

COMPARATIVE INVESTIGATION OF PALYNOLOGICAL CHARACTERS OF *CUSCUTA REFLEXA* AND FEW MEMEBERS OF CONVULVACEAE

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ABSTRACT

Cuscuta reflexa Roxb. belonging to family Cuscutaceae was explored for its pollen morphology and its pollen characters examined with light and scanning electron microscope were compared with six species belonging to family Convolvulaceae. Pollen samples were collected from Districts; Kotli, Bhimber and Mirpur, Azad Jammu & Kashmir, Pakistan. Flowers of *Cuscuta reflexa* Roxb., five species of *Ipomoea* including *Ipomoea carnea* Jacq., *Ipomoea cairica* (L.) Sweet, *Ipomoea hederacea* Jacq., *Ipomoea pes-tigridi* sL., *Ipomoea eriocarpa* R.Br., *Ipomoea arachnosperma* Welw. and *Convolvulus arvensis* L. were collected. Pollen shape of these selected species are generally prolate, subprolate, prolate-spheroidal, oblate spheroidal and perprolate. The apertures of the pollens of these taxa were found scabrate, scabrate-perforate, reticulate and echinate-reticulate. Among the examined taxa, *C. reflexa* Roxb. has smallest size of pollen (2-5×2.5-5µm) while the largest pollen (28.5-37.6 x 24.7-28.9µm) belongs to the *Ipomoea carnea*. It has been founded that pollen of *Cuscuta reflexa* Roxb. is prolate in shape and was similar to *I. carnea* Jacq, *I. pes-tigridis* L, *I. eriocarpa* R.Br pollens. Hierarchical cluster analysis based on pollens observations grouped *I. arachnosperma* and *Cuscuta refelexa* together at maximum similarity 99.86 at a distance level of 0.003. It has been concluded from this study that *Cuscuta refelexa* pollen are exactly similar to *Ipomea arachnosperma* on the bases of pollen morphology and exine appearance.

Keywords: Ipomea, Pollen, SEM, Cuscuta.

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INTRODUCTION

The significance of pollen morphology in the taxonomy of Convolvulaceae has been investigated by many workers (Rajurkar *et al.*, 2011). The morning glory family (Convolvulaceae) is a stunning family which is broadly cultivated for its ornamental values. In tropical and temperate regions almost 55 genera and 1930 species of the Convolvulaceae are extensively dispersed all through and abundant in tropical America and tropical Asia (Sunar *et al.*, 2012). *Ipomoea* is the key genus represented by 600 species (Judd *et al.*, 2002). In temperate region the family is, represented by the best-known weedy representatives, i.e. *Convolvulus* and *Calystegia*. A customary position of the family Convolvulaceae is in the order Solanales (Thorne, 1992). On the basis of molecular phylogenetic studies, it was suggested that family Convolvulaceae is closely related to the Montiniaceae and Solanaceae (Olmstead and Palmer 1992; Olmstead, 2004; Soltis *et al.*, 2000; Stefanovic *et al.*, 2003).

Palynology is the study of pollen and spores. The word palynology was first proposed by Hyde and William (1945). Today, little is known about the pollen morphology of *Cuscuta* species. Species diversity was

evaluated by studying the importance of pollen properties, palynological features such as spine strength spine length and aperture type in the discrimination of taxa. Shape of pollen ranges from spheroidal to prolate spheroidal (Reshmi and Rajalakshmi 2019). The palynological morphological characters are taxonomically important in identification and demarcation of species (Ullah *et al.*, 2018).

According to Welsh *et al* (2010), pollen morphology is used to find out the relationships of members of the Convolvulaceae family. Sudhama *et al.*, (2019) compared eight species of *Ipomea* on the bases of pollen morphology and described oblate spheroidal pollen shape spinose exine with pentaporate aperture. Parveen and Qaiser (2004) investigated eleven species of Cuscutaceae from Pakistan by the use of light and scanning electron microscope and founded three types of pollen shapes.

Convolvulaceae and Cuscutaceae families have characters that are common in genus *Ipomoea* and *Merremia*. This study was carried out to study the variation of selected genera from both families to indicate that whether these two families are homogeneous or heterogenous on the bases of pollen characters.

MATERIALS AND METHODS

Field Surveys and Collecting the Samples: This study was carried out during 2017-2018. Samples of flowers for the study of pollen morphology was collected during September and November when pollens were fully mature belonging to the genus *Cuscuta reflexa* Roxb.,

genus *Ipomoea* including *I. carnea* Jacq., *Ipomoea cairica* (L.) Sweet, *I. hederacia* Jacq., *I. pes-tigridis* L., *I. eriocarpa* R.Br., *I. arachnosperma* Welw. and one species of the genus *Convolvulus* i.e. *Convolvulus arvensis* L., from different geographical regions in district Kotli, District Bhimber and District Mirpur as indicated in the Flora of Pakistan (Davis, 1978) (Table1).

Table1. Geographic distribution of 7 taxa and their flowering period.

Taxon	Collection Site	Flowering period
<i>Ipomoea cairica</i> (L.)	District Bhimber	June-December
<i>I. carnea</i> Jacq.	District Kotli	July–November
<i>I. hederacia</i> Jacq.	District Bhimber	September–October
<i>I. pes-tigridis</i> L	District Bhimber	September–October
<i>I. eriocarpa</i> R.Br.,	District Bhimber	August–October
<i>Convolvulus arvensis</i>	District Kotli	All the year
<i>Cascuta reflexa</i> Roxb	District Mirpur	November-February
<i>I. arachnosperma</i> Welw	District Bhimber	August-November

Light microscopic (LM) and Scanning Electron Microscopic (SEM) characters: LM observations were made on fully mature pollens that were fixed on slide from an anther at anthesis stage, to mount pollen grains glycerine jelly was used and for staining purpose safranin was used. A drop of alcohol was added to remove the oily and resinous substances and then examined using oil emersion. The photograph was taken with Stereo zoom microscope IM-SC-500. Pollen were measured on the bases of equatorial diameter (E) Polar diameter (P) and sphericity (P/E ratio). Twenty pollen grains were considered for each measurement and the arithmetic mean was calculated for every diameter (Erdtman, 1952). Pollen size, was measured according to Walker & Doyle (1975) standards: minute grain <10µm; small grain 10-24 µm; medium grain 25-49µm; large grain 50-99 µm; very large grain 100-199 µm and gigantic grain <or=>200µm.

For scanning electron microscopy (SEM) pollen slides were prepared by using the technique of Jones & Pucci (2012). Pollen grains were suspended in a drop of 40% acetic acid and then transferred to clear metallic stub. Stubs were covered with double-stick tape and pollen grain were placed on to the stubs and samples were stored for 24 hours at 28–30°C. After all the samples were prepared, the stubs were coated with a thin layer of gold by a Polar on SC502 sputter coater. They were examined under the scanning electron microscope (Jeol JSM-840-A, Japan). To measure the size of the pollens equatorial axis and polar axis were considered by the measurement of 30 pollen grains per sample. Pollens were characterized in size as small (10-25 pm) or medium (25-50 pm) according to Erdtman (1952). The terminology used is mainly from Punt *et al.* (2007). Shape classification follows Erdtman (1969), based on

P/E ratios in the Table. The minimum and maximum range values and standard deviations (STD) of the measured grains were also determined.

Hierarchical cluster analysis of variables: Hierarchical cluster analysis of variables was performed on the basis of six observed variables (Polar length (µm±STD) Equatorial length (µm±STD) P/E ratio Exine thickness pm±STD Spine Width (pm) Spine length (pm) with the help of MINITAB Software and maximum similarity level is shown on X-axis and species on Y axis (Abdel Khalik, 2008).

RESULTS

General Morphology of the Flower of the Convolvulaceae: Flower in terminal or axillary simple or compound cymose inflorescence, bisexual mostly actinomorphic. Sepals 5, free corolla of 5 sympetalous stamens 5 epipetalous free anther 2 locular, carpel 2 rarely 3, ovary superior ovule 2, styles 2; stigma capitate or linear.

General Morphology of the Flower of the Cuscutaceae: Flower are arranged in panicle cyme white, pedicellate 5.5 to 7.5 mm long, bract leaf like calyx lobes 5, fleshy margin, corolla lobes 5, ivory white 1-1.5mm, corolla tube 2-3 mm long; scales 1mm long stamens five filamentous linear 2-3 mm long, anther oblong basifixed 1-1.5 mm long ovary conical fleshy, ovule 4, capsule 1-3mm globose style and stigma at the top.

Selected species of Convolvulaceae and Cuscutaceae were collected from District Kotli, Bhimber and Mirpur. Among the examined taxa, *C. reflexa* Roxb. has smallest size of pollen (2-5×2.5-5pm) while the largest pollen (28.5-37.6 x 24.7-28.9pm) belongs to the *Ipomoea carnea* (Fig.1). Pollen shape of these selected

species are generally prolate, subprolate, prolate-spheroidal, oblate spheroidal and perpolate.

Observations: Characteristics of pollens of selected species as observed under SEM and LM are summarized here;

***Cuscuta reflexa* Roxb:** Pollen shape of this species is prolate, (P/E 1.7), size; (2.5×2.5-5pm) categorized as small. Exine thickness is 0.53 pm, spine length 5pm spine 2.5 pm. Exine are scabrate perforate (Table 2; Fig.1).

***Ipomoea cairica* (L.) sweet:** Pollen shape was oblate-spheroidal, (P/E 0.96), size; (27.6–29.4×17.2–20.4 pm) categorized as medium. Exine thickness was 1.04 pm, exine spine width 2.5pm, spine length 5pm. Exine are scabrate (Table2; Fig.1).

***I. carnea* Jacq:** Pollen form of this taxon is prolate, (P/E.1.58), size; (28.5–37.6 x2 4.7–28.9pm) categorized as medium. Exine thickness 1.25 pm, spine width 3.75 pm, spine length 8.75 pm. Exine were reticulate (Table 2; Fig.1).

***I. hederacia* Jacq :** Pollen shape of this taxon was prolate- spheroidal, (P/E 1.02), size; (22.8×22.7 pm) categorized as medium. Spine width 2.5 pm, spine length 5pm. Exine are echinate reticulate (Table 2; Fig.1).

***I. pes-tigridis* L.:** Pollen shape of this taxon is prolate (P/E 1.82), size; (24.3-22.6 × 22.8-20.7 pm) categorized

as medium. Exine thickness 0.51 pm, spine width 1.25 pm, spine length 5 pm. Exine are echinate-reticulate (Table 2; Fig.1).

***I. eriocarpa* R.Br.:** Pollen shape of this taxon is prolate, (P/E 1.98), size: (24.8–30.3×17.1–18.4pm). Exine thickness 0.85 pm, spine width 2.5pm, spine length 3.75. Exine are markedly scabrate(Table2;Fig.1).

***Convolvulu sarvensis* L.:** Pollen shape is oblate spheroidal (P/E 0.94), size: (19.1-26.2×12.8-16.6 pm) categorized as small. Exine thickness 0.37 pm, spine width 2.5 pm, spine length 5pm, Exine are very distinctly scabrate (Table 2; Fig.1).

***I. arachnosperma* Welw:** Pollen shape of this taxon is oblate spheroidal (P/E 1.98), size; (24.8-30.3×17.1-18.4pm) catagorized as medium. Exine thickness 0.37pm, spine length 2.5 pm, width 1.5pm, spine length 2.5pm. Exine are scabrate (Table 2; Fig.1)

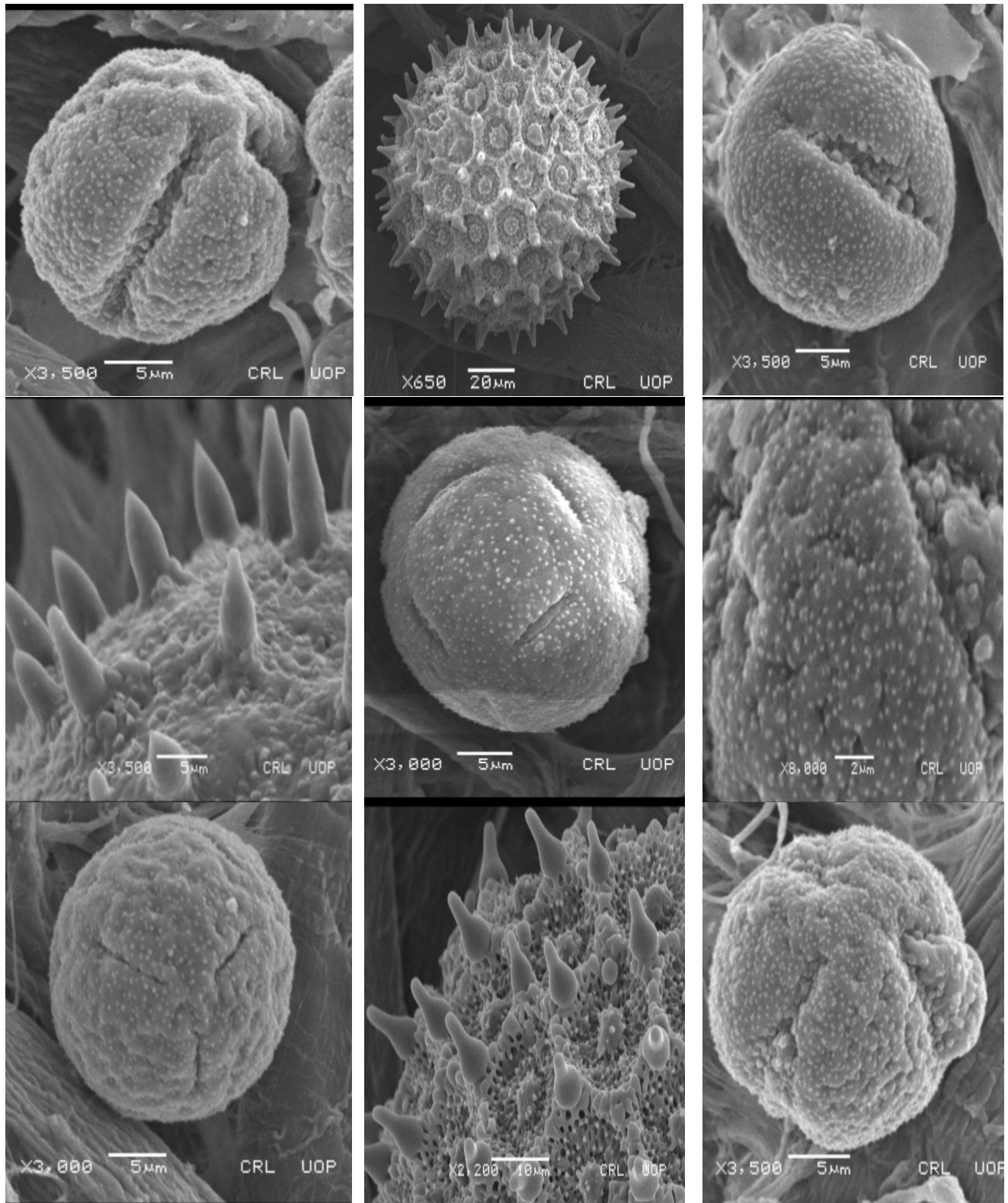
Hierarchical cluster analysis of variables: Hierarchical cluster analysis of variables was done on the basis of six observed variables (Polar length (µm±STD) Equatorial length (µm±STD) P/E ratio Exine thickness pm±STD Spine Width (pm) Spine length(pm) with the help of MINITAB Software maximum similarity observed was 99.86 at a distance level of 0.003 that is between *I. arachnosperma* and *Cuscuta refelexa* (Table3;Fig.2).

Table 2. LM Pollen morphological characteristics of the examined taxa.

Taxa	Polar length ($\mu\text{m}\pm\text{STD}$)	Equatorial length ($\mu\text{m}\pm\text{STD}$)	P/E ratio	Exine thickness $\mu\text{m}\pm\text{STD}$	Spine Width (μm)	Spine length (μm)	Shape
<i>Ipomoea cairica</i> (L.)	4.07	1.85	0.96	1.04	2.5	5	oblate-spheroidal
<i>I. carnea</i> Jacq.	3.33	4.81	1.58	1.25	3.75	8.75	prolate
<i>I. hederacea</i> Jacq.	6.897	4.65	1.02	1.095	1.25	6.75	prolate-spheroidal
<i>I. pes-tigridis</i> L	3.664	2.167	1.82	0.51	1.25	2.5	prolate-
<i>I. eriocarpa</i> R.Br.	7.45	3.06	1.98	0.85	2.5	3.75	prolate
<i>Convolvulus arvensis</i>	8.097	1.85	0.94	0.88	2.5	2.5	oblate-spheroidal
<i>Cascuta reflexa</i> Roxb	4.07	2.91	1.7	0.53-	1.25	2.5	Prolate
<i>I. arachnosperma</i> Welw	3.95	2.70	1.5	0.37	1.25	2.5	oblate-spheroidal

Table 3. SEM Pollen characteristics of the examined taxa.

Characters	Type	Length	Aperture width	Apex	Membrane	Perforated	Tectum	Pore		Sculpture echinase	Apex	Distribution
								shape	size			
<i>Ipomoea cairica</i>	Colpate	Long	Wide	Tapering	Granulated	Perforated	Circular	Circular	Coarse	regular	Acute	Regular
<i>I. carnea</i>	Colpate	Long	Wide	Tapering	Granulated	Perforated	Circular	Circular	Fine	regular	Acute	Regular
<i>I. hederacia</i>	Colpate	Long	Wide	Tapering	Granulated	Perforated	Circular	Circular	Coarse	regular	Acute	Regular
<i>I. pes-tigridis</i>	Colpate	Long	Wide	Tapering	Granulated	Perforated	Circular	Circular	Coarse	regular	Acute	Regular
<i>I. eriocarpa</i>	Colpate	Long	Wide	Tapering	Granulated	Perforated	Circular	Circular	Coarse	regular	Acute	Regular
<i>Convolvulus arvensis</i>	Colpate	Long	Wide	Tapering	Granulated	Perforated	Circular	Circular	Coarse	regular	Acute	Regular
<i>Cascuta reflex</i>	Colpate	Long	Wide	Tapering	Granulated	Perforated	Circular	Circular	Coarse	regular	Acute	Regular



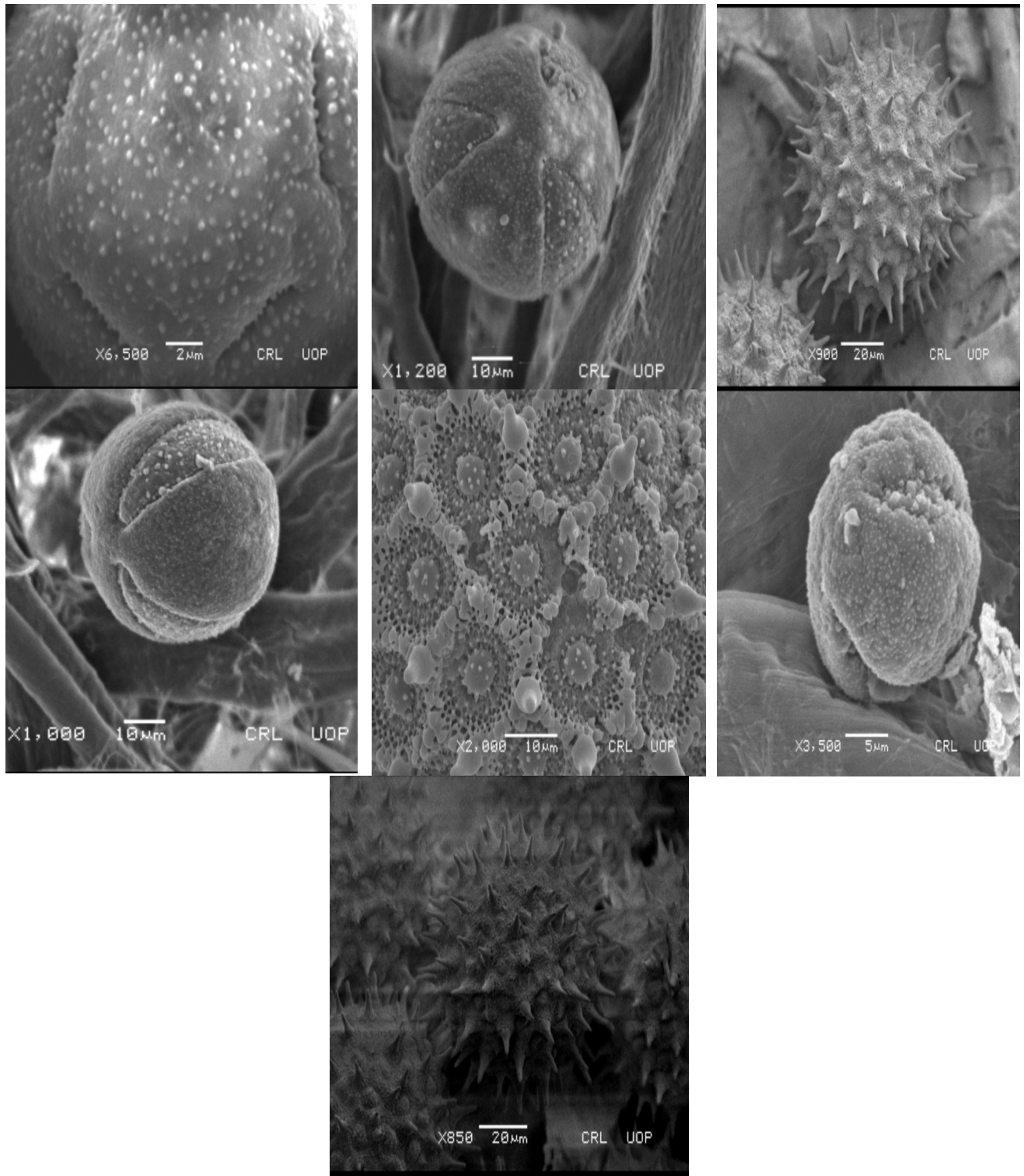


Figure 1. SEM pollen images of : A. *Cuscuta refelexa* a: equatorial view of pollens , B detail of exine showing the perforate, scarbate ornamentation; *Convolvulus arvensis* C: Equatorial view, D detail of exine showing the distinctly scarbate *Ipomoea carnea* E colpus, D: Exine reticulate *Ipomoea cairica* (L.) Sweet G equatorial view Exine scarbate; *I. hederacia* Jacq. I. equatorial view J detail of exine echinate reticulate; *I. eriocarpa* R.Br., K Equatorial view L detail of exine showing the perforate; *I. pes-tigridis* M equatorial view N exine echinate reticulate; *I. arachnosperma* Welw equatorial view P. Exine; markedly scarbate

Table 4. Hierarchical cluster analysis of variables.

Steps	No of Clusters	Similarity level	Distance level	Cluster joined		New cluster	No of observation in new cluster
1	7	99.86	0.003	7	8	7	2
2	6	98.90	0.022	4	7	4	3
3	5	98.11	0.038	5	6	5	2
4	4	97.01	0.060	4	5	4	5
5	3	94.17	0.117	3	4	3	6
6	2	92.96	0.147	1	3	1	7
7	1	90.22	0.196	1	2	1	8

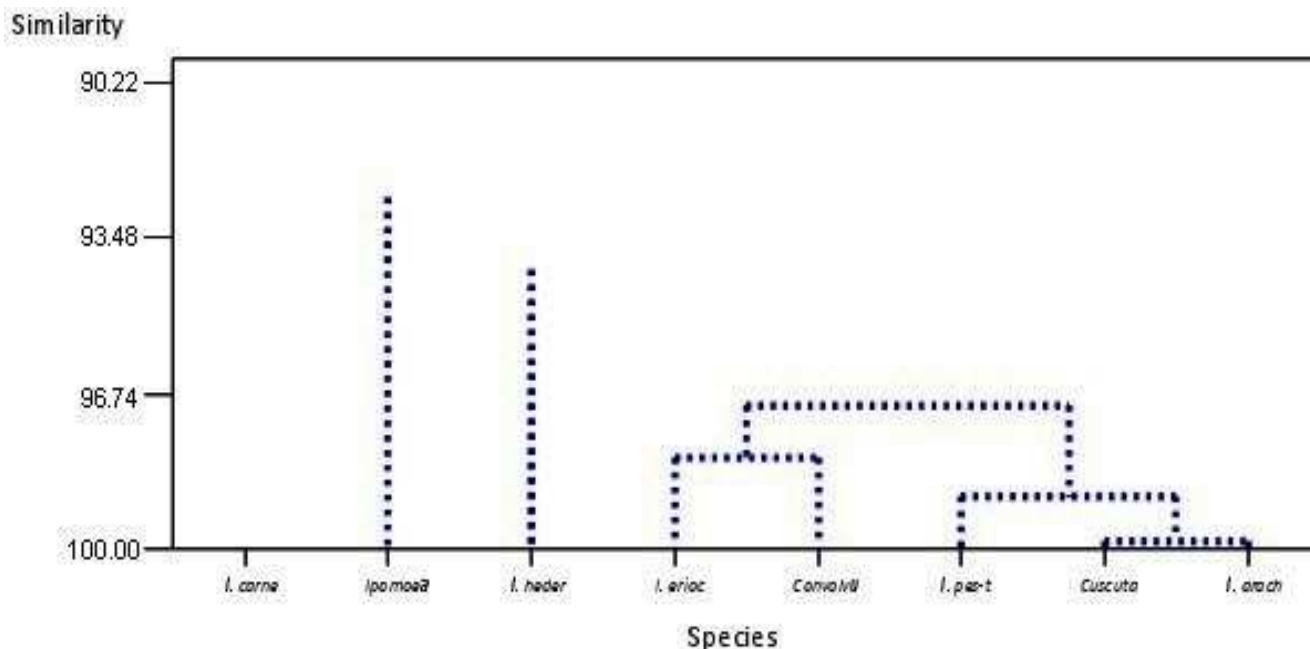


Figure 2. Hierarchical cluster analysis of variables was done on the basis of six observed variables (Polar length ($\mu\text{m}\pm\text{STD}$) Equatorial length ($\mu\text{m}\pm\text{STD}$) P/E ratio Exine thickness $\mu\text{m}\pm\text{STD}$ Spine Width (μm) Spine length (μm) similarity level is shown on X-axis and species on Y axis. Cluster 1 represents *Ipomoea carnea*, cluster 2 *Ipomoea cairica* (L.) Sweet cluster 3, *I. hederacia* Jacq. and *Cuscuta reflexa*, *Convolvulus arvensis*, *Lpes-tigridis*, *I. arachnosperma* are in cluster 4.

DISCUSSION

Palynological identification has its worth since long as found in literature for the multiple applications and uses of pollens by American botanist Wodehouse in 1935 (Wodehouse, 1935). Moreover, Swedish botanist Erdtman developed method of acetolysis for detailed study of pollen morphology and emphasized on their role for the taxonomic classification of taxa (Erdtman, 1952). Pollens are proved important for their use to recognize the vegetal organism which produce them.

This study was also planned to compare the palynological characters of species *Cuscuta reflexa* Roxb with selected species of Convolvulaceae. The pollen morphology of Convolvulaceae is known to be a highly

diverse and has a taxonomic importance (Telleria and Daners, 2003).

The present study noted echinate and pantoporate characters in two *Ipomoea* species i.e in *I. hederacia* and *Ipomea pestigridis*, these finding are exactly similar to that of Erdtman (1971) and Rao and Lee (1970) observations. In five species i.e *Ipomea cairica*, *Ipomea carnea*, *I. arachnosperma* *Convolvulus arvensis* exine are scabrate perforate ornamentation this was in accordance with the studies of Sudhama *et al* (2019). In *Convolvulus arvensis* pollen shape is oblate spheroidal this was in accordance with work of TeKin and Yilmaz (2016).

The smallest pollen grains are those of *Cuscuta reflexa* Roxb. size; (2-5×2.5-5 μm) categorized as small and largest pollen (28.5-37.6 x 24.7-28.9 μm) belongs to

the *Ipomoea carnea*. Pollen size of other taxa ranged between (19.1-26.2×12.8-16.6µm)-(27.6–29.4×17.2–20.4µm). Shape of pollen grains prolate-spheroidal, spheroidal, oblate-spheroidal, sub oblate to subprolate. Similar observations are reported by some other studies that pollen shapes of *Cuscuta* species are oblate, suboblate, subprolate, spheroidal, oblate-spheroidal, and prolate spheroidal (Lia *et al.*, 2000); Perveen and Qaiser, 2004; Welsh *et al.*, 2010).

Pollen observed in this study were colpate with colpi apertures. All the species distinguished on the basis of granulated pore membrane, exine structure and nature of pore. On the bases of pore, exine of *I. eriocarpa* R.Br., was perforated, the grains are perforate and pores are irregular in shape while in *I. hederacia* Jacq echinate reticulate. Based on Hierarchical cluster analysis of species maximum similarity was observed between *I. arachnosperma* and *Cuscuta reflexa* which was also reported by Abdel Khalik (2008).

Conclusion: On the basis of pollen morphology it has been founded that *Cuscuta* has close resemblance with *Ipomea* species. The pollens of these taxa are generally identified as oblate spheroidal prolate, subprolate, prolate spheroidal and perprolate. On the basis of our study it is concluded that genus *Cuscuta* placed in Convolvulaceae due to most of similarities with other genera because prolate in shape and was similar to *I. carnea* Jacq, *I. pestigridis* L., *I. eriocarpa* R.Br pollens as found by Sengupta (1972).

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