimes curved in *C. caroliniana*), cylindrical and green. In studying internodes priority was given to first three ones. First internode is known as epicotyl. In the studied taxa it is found that epicotyl is shorter than the subsequent internodes and vice versa. In *C. benghalensis, C. paludosa, C. cristata* and *M. nudiflora* epicotyl is comparatively shorter then subsequent internodes. Whereas, epicotyl of *C. appendiculata, C. caroliniana and Cyanotis axillaris* is longer than the other internodes. Surface of internodes are hairy in the *Cyanotis* spp., *T. spathacea* and glabrous in *C. caroliniana, C. paludosa and M. nudiflora*. In *C. appendiculata* and *C. benghalensis* epicotyl is glabrous but subsequent internodes are hairy.

First leaf: First leaf is simple, petiolate (*Commelina* spp., *M. nudiflora*) or sessile (*Cyanotis* spp., *T. spathacea*) *and* sheathing. Petiole is mostly varied in *C. benghalesis* with 2.5 – 20 mm in length and hairy surface. In *C. appendiculata, C. caroliniana and C. paludosa* petiole is 5 – 8 mm, 5 – 7 mm and 15 – 18 mm, respectively with glabrous surface. In *M. nudiflora* petiole is short 2 – 3 mm. The sheath is hairy and oblique in all the eight taxa but their length differs. In *Commelina* spp. length varies between 8 – 15 mm, in *Cyanotis* spp. 3 – 5 mm and in *M. nudiflora* and *T. spathacea* 5 – 7 mm.

First leaf shape is much diversified as eight different types of shapes are noticed. And these definitely help their identification in the natural condition. In the four species of *Commelina* four different types of shapes are observed, *C. appendiculata* – oblanceolate (30-40 mm x 7-10 mm), *C. benghalensis* – obovate or elliptic (13-33 mm x 7-16 mm), *C. caroliniana* – narrowly elliptic (24-38 mm x 6.5-11 mm) and *C. paludosa* – elliptic-lanceolate (25-31 mm x 7-9 mm). In *C. axillaris* (40-50 mm x 4-5 mm) and *M. nudiflora* (26-50 mm x 2-3 mm) shape is linear. First leaf shape of *C. cristata* and *T. spathacea* is oblanceolate or narrowly elliptic (23-42 mm x 2.5-4 mm) and oblanceolate or oblanceolate-ovate (33-51 mm x 12-15 mm) respectively. Apex is obtuse in *C. appendiculata and C. caroliniana*; obtuse or rounded in *C. benghalensis, C. paludosa, C. cristata* and *T. spathacea*; acute in *C. axillaris*; narrowly acute in *M. nudiflora*. Similarly, base is cuneate in *C. benghalensis* and *C. paludosa;* narrowly cuneate in *C. appendiculata*; attenuate in *C. caroliniana* and *T. spathacea*; truncate in *Cyanotis* spp.; rounded in *M. nudiflora*. Margin of the eight different leaf types is solely entire, surface glabrous and venation pattern is exclusively parallelodromous. Colour of the first leaf on adaxial and abaxial surface is green in the investigated taxa except *T. spathacea*, where abaxial surface is purple.

Subsequent leaves: Subsequent leaves with green expanded blades after first leaf are developed. Subsequent leaves also bear significant characters as those of first leaf. Subsequent leaves in Commelinaceae are started from the second leaf stage up to the stage of the matured leaves. Subsequent leaves are petiolate (*C. appendiculata, C. benghalensis, C. caroliniana, C. paludosa*) or sessile (*Cyanotis* spp., *M. nudiflora*, *T. spathacea*), with alternate (*Commelina* spp., *Cyanotis* spp. and *M. nudiflora*) or spiral (*T. spathacea*) phyllotaxy. In *Commelina* spp., *C. cristata* and *T. spathacea*, subsequent leaves bear noticeable variations with the first leaf, but in *C. axillaris* and *M. nudiflora* first leaf and subsequent leaves are almost identical. In *C. appendiculata* shape of the leaves are narrowly elliptic to lanceolate (50-65 mm x 8-15 mm) with cuneate base, acute apex, entire margin (light hairy apical margin) and glabrous surface (Fig. 2A-C). *C. benghalensis* reveals ovate or lanceolate-ovate (20-55 mm x 7-23 mm) blade with obtuse base, acute apex, entire or wavy margin and hairy surface (Fig. 2D-F). Presence of oblong-lanceolate (46-82 mm x

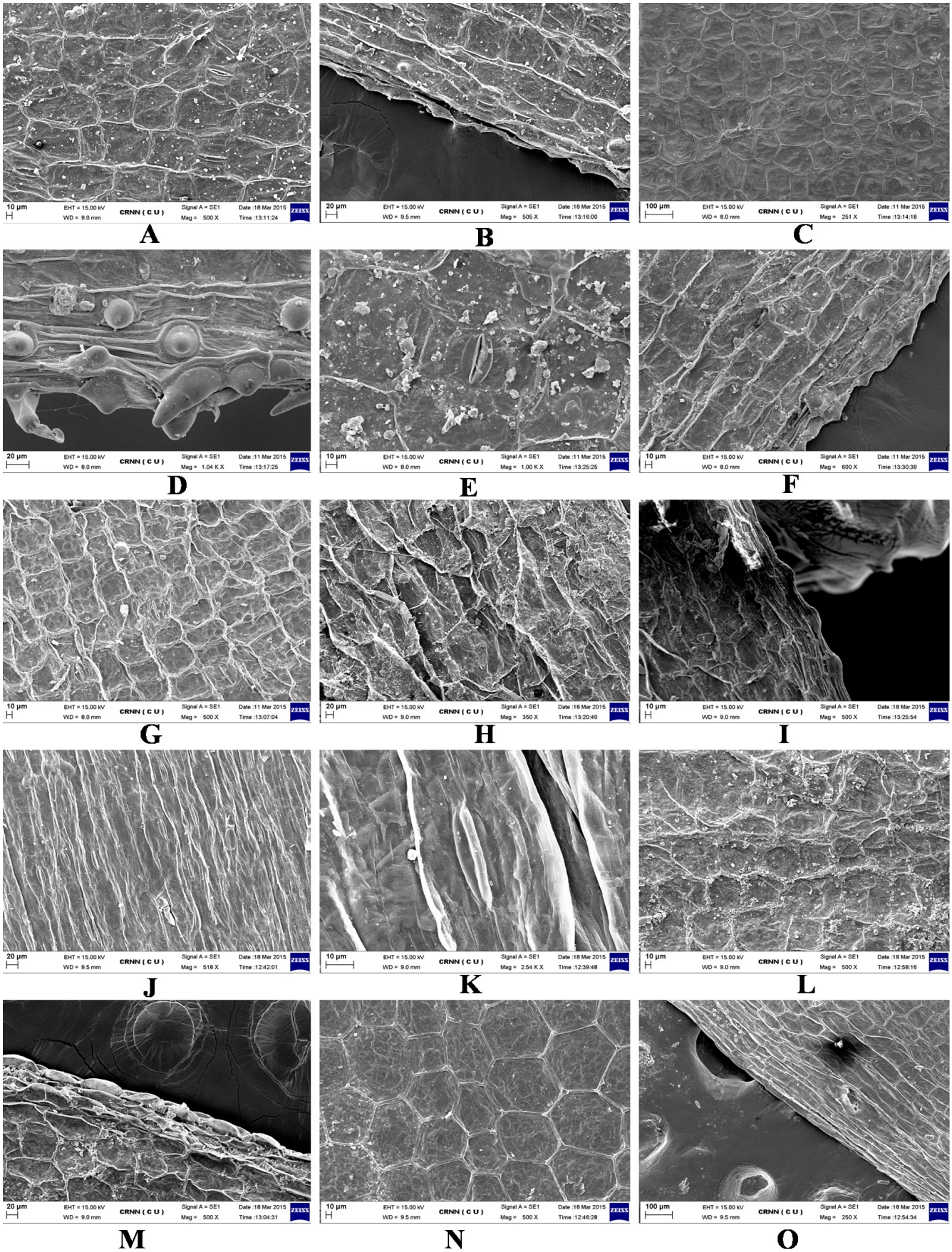

Fig. 6. SEM micrographs of upper epidermal surface of first leaf, **A,B**: *Commelina appendiculata* - **A**: hexagonal cells with 6 celled stomata (2 sls, 2 als, 2 ts), **B**: pointed papillate margin; **C,D**: *Commelina benghalensis* - **C**: hexagonal cells with 6 celled stomata (2 sls, 2 als, 2 ts), **D**: pointed papillate margin with 2 celled prickle hairs; **E,F**: *Commelina caroliniana* - **E**: polygonal cells with 6 celled stomata (2 sls, 2 als, 2 ts), **F**: pointed papillate margin; **G**: *Commelina paludosa* - rectangular cells with 6 celled stomata (2 sls, 2 als, 2 ts); **H,I**: *Cyanotis axillaris* - **H**: longitudinally extended rectangular cells with striae, **I**: margin without papillae; **J,K**: *Cyanotis cristata* - **J**: longitudinally extended rectangular cells with striae, and 2 celled stomata on longitudinal veins (2 ls), **K**: margin without papillae; **L,M**: *Murdannia nudiflora* - **L**: polygonal cells; **M**: papillate margin not pointed; **N,O**: *Tradescantia spathacea* - **N**: hexagonal cells, **O**: margin without papillae. [sls: small lateral subsidiary cells, als: additional lateral subsidiary cells, ts: terminal subsidiary cells].

7.5-15 mm) blade with cuneate base, narrowly acute apex, entire margin and glabrous surface is observed in subsequent leaves of *C. caroliniana* (Fig. 2G-I). For *C. paludosa* blade shapes are lanceolate or elliptic-lanceolate (40-90 mm x 9-25 mm) having cuneate base, acuminate apex, entire margin and glabrous surface (Fig. 2J-L). For *Cyanotis* spp. some interesting observations are noted. In *C. axillaris* subsequent leaves are almost identical with the first leaf, except size, apex, base and surface (Fig. 2M-O). Size is larger than first leaf, *i.e.* 50 – 70 mm x 5 – 6 mm with narrowly acute apex, rounded base and glabrous surface (light hairy apical margin). *C. cristata* shows noticeable variations in respect to first leaf. Its shape is oblong or oblong-lanceolate (44 – 90 mm x 5 – 16 mm), having acute apex, rounded base and hairy surface (Fig. 2P-R). *M. nudiflora* reveals identical features for both first leaf subsequent leaves (Fig. 2S-U). In *T. spathacea* subsequent leaves are narrowly elliptic or lanceolate (94 – 105 mm x 12 – 16 mm) with narrowly acute apex, cuneate base and glabrous surface (Fig. 2V-X).

Upper surface of First leaf under SEM: The first leaf has been given priority for SEM study as after germination this is the primary leaf which faces the environment. From the light microscope observation, it is found that in seven of the studied eight taxa the first leaf has some character differences in contrast to the subsequent leaves. So, the first leaf characters are conservative and after facing the environment the seedling plant develops some adaptive strategies that exposes through the changes in characters of subsequent leaves. Under SEM, upper surface of first leaf of the investigated taxa reveals some significant taxonomically useful characters (Table 4).

Primarily, the cuticular deposition is comparatively thick in *Cyanotis* spp. and *T. spathacea* in contrast to the other taxa. The epidermal cell arrangement is in longitudinal file in six taxa, while in *C. benghalensis* and *M. nudiflora* cells are not in longitudinal file. Striae are present within the longitudinal file of cells in *Cyanotis* spp. Shape of epidermal cells is rectangular in *C. paludosa, C. axillaris and C. cristata*; hexagonal in *C. appendiculata, C. benghalensis and T. spathacea*; and polygonal in *C. caroliniana* and *M. nudiflora*. Two types of stomata depending on number of subsidiary cells are found in the investigated four genera (20). *Commelina* spp. and *M. nudiflora* have six celled stomata, *i.e.* with 2 small lateral, 2 additional lateral and 2 terminal subsidiary cells. *Cyanotis* spp. and *T. spathacea* reveals 4 celled stomata, *i.e.* with 2 terminal and 2 lateral subsidiary cells. In *Cyanotis* spp., stomata are present on longitudinal veins. Stomatal apparatus is comparatively large in the *Commelina* spp., than in the other three genera. As in the investigated taxa guard cells length is between 43 – 61 µm in *Commelina* spp., 34 – 45 µm in *Cyanotis* spp., 37 – 41 µm in *M. nudiflora* and 36 – 38 µm in *T. spathacea*. Silica deposition is a regular phenomenon for the epidermal cells of Commelinaceae (20). Fine deposition of silica is found in *C. appendiculata and C. benghalensis*. In the other taxa coarsely or densely (*M. nudiflora*) deposition of silica is noticed. Within silica some waxy substances with non-articulate raphide canals are found in *C. paludosa*. Similarly, crystals and starch in addition to articulate raphide canals within silica are observed in *C. axillaris*. Marginal cells are longitudinally extended for all eight taxa. An interesting observation is the presence of papillae on the margin of leaves in some Commelinaceae (20). In this study, papillate margin is noticed in *Commelina* spp. and *M. nudiflora*. *C. benghalensis* leaf bears 2-celled prickle hairs on the margin. Outside region of papillae is pointed in *C. appendiculata, C. benghalensis*, *C. caroliniana* and blunt in *C. paludosa, M. nudiflora.* (Fig. 6A-X).

Artificial key (Valid for investigated taxa only)

1a. Cotyledonary sheath long, more than 3 mm; apocole arises from the apical or middle region of the sheath 2

1b. Cotyledonary sheath short, less than or equal to 3 mm; apocole arises from the basal region of the sheath 6

2a. First leaf petiolate, stomata 6-celled, margin papillate under SEM 3

2b. First leaf sessile, stomata 4-celled, margin not papillate ..... *Tradescantia spathacea*

3a. First leaf oblanceolate or obovate or elliptic, surface cells hexagonal; first internode glabrous, subsequent ones hairy 4

3b. First leaf narrowly elliptic to elliptic-lanceolate, surface cells polygonal or rectangular; internodes glabrous 5

4a. Subsequent leaves sessile, narrowly elliptic or lanceolate; first leaf margin glabrous .... *Commelina appendiculata*

4b. Subsequent leaves petiolate, ovate or lanceolate-ovate, first leaf margin hairy .... *Commelina benghalensis*

5a. Cotyledonary sheath with constriction at apex; hypocotyl 10 – 18 mm long; first leaf base cuneate ..... *Commelina paludosa*

5b. Cotyledonary sheath with no constriction at apex; hypocotyl 2 – 5 mm long; first leaf base attenuate ..... *Commelina caroliniana*

6a. First leaf surface cells in longitudinal files, rectangular longitudinally extended, stomata 2-celled, margin not papillate 7

6b. First leaf surface cells not in longitudinal files, polygonal, stomata 6-celled, margin papillate ..... *Murdannia nudiflora*

7a. First leaf linear, apex acute .... *Cyanotis axillaris*

7b. First leaf oblanceolate or narrowly elliptic, apex obtuse or rounded ..... *Cyanotis cristata*

Table 3. Seed morphological data of the investigated species of Commelinaceae

Taxa Characters	***Commelina appendiculata* C.B.Clarke (*Fig. 5A, B*)**	***Commelina benghalensis* L. (*Fig. 5C, D*)**	***Commelina caroliniana* Walter (*Fig. 5E, F*)**	***Commelina paludosa* Blume (*Fig. 5G, H*)**	***Cyanotis axillaris* (L.) D. Don ex Sweet (Fig. 5I, J)**	***Cyanotis cristata* (L.) D. Don (*Fig. 5K, L*)**	***Murdannia nudiflora* (L.) Brenan (*Fig. 5M, N*)**	***Tradescantia spathacea* Sw. (*Fig. 5O, P*)**
shape	oblong-cylindrical	oblong	ellipsoid	subglobose	oblong	oblong	ovoid	oblong-cylindrical
size (l x b x w) mm	3.5 – 5 x 1.5 – 2 x 1.2 – 1.8	2.5 – 3 x 1.5 – 2.2 x 0.8 – 1.2	3 – 4.5 x 2 – 2.8 x 1.5 – 2	2.5 – 3 x 2 – 2.5 x 1.8 – 2	1.8 – 2.5 x 1.2 – 1.4 x 1.8 – 2	1.2 – 1.5 x 0.8 – 1 x 0.6 – 0.8	1 – 1.2 x 0.9 – 1 x 0.5 – 0.6	3 – 4 x 1.4 – 1.8 x 1 – 1.5
surface	smooth with white farinose	upper-ridges-furrows and lower - smooth	smooth with white farinose	smooth with white farinose	striate, reticulations and puncticulate	striate, reticulate with 1 – 4 circular pits	striate, punctuate with white farinose	irregularly striate
operculum	lateral, circular	lateral, ovate	lateral, circular	lateral, circular	terminal, circular	terminal, circular	lateral, circular	lateral, circular
hilum (mm)	central, linear (2.5 – 3)	central, linear (1.1 – 1.9)	central, linear (2.5 – 3.1)	central, slightly curved (1.5- 2.1)	terminal, basal, punctiform	terminal, basal, punctiform	central, linear (0.2 – 0.3),	central, linear (2.5 – 3)
colour	grey brown	grey brown	brown	grey	grey brown	Brown	grey brown	grey

Table 4. Characters of upper surface of first leaf under SEM of the investigated species of Commelinaceae

Taxa Characters	***Commelina appendiculata* C.B.Clarke (*Fig. 6A, B*)**	***Commelina benghalensis* L. (*Fig. 6C, D*)**	***Commelina caroliniana* Walter (*Fig. 6E, F*)**	***Commelina paludosa* Blume (*Fig. 6G*)**	***Cyanotis axillaris* (L.) D.Don ex Sweet (*Fig. 6H, I*)**	***Cyanotis cristata* (L.) D. Don (*Fig. 6J, K*)**	***Murdannia nudiflora* (L.) Brenan (*Fig. 6L, M*)**	***Tradescantia spathacea* Sw. (*Fig. 6N, O*)**
Cell arrangement	longitudinal files	not in longitudinal files	longitudinal files	longitudinal files	longitudinal files	longitudinal files	not in longitudinal files	longitudinal files
shape	hexagonal	hexagonal	polygonal	rectangular	rectangular longitudinally extended	rectangular longitudinally extended	polygonal	hexagonal
Stomata cell no.	6 celled	6 celled	6 celled	6 celled	4 celled	4 celled	6 celled	4 celled
guard cell length	51 – 53 µm	43 – 50 µm	58 – 61 µm	43 – 46 µm	37 – 45 µm	34 – 41 µm	37 – 41 µm	36 – 38 µm
Silica deposition	finely	finely	coarsely	coarsely	coarsely	coarsely	densely	coarsely
Marginal cells	longitudinally extended	longitudinally extended	longitudinally extended	longitudinally extended	longitudinally extended	longitudinally extended	longitudinally extended	longitudinally extended
Margin	papillate	papillate, 2 celled hairs	papillate	papillate	not papillate	not papillate	papillate	not papillate
Papillae	pointed	pointed	pointed	blunt	---	---	blunt	---

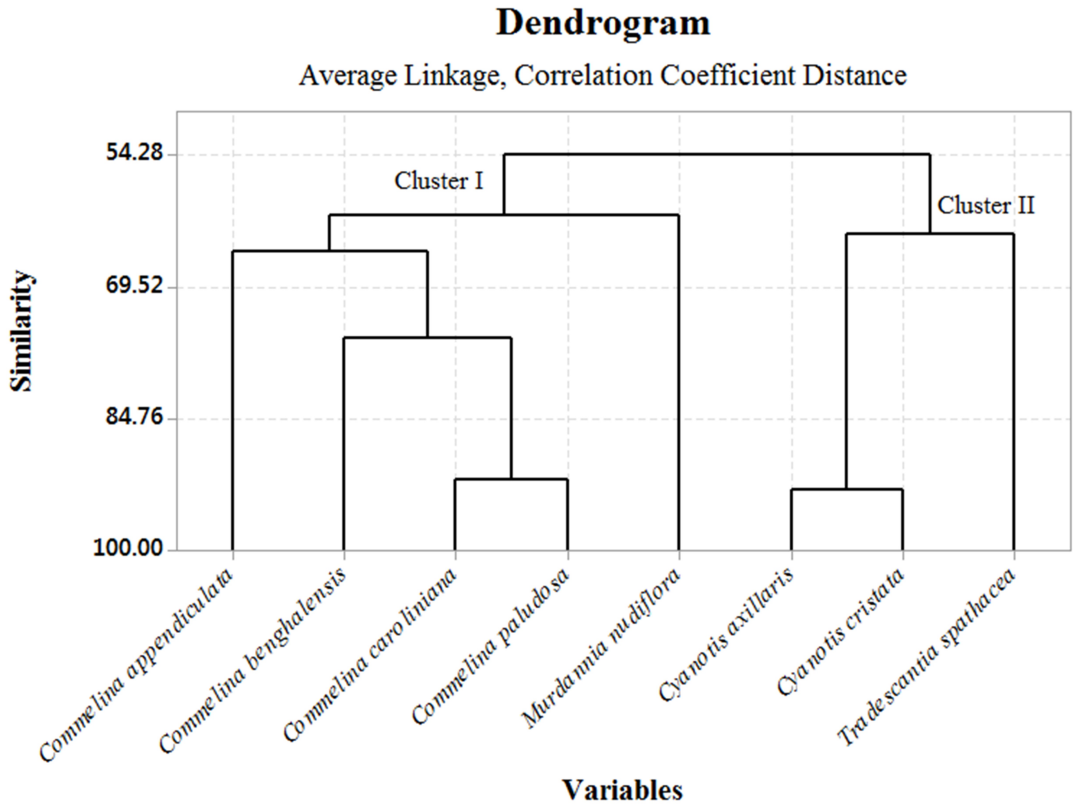

Fig. 7. Dendrogram of the eight investigated taxa (based on 45 seedling morphological characters following average linkage method with correlation coefficient distance).

Multivariate analysis: Multivariate analysis reveals a dendrogram (Fig. 7) that separates the investigated taxa into two clusters. Cluster I contains 5 taxa, of which *M. nudiflora* is sister to the four species of *Commelina*. Among the *Commelina* spp., *C. caroliniana* and *C. paludosa* are clustered together due to the similarities in seed, apocole, first leaf and subsequent leaves. *C. benghalensis* is related with *C. caroliniana* and *C. paludosa. C. appendiculata* is further related with the three species. *M. nudiflora* though in cotyledon structures is like the *Cyanotis* spp. but in the characters of hypocotyl, first leaf, subsequent leaves and upper surface of first leaf it is similar with *Commelina* spp. In cluster II, the two species of *Cyanotis*, *i.e. C. axillaris* and *C. cristata* exhibit strong relationship due to similarities in the characters of seed, hypocotyl, cotyledon, internode, first leaf, subsequent leaves and upper surface of first leaf. *T. spathacea* is represented as sister of *Cyanotis* spp. in the dendrogram.

Discussion

The present paper deals with the seedling morphology of eight species belonging to four genera of the family Commelinaceae. The seedlings are not so diversified in respect of germination pattern and cotyledon type, as hypogeal and remote germination with tubular cotyledon is the common pattern in the eight taxa. This result supported the view of Boyd (11) in having a uniform germination and seedling structure for the Commelinaceae. According to Tillich (13) remote germination is the development of seedlings at some distance from the seed. The term remote and admotive was introduced by Martius (21) to describe seedling types of palms. Tubular cotyledon is characterized by a non-haustorial part of the cotyledonary hyperphyll *(i.e.* apocole), long tubular cotyledonary sheath, or a secondary tubular elongation (coleoptile) produced as a tubular protective organ for the first plumular leaves (22). The tubular cotyledon is followed by the manifestation of hypocotyl, first internode, first leaf and subsequent leaves. In addition to that seed, root and periblast have also been characterized in this investigation. Character diversity is mainly concentrated in apocole, cotyledonary sheath, internodes, first leaf and subsequent leaves, which are summarised in Table 2, 3 and 4. As revealed in the artificial key, these features can be utilised as key characters to identify the investigated Commelinaceae at seedling stage.

The objective of the investigation is not only concentrated on mere characterization of the taxa but also put some insight on their phylogenetic trends depending on seedling characters. Phylogenetic observations has been carried out based on the comments by Tillich (9), that remote-tubular cotyledon, combined with a well-developed sheath, existence of adventitious roots and absence of cataphyll represents apomorphy whereas, long-lived, freely branching primary root is the ancestral condition and

reduction in branching, shortly elongated, small life-span primary root is rather derived. As such, the investigated eight taxa reveal hypogeal, remote germination pattern with tubular cotyledon. Remote germination is due to the cotyledon type, *i.e*., long thread-like apocole which keep the seed away from the seedling axis. Presence of apocole is advantageous as it helps in providing nutrient to the growing plumule. Presence of cotyledonary sheath is helpful in protecting the growing plumule, though its length may vary depending on the deepness of the seed inside the soil. Presence of apocole helps in effective assimilation of nutrient and maintaining balance of the growing seedling axis. Even an increase in the volume of root collar or collet region, *i.e.* periblast in Commelinaceae is also a derived state. Numerous adventitious roots and voluminous collar region, *i.e.* periblast not only increase the chance of successful early establishment but also function effectively in maintaining a firm contact between young seedling and substrate. In addition, cataphyll is also lacking in the investigated seedlings. However, presence of primary root and hypocotyl in all the investigated taxa represents the ancestral state. Though, primary root is small and short-lived and hypocotyl is also very short (length 2 – 28 mm), exhibiting a trend toward advancement. So, the Commelinaceae seedlings investigated here are advanced type as a result of their noteworthy adaptive capability in an environment. Presence of long apocole (5 – 20 mm), elongated cotyledonary sheath (4 – 12 mm) and prominent periblast region are synapomorphic for *Commelina* spp. and *T. spathacea*. Whereas short apocole (1 – 4 mm), small cotyledonary sheath (1 – 3 mm) and lack of periblast are comparatively plesiomorphic for *Cyanotis* spp. and *M. nudiflora*.

The dendrogram reveals that cluster I contains *Commelina* spp., *M. nudiflora* of the tribe Commelineae and cluster II includes *Cyanotis* spp., *T. spathacea* of the tribe Tradescantieae, *i.e.* according to the established classification of Commelinaceae by Faden and Hunt (7). Further, petiolate first leaf, glabrous epicotyl, papillate margins of upper surface of first leaf with 6-celled stomata (2 small lateral, 2 additional lateral and 2 terminal subsidiary cells) are diagnostic seedling characters for the investigated members of the tribe Commelineae and sessile first leaf, hairy epicotyl, 4-celled stomata (2 terminal subsidiary cells and 2 lateral subsidiary cells) of upper surface of first leaf with lack of papillae on the margin are specific for the members of the tribe Tradescantieae.

However, the segregation of *Commelina* spp., *M. nudiflora* of Commelineae and *Cyanotis* spp., *T. spathacea* of Tradescantieae in the dendrogram can be correlated with some previous investigations. In pollen morphology, Rowley (23) and, Poole and Hunt (24) reported spinulose exine, perforate tectum and tuberculate sulcus in pollen grains of *Commelina* spp. and *M. nudiflora*, whereas cerebroid tectum, coarsely granulate sulcus and lack of spine on exine region is noticed in genera *Cyanotis* and *Tradescantia.* In floral morphology, Evans et al. (3) stated well developed cincinnus peduncle and sterile inner antepetalous stamens in *Commelina* spp. and *M. nudiflora*, but in *Cyanotis* spp. and *T. spathacea* cincinnus peduncle is absent and antepetalous stamens are fertile. The close proximity of *Cyanotis* and *Tradescantia* in the dendrogram also confirms the cladistic analyses by rbc-L (chloroplast DNA region) data provided by Evans et al. (4) and 5S NTS (nuclear ribosomal DNA region), trnL-trnF (chloroplast DNA region) data provided by Burns et al. (25). So, a positive scenario about the interrelationship among the eight taxa is coming out from the correlation of the dendrogram with the above mentioned other botanical disciplines and Commelinaceae classification of Faden and Hunt (7).

Authors Contribution

AB carried out the collection of specimens, critical analysis of different seedling parts, drawing the different stages, LM and SEM photographs and herbarium preparation part. Whereas, NDP participated in the design of the study, preparation of artificial key, statistical work, involved in drafting and gave final approval of the version of the manuscript.

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Competing Interests

The authors have no conflict of interests.

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