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THE SEED ATLAS OF PAKISTAN-IX. OROBANCHACEAE

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Abstract

Numerical analysis based on seed morphological characters of 38 taxa belonging to the family Orobanchaceae was carried out. Seed macro and micro-morphological characters were found useful to trace the phylogenetic relationship within the family Orobanchaceae. The seed morphological data fully support the placement of Scrophs genera viz., *Euphrasia, Leptorhabdos, Lindenbergia, Pedicularis* and *Striga* within the family Orobanchaceae.

Introduction

Orobanchaceae is the family of facultative or obligate root parasites (Plaza *et al*., 2004; Bennett & Mathew, 2006) and recognized as the biggest parasitic family amongst the angiosperms (Nickrent, 2006; Bennett & Mathew, 2006). The family Orobanchaceae comprises 96 genera and 2100 species distributed in Northern Hemisphere to tropics (Mabberley, 2008). In Pakistan it is represented by 7 genera viz., *Cistanche* Hoffm. & Link, *Euphrasia* L., *Leptorhabdos* Schrenk, *Orobanche* Linn., *Pedicularis* L., *Striga* Lour., *Lindenbergia* Lehm (Stevens, 2003). The family Orobanchaceae is closely related to the family Schrophulariaceae and the relationship between the parasitic and non parasitic genera of the family Scrophulariaceae and Orobanchaceae is evident by various reports (Boeshore, 1920; Kuijt, 1969; Weber, 1980; Stace, 1985; Minkin & Eshbaugh, 1989; Plaza *et al*., 2004). In contrast to these reports Hutchinson (1969), Takhtajan (1980) and Cronquist (1981) treated Orobanchaceae as a separate family which is believed to originate from the family Scrophulariaceae. The recent reports on molecular studies also strengthen the placement of Scrophularious genera within the family Orobanchaceae (Young *et al*., 1999; Olmstead *et al*., 2001; Bennett & Mathew, 2006). Recently Qaiser *et al*., (2011) described two new species of *Euphrasia* from Pakistan and treated the genus under Orobanchaceae. In the modern approach of plant systematics, the numerical taxonomy is recognized as a method to quantify the diversity and phylogenetic relationship in more comprehensive manner. Concerning to the seed morphology of the family Orobanchaceae few workers gave the attention (Zhang, 1990; Plaza *et al*., 2004; Shavvon & Mehrvarz, 2010) but no detailed report is available on the family Orobanchaceae. In the present studies seed morphological investigations of the family Orobanchaceae from Pakistan are carried out in order to find out additional micro-morphological characters of various taxa and also to strengthen their taxonomic status.

Material and Methods

Mature and healthy seeds of 38 taxa of the family Orobanchaceae were collected from the herbarium specimens. Mostly 10 plants/species and 10 seeds/plant were studied (Appendix-I) and examined under stereomicroscope (Nikon XN Model), compound microscope (Nikon type 102) and scanning electron microscope (JSM-6380A). For scanning electron microscopy dry seeds were directly mounted on metallic stub using double adhesive tape and coated with gold for a period of 6 minutes in sputtering chamber and observed under SEM. The terminology used is in accordance to Lawrence (1970), Radford (1974) and Stearn (1983) with slight modifications. Numerical analysis was carried out to recognize the relationship and dissimilarities of species within the family Orobanchaceae. Hierarchical clustering was performed by using Euclidean distance index with the computer package (IBM SPSS Statistics. Vol. 19). Each taxon was treated as operational taxonomic unit (OTU). Macro and micro-morphological characters of seeds viz., size, shape, colour and surface patterns were used. Characters were recorded as presence or absence and coded as 1 or 0 respectively (Tables 2, 3) and the average values of the quantitative characters viz., seed length and breadth were directly used (Tables 2, 3).

General seed characters of the family Orobanchaceae

Seeds 0.2-4 x 0.1-2mm, angular or non-angular, oblong, obovate, obliquely obovate, elliptic, obliquely elliptic, elliptic pyriform, transversally cuneate or deltoid, apex rounded, obtuse, acute, acuminate, oblique, truncate, obliquely truncate or beaked, base rounded, obtuse, cuneate, oblique, truncate, obliquely truncate or beaked, dark brown, light brown, greyish, dusty brown with or without white or creamy lines, greenish brown with or without dark brown spots, black, brown, yellowish brown, orange brown, creamy, yellow, green, whitish, greenish white, surface reticulate and reticulately foveate with or without foveolation or punticulation, reticulately sulcate, foveate, foveolate or reticulate, glebulate, ribbed along with verrucation, ridged, scalariform with or without murication or foveolation, undulately grooved, ruminate with alveolate, ruminately foveate, laterally reticulate foveate or lineate, hilum basal (Table 1; Figs. 1-10).

Represented by 7 genera viz., *Cistanche* Hoffm. & Link, *Euphrasia* L., *Leptorhabdos* Schrenk, *Lindenbergia* Lehm., *Orobanche* L., *Pedicularis* L., and *Striga* Lour.

Cistanche Hoffm. & Link.

Seeds 1-1.2 x 0.4-0.5mm, angular oblong, apex truncate, base obliquely truncate, black, surface reticulately sulcate, hilum basal (Table 1; Fig. 1A-B).

Represented by a single species viz., *Cistanche tubulosa* (Schrenk) Hook.f.

Table 1. Seed morphological characters of the family Orobanchaceae.

Name of taxa	Size (mm)		Shape	Apex	Base	colour	Surface	Hilum
	Length	Breadth						
Cistanche tubulosa	0.9-1	0.4-0.5	Angular, oblong	Truncate	Obliquely truncate	Black	Reticulate with sulcate	Basal
Euphrasia alii	1.4-1.5	0.4-0.5	Non-angular, elliptic	Acuminate	Obtuse	Yellow brown	Longitudinally ridged and scalariform along with foveolation	Basal
E. aristulata	1.2-1.5	0.4-0.8	Non-angular, obovate	Obtuse	Slightly truncate	Dark brown with creamy lines	Longitudinally ridged and scalariform along with densely muricate	Basal
E. densifolia	1.5-1.8	0.4-0.5	Non-angular, elliptic	Obliquely truncate	Obtuse	Dark brown with white line	Longitudinally ridged and scalariform along with muricate and laterally reticulate-foveate	Basal
E. foliosa	1.2-1.5	0.4-0.5	Non-angular, elliptic	Acuminate	Cuneate	Dark brown	Longitudinally ridged and scalariform along with foveolation	Basal
E. himalayica	1-1.2	0.4-0.5	Non-angular, elliptic	Obtuse	Obtuse	Dark brown with white lines	Longitudinally ridged and scalariform along with densely muricate	Basal
E. incisa	1.2-1.5	0.4-0.5	Non-angular, obliquely elliptic	Obtuse	Obtuse	Greyish-light brown with white lines	Longitudinally ridged and scalariform along with densely muricate	Basal
E. jaeschkei	1.5-1.8	0.6-0.8	Non-angular, elliptic	Obtuse-beaked	Obtuse	Light brown with white lines	Longitudinally ridged and scalariform along with densely muricate	Basal
E. kashmiriana	1.2-1.5	0.4-0.5	Non-angular, obliquely elliptic	Oblique	Cuneate	Greyish or dusty brown with white lines	Longitudinally ridged and scalariform along with densely muricate	Basal
E. kurramensis	1.5-1.8	0.5-0.8	Non-angular, elliptic	Obtuse	Beaked	Dark brown with white line	Longitudinally ridged and scalariform along with densely muricate	Basal
E. laxa	1-1.2	0.4-0.5	Non-angular, elliptic	Oblique	Cuneate	Dark brown with white line	Longitudinally ridged and scalariform along with densely muricate	Basal
E. paucifolia	1-1.2	0.4-0.5	Non-angular, elliptic	Obtuse	Obtuse	Dark brown with white line	Longitudinally ridged and scalariform along with foveolation	Basal
E. platyphylla	1-1.2	0.4-0.5	Non-angular, elliptic	Obtuse	Obtuse	Creamy	Longitudinally ridged and scalariform along with murication	Basal
E. remota	1.2-1.5	0.4-0.5	Non-angular, obliquely elliptic	Oblique	Obtuse	Creamy	Longitudinally ridged and scalariform along with densely muricate	Basal
E. schlagintweitii	1.2-1.5	0.6-0.8	Non-angular, oblong	Obtuse	Obliquely truncate	Dark brown	Longitudinally ridged and scalariform along with murication	Basal
Leptorhabdos parviflora	2.2-2.5	1.2-1.5	Non-angular, obliquely obovate	Obtuse	Oblique	Black	Transversally undulately grooved and transversally scalariform	Basal
Lindenbergia abyssinica	2.2-2.5	0.9-1	Non-angular, transversally cuneate	Rounded	Oblique	Black	Ruminate along with alveolate	Basal
L. indica	2.2-2.5	0.9-1	Non-angular, obovate	Truncate	Obtuse	Black	Ruminate along with irregularly alveolate	Basal
Orobanche aegyptiaca	0.2-0.3	0.1-0.2	Angular, obovate	Rounded	Cuneate	Orange brown	Reticulate	Basal

Table 1. (Cont'd.).

Name of taxa	Size (mm)		Shape	Apex	Base	colour	Surface	Hilum
	Length	Breadth						
O. alba	0.3-0.4	0.1-0.2	Angular, oblong	Truncate	Obliquely truncate	Black	Reticulate	Basal
O. amethystea	0.3-0.4	0.1-0.2	Angular, obovate	Rounded	Truncate	Dark brown	Reticulate	Basal
O. cernua var. *cernua*	0.4-0.5	0.1-0.2	Angular, obovate	Truncate	Obtuse	Light brown	Reticulately foveate	Basal
O. cernua var. *pseudo-clarkei*	0.4-0.5	0.1-0.2	Angular, obliquely obovate	Rounded	Oblique	Light brown	Reticulate	Basal
O. coelistis	0.3-0.4	0.1-0.2	Angular, obliquely obovate	Rounded	Oblique	Light brown	Reticulately reticulate	Basal
O. solmsii	0.2-0.3	0.1-0.2	Angular, obovate	Rounded	Obtuse	Greenish brown	Reticulately reticulate	Basal
O. stocksii	0.2-0.3	0.1-0.2	Angular, obovate	Rounded	Truncate	Black	Reticulately foveolate	Basal
Pedicularis bicornuta	2-2.2	0.9-1	Non-angular, obliquely elliptic	Acuminate	Obtuse	Green	Longitudinally ridged and reticulate along with foveolation	Basal
P. kashmiriana	3.8-4	1.8-2	Non-angular, obliquely elliptic	Acuminate	Rounded	Whitish	Longitudinally ridged and reticulate along with puncticulation, laterally lineate	Basal
P. multiflora	3.8-4	1.8-2	Non-angular, obliquely elliptic	Acuminate	Rounded	Greenish white	Longitudinally ridged and reticulate-foveate along with puncticulation, laterally lineate	Basal
P. pectinata subsp. *palans*	2.5-2.8	0.9-1	Non-angular, elliptic	Acuminate	Cuneate	Dark brown	Longitudinally ridged and reticulate along with puncticulation	Basal
P. punctata	1.5-2	0.8-1	Non-angular, elliptic	Obtuse	Obtuse	Black	Longitudinally ridged in upper portion reticulate	Basal
P. pycnantha	2.5-2.8	1.2-1.5	Non-angular, obovate	Beaked	Obtuse	Greenish brown with dark brown spots	Undulately ridged and ruminately foveate	Basal
P. pyramidata	1.8-2	0.9-1	Non-angular, elliptic	Acute	Cuneate	Brown with creamy lines	Longitudinally ridged and reticulate along puncticulation	Basal
P. stewartii	3.8-4	1.8-2	Non-angular, elliptic pyriform	Beaked	Truncate	Dusty brown	Longitudinally ridged and reticulate-foveate along with foveolation, laterally lineate	Basal
P. tenuirostris	3-3.2	1-1.2	Non-angular, elliptic pyriform	Beaked	Cuneate	Yellow	Longitudinally ridged and reticulate along with foveolation	Basal
Striga asiatica	0.2-0.3	0.1-0.2	Angular, elliptic-deltoid	Acute-rounded	Obliquely truncate-rounded	Dark brown	Ribbed, along with verrucation dorsally glebulate	Basal
S. euphrasioides	0.2-0.3	0.1-0.2	Angular, elliptic	Obliquely truncate	Obtuse-cuneate	Light brown	Ribbed, along with verrucation dorsally glebulate	Basal
S. lutea	0.2-0.3	0.1-0.2	Angular, obovate	Truncate	Cuneate	Dark brown	Ribbed along with verrucation dorsally glebulate	Basal

Table 2. List of characters, scored for cluster analysis for the taxa of Orobanchaceae listed in Table 1.

		Character description		
	1	Length of Seeds (mm)		
	2	Breath of Seeds (mm)		
Shape	3	Angular or Non-angular	Angular=1	Non-angular=0
	4	Obovate	Present=1	Absent=0
	5	Obliquely obovate	Present=1	Absent=0
	6	Elliptic	Present=1	Absent=0
	7	Obliquely elliptic	Present=1	Absent=0
	8	Elliptic pyriform	Present=1	Absent=0
	9	Transversally cuneate	Present=1	Absent=0
	10	Oblong	Present=1	Absent=0
	11	Deltoid	Present=1	Absent=0
Apex	12	Acute	Present=1	Absent=0
	13	Acuminate	Present=1	Absent=0
	14	Beaked	Present=1	Absent=0
	15	Obtuse	Present=1	Absent=0
	16	Rounded	Present=1	Absent=0
	17	Truncate	Present=1	Absent=0
	18	Oblique	Present=1	Absent=0
	19	Obliquely truncate	Present=1	Absent=0
Base	20	Cuneate	Present=1	Absent=0
	21	Beaked	Present=1	Absent=0
	22	Obtuse	Present=1	Absent=0
	23	Rounded	Present=1	Absent=0
	24	Oblique	Present=1	Absent=0
	25	Truncate	Present=1	Absent=0
	26	Obliquely truncate	Present=1	Absent=0
Colour	27	Black	Present=1	Absent=0
	28	Dark brown	Present=1	Absent=0
	29	Greyish-Light brown	Present=1	Absent=0
	30	Dusty brown	Present=1	Absent=0
	31	Yellow brown	Present=1	Absent=0
	32	Yellow	Present=1	Absent=0
	33	Orange brown	Present=1	Absent=0
	34	Greenish brown	Present=1	Absent=0
	35	Green	Present=1	Absent=0
	36	Greenish white	Present=1	Absent=0
	37	Creamy	Present=1	Absent=0
	38	White	Present=1	Absent=0
Surface	39	Ridged or Non ridged	Non ridged=0	Ridged=1
	40	Reticulate	Present=1	Absent=0
	41	Reticulately foveate	Present=1	Absent=0
	42	Reticulately foveolate	Present=1	Absent=0
	43	Reticulately reticulate	Present=1	Absent=0
	44	Reticulate along with foveolate	Present=1	Absent=0
	45	Reticulate along with punctuate	Present=1	Absent=0
	46	Reticulate with sulcate	Present=1	Absent=0
	47	Reticulate-foveate along with punctate	Present=1	Absent=0
	48	Reticulate-foveate along with foveolate	Present=1	Absent=0
	49	Scalariform along with foveolation	Present=1	Absent=0
	50	Scalariform along with muricate	Present=1	Absent=0
	51	Transversally undulately groove	Present=1	Absent=0
	52	Ruminate along with alveolate	Present=1	Absent=0
	53	Ruminately foveate	Present=1	Absent=0
	54	Ribbed or Non Ribbed	Ribbed=1	Non-ribbed=0

Table. 3. Data matrix of the family Orobanchaceae scored for 54 characters present in Table 2.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
Cistanche tubulosa	1	0.5	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Euphrasia alti	1.5	0.5	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
E. aristulata	1.5	0.8	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
E. densifolia	1.8	0.5	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
E. foliosa	1.5	0.5	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
E. himalayica	1.2	0.5	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
E. incisa	1.5	0.5	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
E. jaeschkei	1.8	0.8	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
E. kashmiriana	1.5	0.5	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
E. kurramensis	1.8	0.8	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
E. laxa	1.2	0.5	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
E. paucifolia	1.2	0.5	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
E. platyphylla	1.2	0.5	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
E. remota	1.5	0.5	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
E. schlagintweitii	1.5	0.8	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Leptorhabdos parviflora	2.5	1.5	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Lindenbergia abyssinica	2.5	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
L. indica	2.5	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Orobanche aegyptiaca	0.2	0.1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O. alba	0.3	0.1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O. amethystea	0.3	0.2	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O. cernua	0.4	0.2	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O. coelistis	0.3	0.2	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
O. solmsii	0.2	0.1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
O. stocksii	0.2	0.1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Pedicularis bicornuta	2.2	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
P. kashmiriana	4	2	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
P. multiflora	4	2		0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
P. pectinata subsp. *palans*	2.8	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
P. punctata	2	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. pycnantha	2.8	1.5	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
P. pyramidata	2	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
P. stewartii	4	2	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
P. tenuirostris	3.2	1.2	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Striga asiatica	0.2	0.1	1	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S. euphrasioides	0.2	0.1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S. lutea	0.2	0.1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Appendix-I. List of voucher specimens.

S. No.	Taxa	Collector, number and herbarium
1.	*Cistanche tubulosa*	Shoaib Ismail *s.n.* (KUH); A. Ghafoor & Steve M. Goodman 4422 (KUH); M. Qaiser & Ajmal Khan *s.n*. (KUH).
2.	*Euphrasia alii*	S. Omer & M. Qaiser 2367 (KUH).
3.	*E. aristulata*	R. R. Stewart 13957, 14020 (KUH); R.R. Stewart & E. Nasir 22028 (KUH); E. Nasir 2936 (KUH); S. Omer 309 (KUH).
4.	*E. densiflora*	S. Abedin & M. Qaiser 8891 (KUH).
5.	*E. foliosa*	R. R. & I. D. Stewart 2160A (RAW).
6.	*E. himalayica*	S. W. Khan *et al*., 328 (KUH).
7.	*E. incisa*	R. R. Stewart 24574, 18934 (RAW); R. R. Stewart & E. Nasir 22327 (RAW).
8.	*E. jaeschkei*	S. Omer & M. Qaiser 2367 (KUH); S. Abedin 8441 (KUH); S. M. SarwarAlam *s.n.* (KUH).
9.	*E. kashmiriana*	R. R. Stewart *s.n.* (RAW).
10.	*E. kurramensis*	S. Omer & M. Qaiser *s. n.* (KUH).
11.	*E. laxa*	R. R. & I. D. Stewart 18801A (KUH).
12.	*E. paucifolia*	G. R. Sarwar & S. Omer 229 (KUH).
13.	*E. platyphylla*	S. W. Khan 815, 924 (KUH).
14.	*E. remota*	R. R. Stewart 22847, 22970 (RAW); S. Omer & M. Qaiser 2325, 2353, 2361 (KUH).
15.	*E. schlagintweitii*	R. R. Stewart & E. Nasir 24274 (RAW); A. Rashid Khan *s. n.* (KUH); R. R. Stewart 8434 (RAW); S. Omer 232 (KUH).
16.	*Leptorhabdos parviflora*	S. W. Khan 782(A) (KUH); Haider Ali 2511, 2818 (KUH); Jan Alam & Noor Ali 3803 (KUH).
17.	*Lindenbergia abyssinica*	Jan Alam & Saleem 4176 (KUH).
18.	*L. indica*	K. A. Malik & S. Nazimuddin 1039 (KUH); M. Qaiser *et al.* 7154, 7213 (KUH); S. Abedin 3813 (KUH); S. I. Ali *et al s. n.* (KUH); A. Ghafoor &Tahir Ali 3626 (KUH).
19.	*Orobanche aegyptiaca*	S. M. H. Jafri 2884 (KUH); S. A. Farooqi & M. Qaiser 2341 (KUH); S. Abedin & A. Hussain 6893, 6949 (KUH), S. Omer *et al* 2035 (KUH).
20.	*O. alba*	Jan Alam & M. S. Islam 520 (KUH); S. W. Khan *s.n*. (KUH)
21.	*O. amethystea*	S. Omer *et al*., 1060 (KUH); M. Qaiser *et al* 8571 (KUH)
22.	*O. cernua* var.*cernua*	Jan Alam & Naeem Khan 2750 (KUH); Jan Alam & Mir Alam 2278 (KUH); Jan Alam & Nasir Alam 4089 (KUH); Jan Alam 2263 (KUH)
23.	*O. cernua* var. *pseudo-clarkei*	T. Ali & G. R. sarwar 2561 (KUH)
24.	*O. coelistis*	Jafri & Akbar 2194A (KUH)
25.	*O. solmsii*	Sher Wali Khan &Shabbir Hussain 664 (KUH)
26.	*O. stocksii*	A. Ghafoor & Steve M. Goodman 5177, 5236 (KUH); S. Abedin 3342 (KUH).
27.	*Pedicularis bicornuta*	Jan Alam & Fazal Karim 1335, 1394 (KUH); Jan Alam & R. Karim 2415 (KUH).
28.	*P. kashmiriana*	M. Qaiser & A. Ghafoor 5391 (KUH); Jan Alam 2144A (KUH).
29.	*P. multiflora*	Tahir Ali *et al*. 556 (KUH).
30.	*P. pectinata* subsp. *palans*	Sher Wali Khan 440, 504 (KUH).
31.	*P. punctata*	Jan Alam & Fazal Karim 1305, 1546 (KUH); S. Abedin 3425 (KUH).
32.	*P. pycnantha*	Haider Ali 3909, 5357, 5688 (KUH).
33.	*P. pyramidata*	*Coll. ignot. s. n.* (KUH).
34.	*P. stewartii*	R. R. Stewart *s. n*. (KUH).
35.	*P. tenuirostris*	M. Qaiser & A. Ghafoor 5222 (KUH); S. Omer & M. Qaiser 2747 (KUH).
36.	*Striga asiatica*	A. Rashid 26981 (RAW); Jafri 1144 (KUH); A. Ghafoor &Tahir Ali 4332 (KUH).
37.	*S. euphrasioides*	A. Ghafoor & Tahir Ali 3792 (KUH); R. R. Stewart 16211 (RAW); S. Abedin 4035 (KUH); S. A. Farooqi & M. Qaiser 2276 (KUH).
38.	*S. lutea*	R. R. Stewart 14443, 21265 (RAW).

Key to the genera

1 + Seeds 0.2-0.5mm long ……..……..……... 2
- Seeds 1-4mm long ……..……..…… 3
2 + Seed surface ribbed with verrucation ..……..……..…..……... *Striga*
- Seed surface reticulate, reticulately foveate, foveolate or reticulate ....……..... *Orobanche*
3 + Seeds ridged ……..……...…….. 4
- Seeds non ridged ……..……...…….. 5
4 + Seeds scalariform ……..…… *Euphrasia*
- Seeds reticulate or ruminately foveate ……..…….. *Pedicularis*
5 + Seeds obliquely obovate, obovate, transversally cuneate ……..……..... 6
- Seeds oblong ……..……..... *Cistanche*
6 + Seed surface grooved ..……..……..... *Leptorhabdos*
- Seed surface ruminately alveolate ……..……..……..……. *Lindenbergia*

Euphrasia L.

Seeds 1.2-1.8x0.4-0.8mm, non-angular, elliptic, obliquely elliptic, obovate and oblong, apex acuminate, obtuse, beaked, obliquely truncate or oblique, base cuneate, obtuse, beaked, obliquely truncate or truncate, creamy, greyish, dusty brown, yellow brown, greyish brown, light brown, or dark brown with or without creamy or white lines, surface longitudinally ridged and scalariform along with foveolation or murication, laterally reticulate-foveate, hilum basal (Table1; Figs. 1 C-R, 2 A-N).

Represented by 14 species viz., *Euphrasia alii* Qaiser & T. Siddiqui, *E. aristulata* Penn., *E. densiflora* Penn., *E. foliosa* Penn., *E. himalayica* Wettst., *E. incisa* Penn., *E. jaeschkei* Wettst., *E. kashmiriana* Pugsley, *E. kurramensis* Penn., *E. laxa* Penn., *E. paucifolia* Wettst., *E. platyphylla* Penn., *E. remota* Penn. and *E. schlagintweitii* Wettst.

Key to the species

1 + Seeds oblong or obovate ……..... 2
- Seeds elliptic or obliquely elliptic …… 3
2 + Seeds obovate, base slightly truncate ……..…….. *E. aristulata*
- Seeds oblong, base obliquely truncate …… *E. schlagintweitii*
3 + Seeds elliptic ……...…….. 4
- Seeds obliquely elliptic ……... 12
4 + Seed surface scalariform along with foveolation …….. 5
- Seed surface scalariform along with murication ……..……..…… 7
5 + Seeds dark brown, or yellow brown apically acuminate …..…… 6
- Seeds dark brown with white lines, apically obtuse …….. *E. paucifolia*
6 + Seeds yellow brown, base obtuse ……..……..... *E. alii*
- Seeds dark brown, base cuneate …….. *E. foliosa*
7 + Seed apex obtuse or beaked ……..... 8
- Seed apex oblique or obliquely truncate ……..…… 11
8 + Seed base obtuse ……...…… 9
- Seed base beaked ……..……..…… *E. kurramensis*
9 + Seeds creamy ……...……. *E. platyphylla*
- Seeds brown ……..……... 10
10 + Seeds light brown …… *E. jaeschkei*
- Seeds dark brown ……..…… *E. himalayica*
11 + Seeds c. 1.5-1.8mm long, apex obliquely truncate, base obtuse ……..... *E. densiflora*
- Seeds c. 1-1.2mm long, apex oblique, base cuneate ……..……... *E. laxa*
12 + Seeds apically oblique ……..……... 13
- Seeds apically obtuse ……..……..…. *E. incisa*
13 + Seeds dusty brown with white lines, base cuneate …… *E. kashmiriana*
- Seeds creamy, base obtuse ……..……..…. *E. remota*

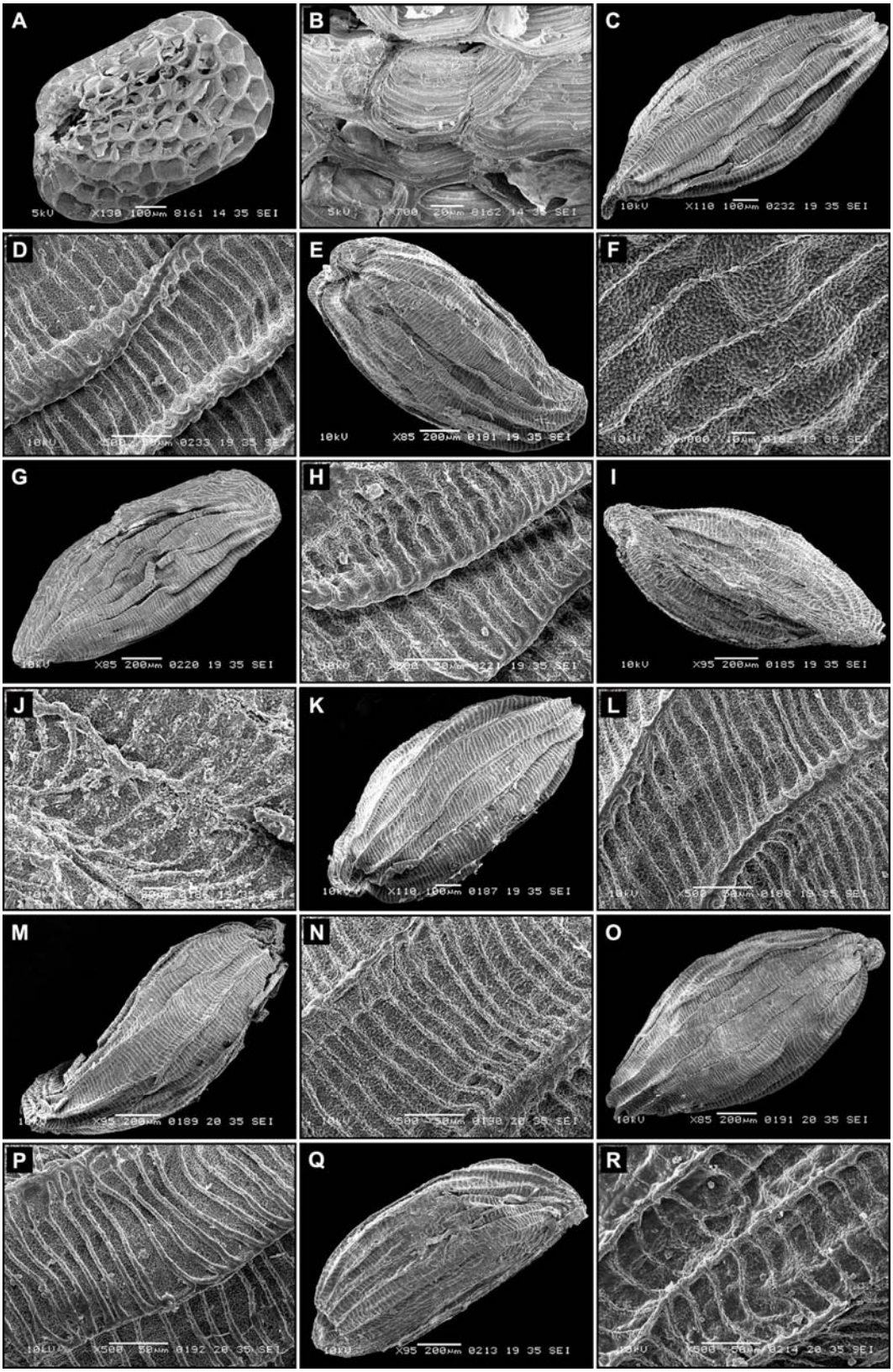

Fig. 1. Scanning electron micrographs. *Cistanche tubulosa*: A, seed; B, surface. *Euphrasia alii* : C, seed; D, surface. *E. aristulata*: E, seed; F, surface. *E. densiflora*: G, seed; H, surface. *E. foliosa*: I, seed; J, surface. *E. himalayica*: K, seed; L, surface. *E. incisa*: M, seed; N, surface. *E. jaeschkei*: O, seed; P, surface. *E. kashmiriana*: Q, seed; R, surface. (Scale bars: E, G, I, M, O, Q= 200µm; A,C, K= 100µm; D, H, L, N, P, R= 50µm; B, J= 20µm; F= 10 µm).

Leptorhabdos Schrenk.

Seeds 2.2-2.5x1.2-1.5mm, non-angular obliquely obovate, apex obtuse, base oblique, black, surface transversally undulately grooved and transversally scalariform, hilum basal (Table 1; Fig. 2 O-P).

Represented by a single species viz., *Leptorhabdos parviflora* (Bth.) Bth.

Lindenbergia Lehm.

Seeds 2.2-2.5x 0.9-1mm, non-angular transversally cuneate or obovate, apex truncate or rounded, base oblique or obtuse, black, surface ruminate along with alveolate or irregular alveolate, hilum basal (Table1; Figs.2 Q-R, 3 A-B).

Represented by 2 species viz., *Lindenbergia abyssinica* Hochst. ex Bth. and *L. indica* (L.) Vatke

Key to the species

1 + Seeds tranversaly cuneate, apically rounded, base oblique ….....…… *L. abyssinica*
- Seeds obovate, apically truncate, base obtuse ……..……..… *L. indica*

Orobanche Linn.

Seeds 0.2-0.4 x 0.1-0.2mm, angular, oblong, obovate or obliquely obovate, apex rounded or truncate, base cuneate, obtuse, oblique, obliquely truncate or truncate, black, brown, greenish brown or orange brown, surface reticulate, reticulately foveate, reticulately foveolate or reticulately reticulate, hilum basal (Table1; Fig. 3 C-R).

Represented by 8 taxa viz., *Orobanche aegyptiaca* Pers., *O. alba* Steph., *O. amethystea* Thuill.,*O. cernua* Loefl. var. *cernua, O. cernua* var. *pseudo-clarkei* Jafri, *O. coelistis* (Reut.) Beck, *O. solmsii* Clarke ex Hook.f. and *O. stocksii* Boiss.

Key to the species

1 + Seeds oblong ……..……..…… *O. alba*
- Seeds obovate or obliquely obovate ……..…… 2
2 + Seeds base obtuse or oblique ……..……... 3
- Seeds base truncate or cuneate ……..……..... 5
3 + Seeds greenish brown ……..…… *O. solmsii*
- Seeds light brown ……. 4
4 + Seed surface reticulate or reticulately foveate ……..……..……..... *O. cernua*
- Seed surface reticulately reticulate ……..……..…….. *O. coelistis*
5 + Seeds dark brown or orange brown with reticulate surface pattern …….. 6
- Seeds black with reticulately foveolate ……...……..……..... *O. stocksii*
6 + Seeds orange brown, base cuneate ……..……..……..…… *O. aegyptiaca*
- Seeds dark brown, base truncate …..……..……..…….. *O. amethystea*

Pedicularis L.

Seeds 1.5-4x0.8-2mm, non-angular, elliptic, obliquely elliptic, elliptic pyriform or obovate, apex acute, obtuse, acuminate or beaked, base cuneate, obtuse, rounded or truncate, green, whitish, greenish white, greenish brown with dark brown spots, dark brown, brown with creamy lines, dusty brown, yellow or black, surface longitudinally ridged and reticulate or reticulate foveate along with foveolation or punticulation, laterally lineate or ruminately foveate, hilum basal (Table1; Fig. 4 A-R).

Represented by 9 taxa viz., *Pedicularis bicornuta* Kl., *P. kashmiriana* Penn., *P. multiflora* Penn., *P. pectinata* Wall. ex Bth. subsp. *palans* Prain, *P. punctata* Dene., *P. pycnantha* Boiss., *P. pyramidata* Royle, *P. stewartii* Penn. and *P. tenuirostris* Bth.

Key to the species

1 + Seeds elliptic or obovate ……..…….. 2
- Seeds obliquely elliptic or elliptic pyriform ……..…….. 5
2 + Seeds c. 1.5-2 mm long, apically acute or obtuse ……..……..……..... 3
- Seeds c. 2.5-2.8 mm long, apically acuminate or beaked ……..…… 4
3 + Seeds black, apex and base obtuse ……..……..…… *P. punctata*
- Seeds brown with creamy lines, apex acute, base cuneate ……..……..….. *P. pyramidata*
4 + Seeds dark brown, elliptic, apically acuminate ……..... *P. pectinata* subsp. *palans*
- Seeds greenish brown, obovate, apically beaked ……..…… *P. pycnantha*
5 + Seeds obliquely elliptic, apex acuminate ……..……..... 6
- Seeds elliptic pyriform, apex beaked ……..……..……..…… 8
6 + Seeds c. 2-2.2 mm long, surface pattern reticulate along with foveolation …… *P. bicornuta*
- Seeds 3.8-4 mm long, surface pattern reticulate or reticulate foveate along with punticulation …….. 7
7 + Seeds whitish ……..……..……..…. *P. kashmiriana*
- Seeds greenish white ……..……..…….... *P. multiflora*
8 + Seeds dusty brown, base truncate ……..…… *P. stewartii*
- Seeds yellow, base cuneate ...……..……..…… *P. tenuirostris*

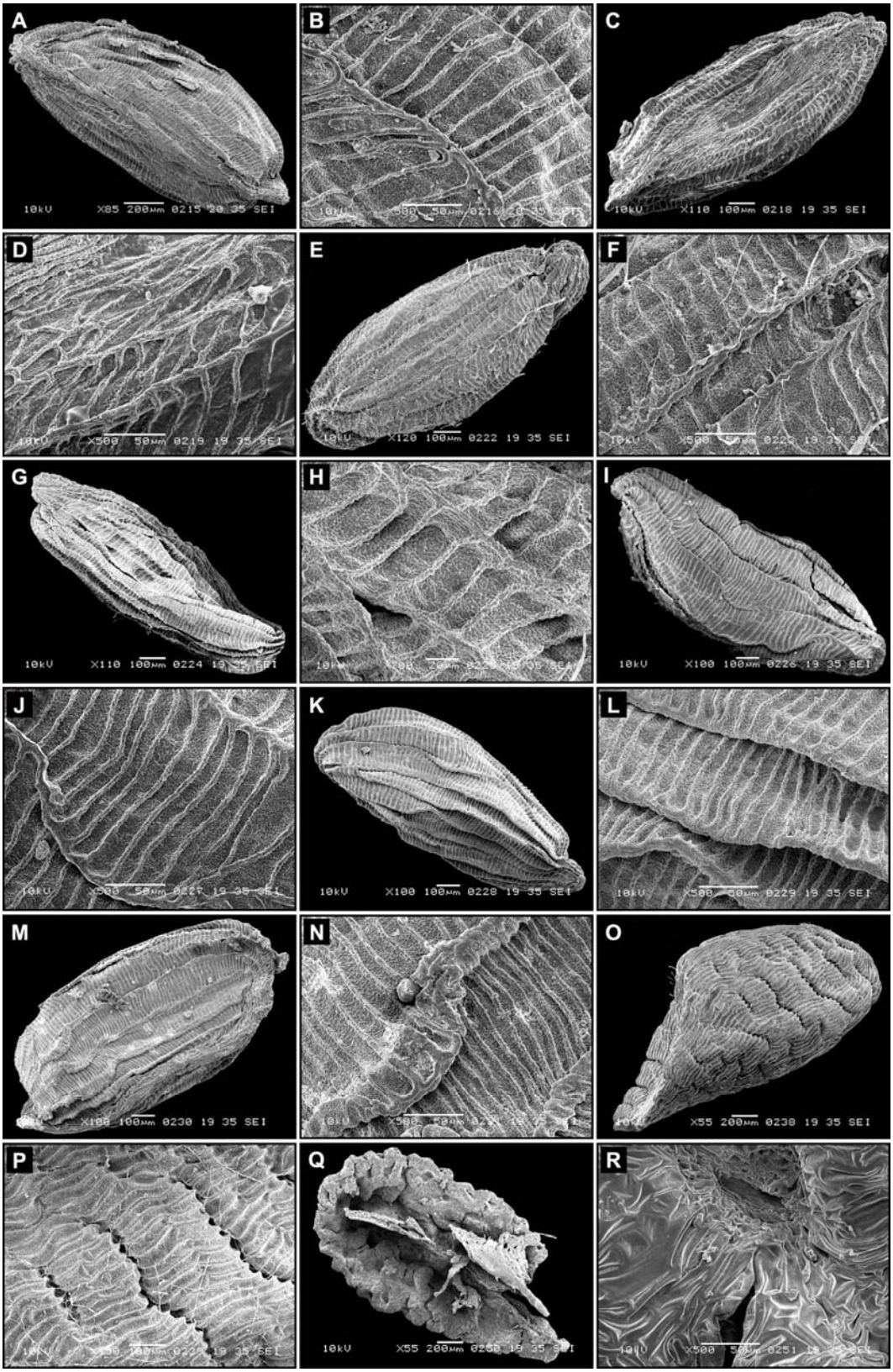

Fig. 2. Scanning electron micrographs. *E. kurramensis*: A, seed; B, surface. *E. laxa*: C, seed; D, surface. *E. paucifolia*: E, seed; F, surface. *E. platyphylla*: G, I, seed; H, J, surface. *E. remota*: K, seed; L, surface. *Euphrasia schlagintweitii*: M, seed; N, surface. *Leptorhabdos parviflora*: O, seed; P, surface. *Lindenbergia abyssinica* : Q, seed; R, surface. (Scale bars: A, O, Q= 200µm; C, E, G, I, K, M, P= 100µm; B, D, F, J, L, N, R= 50µm; H=20µm).

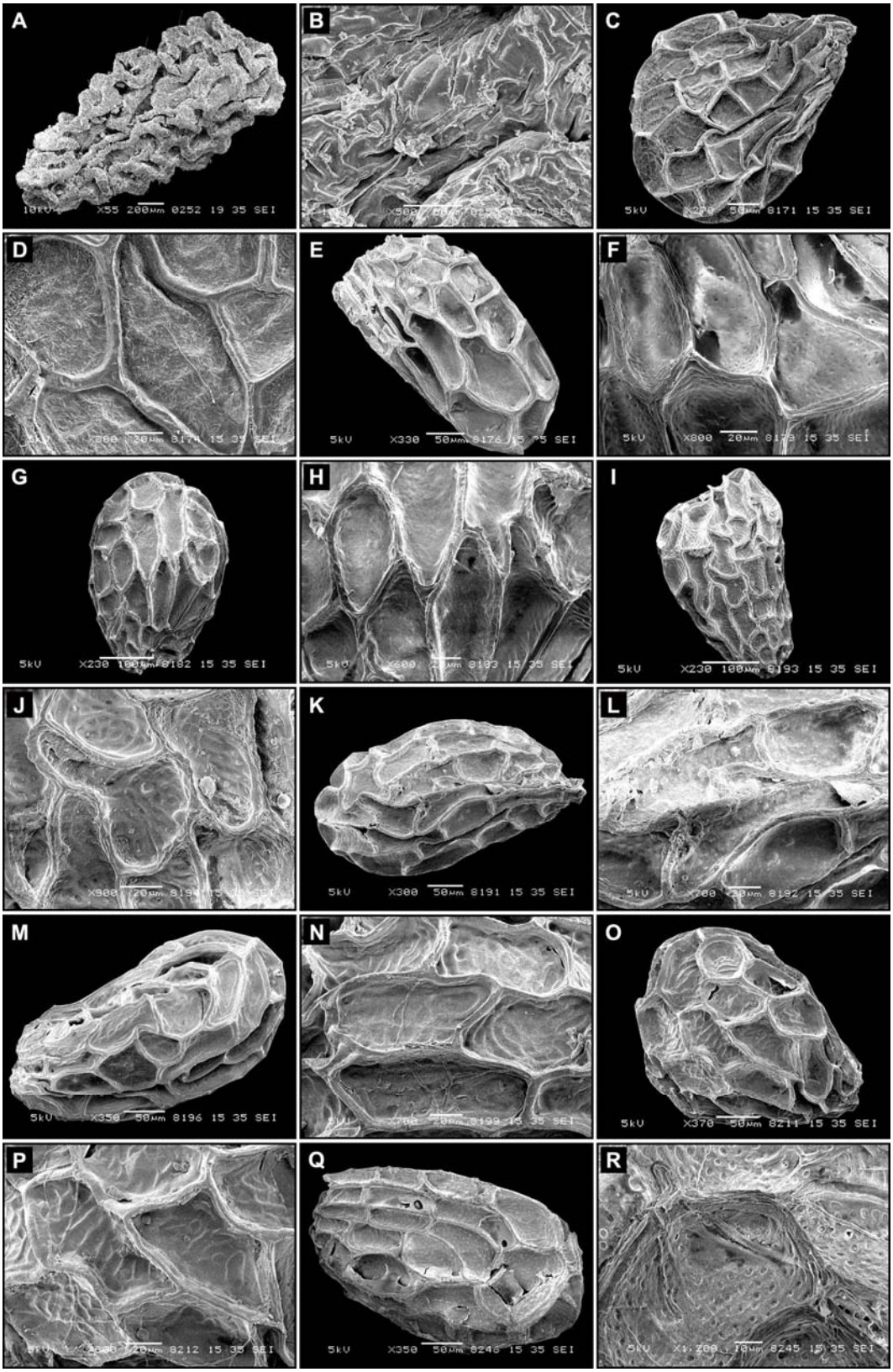

Fig. 3. Scanning electron micrographs. *Lindenbergia indica*: A, seed; B, surface. *Orobanche aegyptiaca*: C, seed; D, surface. *O. alba*: E, seed; F, surface. *O. amethystea*: G, seed; H, surface. *O. cernua* var. *cernua*: I, seed; J, surface. *O. cernua* var. *pseudo-clarkei*: K, seed; L, surface. *O. coelistis*: M, seed; N, surface. *O. solmsii*: O, seed; P, surface. *O. stocksii*: Q, seed; R, surface. (Scale bars: A= 200µm; G, I= 100µm; B, C, E, K, M, O, Q= 50µm; D, F, H, J, L, N, P= 20µm; R= 10µm).

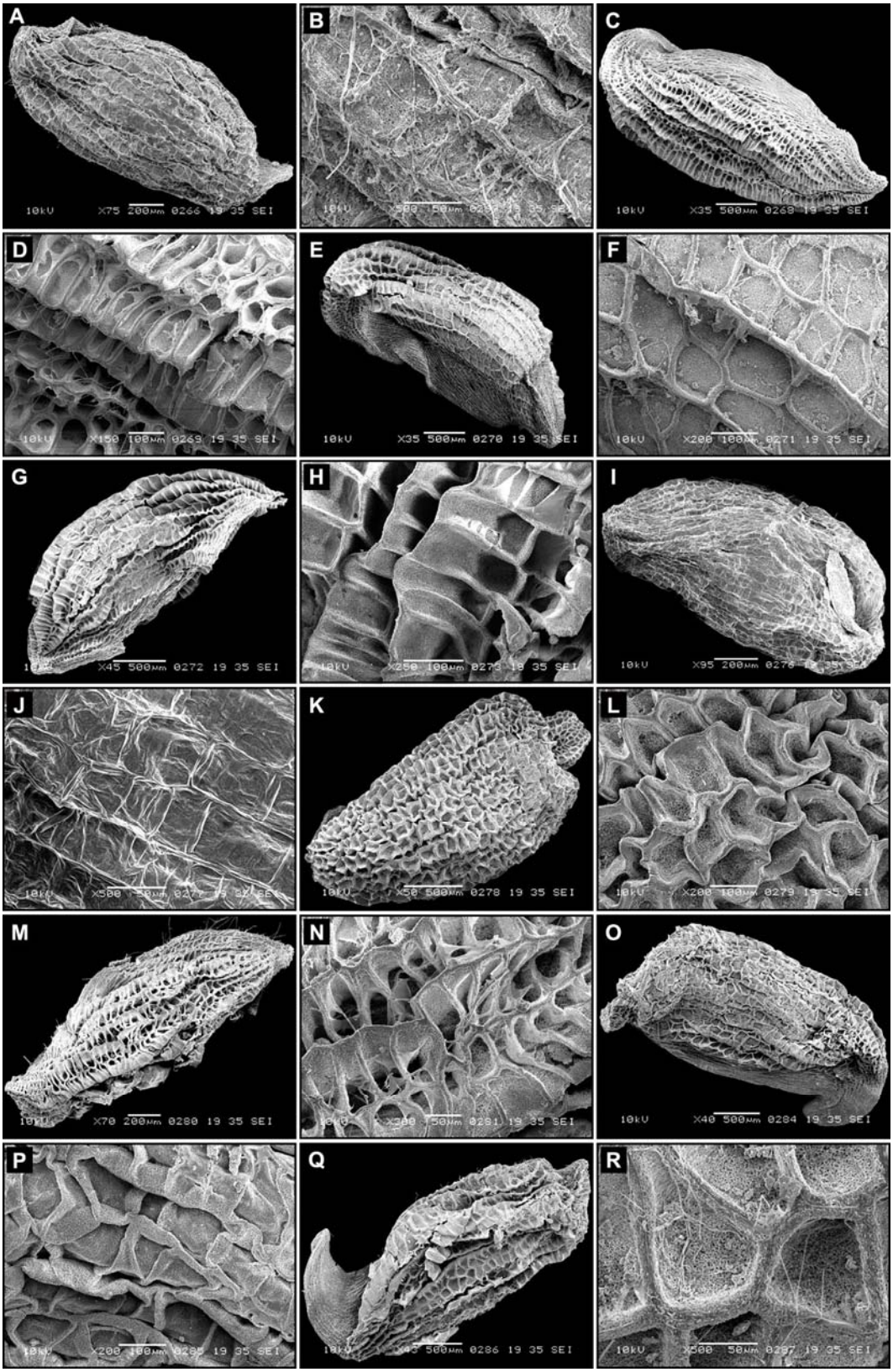

Fig. 4. Scanning electron micrographs. *Pedicularis bicornuta*: A, seed; B, surface. *P. kashmiriana*: C, seed; D, surface. *P. multiflora*: E, seed; F, surface. *P. pectinata* subsp. *palans*: G, seed; H, surface. *P. punctata*: I, seed; J, surface. *P. pycnantha*: K, seed; L, surface. *P. pyramidata*: M, seed; N, surface. *P. stewartii*: O, seed; P, surface. *P. tenuirostris*: Q, seed; R, surface. (Scale bars: C, E, G, K, O, Q= 500µm; A, I, M= 200µm; D, F, H, L, P= 100µm; B, J, N, R= 50µm).

Striga Lour.

Seeds 0.2-0.3x 0.1-0.2mm, angular, elliptic, deltoid or obovate, apex acute, rounded, obliquely truncate or truncate, base cuneate, obtuse, rounded or obliquely truncate, light brown or dark brown, surface ribbed along with verrucation, dorsally glebulate, hilum basal (Table1; Fig. 5 A-I)

Represented by 3 species viz., *Striga asiatica* (L.) O. Ktze., *S. euphrasioides* (Vahl) Bth. and *S. lutea* Lour.

Key to the species

1 + Seeds obovate ……..…… *S. lutea*
- Seeds elliptic or elliptic-deltoid ……..…….. 2
2 + Seeds dark brown, apically acute-rounded, base obliquely truncate ……..… *S. asiatica*
- Seed light brown, apically obliquely truncate, base obtuse-cuneate …… *S. euphrasioides*

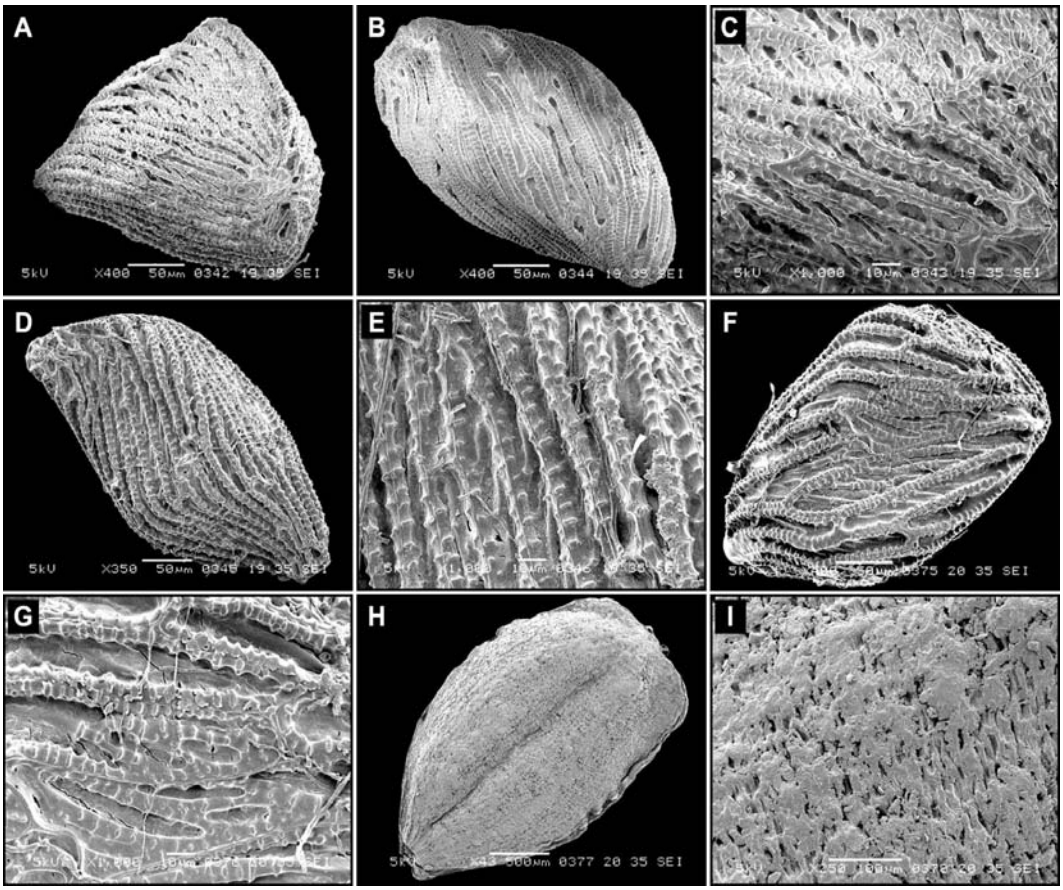

Fig. 5. Scanning electron micrographs. *Striga asiatica*: A, B, seeds; C, surface. *Striga euphrasioides*: D, seed; E, surface. *S. lutea*: F, H, seeds; G, I, surfaces. (Scale bars: H=500µm; I= 100µm; A, B, D, F= 50µm; C, E, G=10µm).

Results and Discussion

The dendrogram shows the significant grouping within the family Orobanchaceae (Fig. 6), the first group of parasitic genera viz., *Cistanche, Orobanche* and *Striga* occupy the terminal position within Clusters which may predict its advanced nature as compared to remaining genera by having spicate inflorescences (Jafri, 1976; Hong *et al*., 1998; Zhang & Tzvelev, 1998), smaller (Figs. 7, 8) and angular seeds with ribbed or various reticulate surface patterns. Similar surface patterns were also previously observed in *Orobanche* (Abu Sbaih & Jury, 1994a, b; Plaza *et al.*, 2004). Amongst the genera of this group *Cistanche* having smallest seeds and are regarded as dust seeds (Musselman & Mann, 1976; Pieterse, 1979; Plaza *et al.*, 2004). The second group with hemiparasitic genera viz., *Pedicularis, Leptorhabdos* and *Euphrasia* and non-parasitic genus *Lindenbergia* occupy more or less intermediate position as they are characterized by the presence of non-angular seeds with ridged, ruminate or undulately grooved and scalariform surface patterns and various types of inflorescence like raceme, terminal spike, axillary or solitary inflorescences (Hong *et al*., 1998). Moreover, the placement of *Cistanche tubulosa* and *Orobanche alba* in the first group is also supported by having similar corolla i.e., campanulate, tubular or infundibuliform type (Jafri, 1976) and black, oblong or obovate seeds with truncate apex. While, all the species of

Striga fall in a common cluster by having slaverform corolla (Hong *et al*., 1998) and elliptic deltoid or obovate, brown seeds with obliquely truncate, acute or rounded apex. All the remaining species of *Orobanche* form a common cluster by having obovate or obliquely obovate seeds with rounded or truncate apex. Within this cluster *O. cernua* and *O. coelistis* show more affinity by having obliquely obovate, light brown seeds with oblique base and tubulose corolla (Jafri, 1976) while the remaining species of this cluster have different seed shapes and colour. The species of the genera of second group viz., *Pedicularis, Leptorhabdos, Lindenbergia* and *Euphrasia* may be further divided on the basis of different surface patterns. *Leptorhabdos parviflora, Lindenbergia abyssinica* and *L. indica* group separately due to non ridged seeds and usually tubular-funnel shaped corolla (Hong *et al*., 1998), while, *Pedicularis bicornuta, P. kashmiriana, P. multiflora, P. pycnantha, P. stewartii* and *P. tenuirostris* are characterized by ridged seeds and bilabiate corolla (Hong *et al*., 1998). Moreover, all species remain distinct with each other on the basis of seed shape. It is also noteworthy that the three species of *Pedicularis* form a common cluster along with the species of *Euphrasia* as these species having common seed characters such as elliptic seeds with obtuse, acute or acuminate apex. There is a general agreement between seed morphology, molecular data (Bennett & Mathews, 2006) and pollen morphological data (Minkin & Eshbaugh, 1989) for the inclusion of the hemiparasitic genera viz., *Euphrasia, Leptorhabdos,* and *Pedicularis* and non parasitic genus *Lindenbergia* within the parasitic family Orobanchaceae.

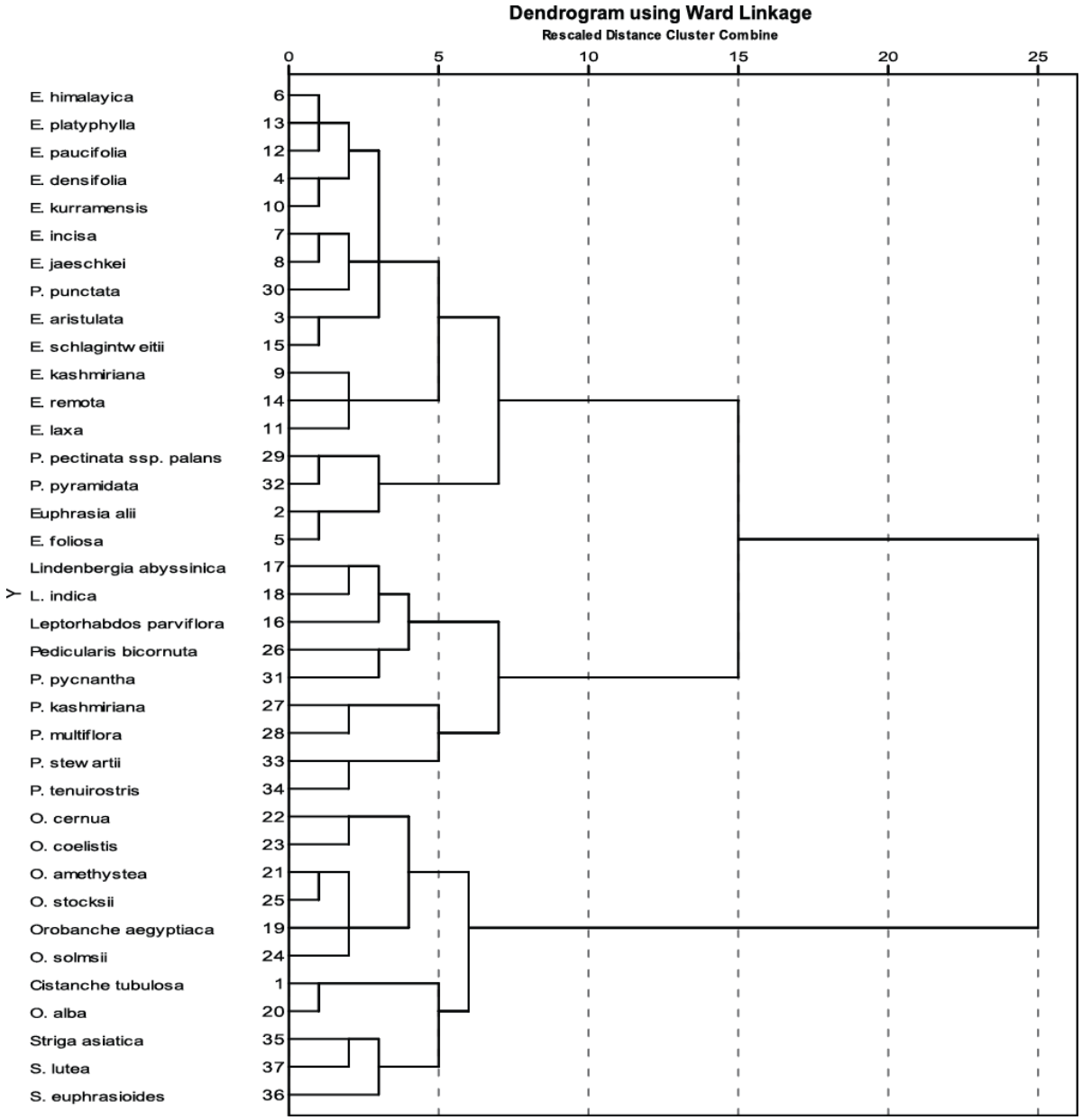

Fig. 6. Dendrogram showing the relationships of the species of the family Orobanchaceae.

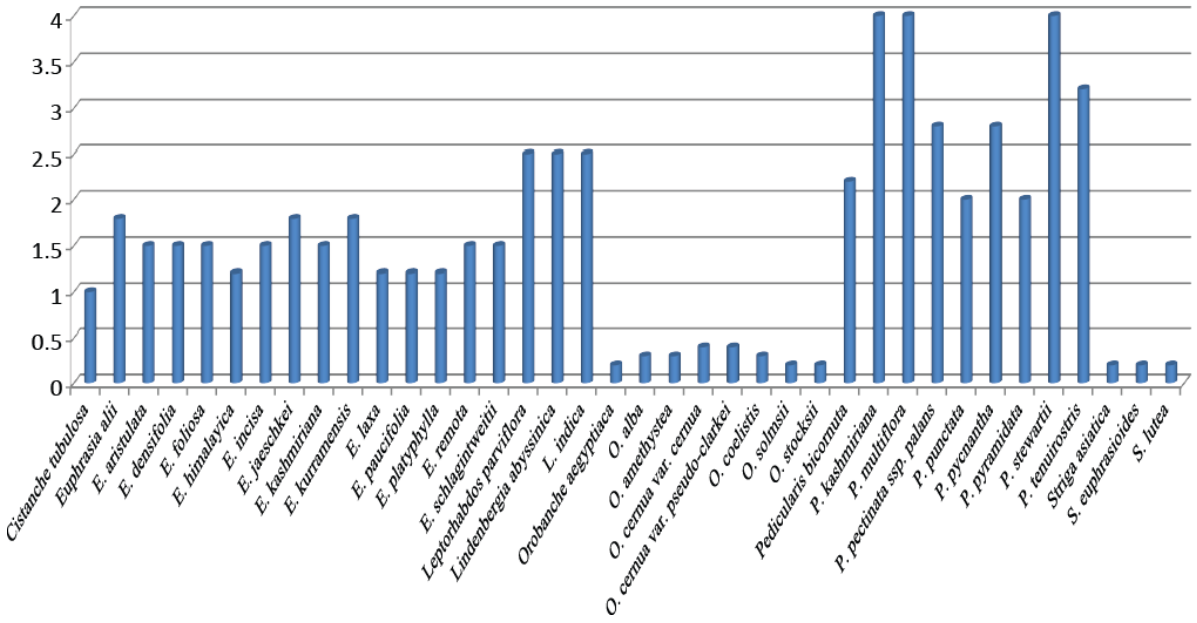

Fig. 7. Bar diagram showing the variation in the length (cm) of the seeds of the family Orobanchaceae.

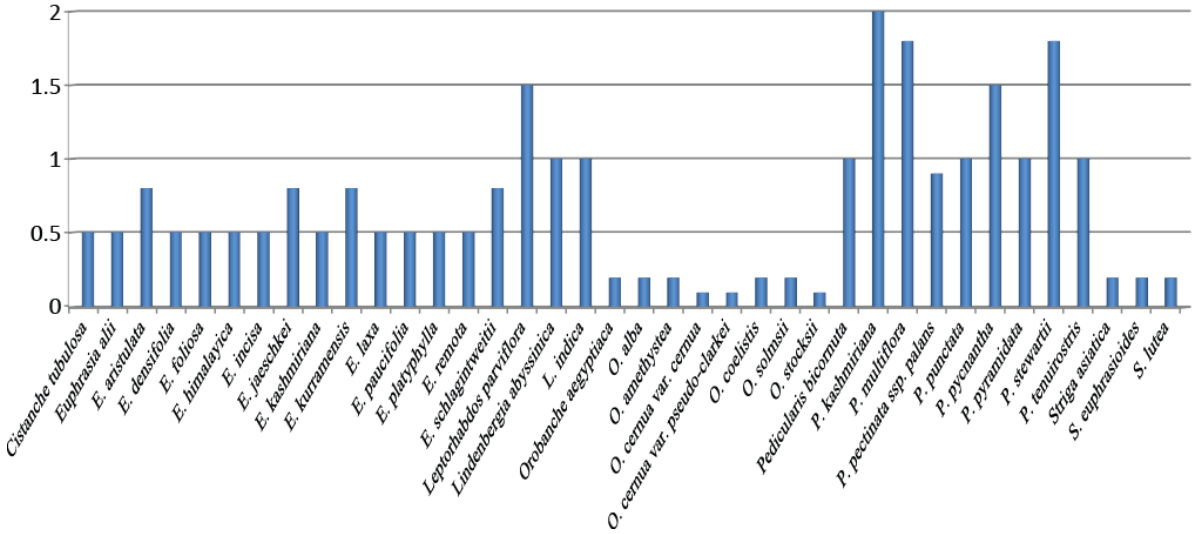

Fig. 8. Bar diagram showing the variation in the breadth (cm) of the seeds of the family Orobanchaceae.

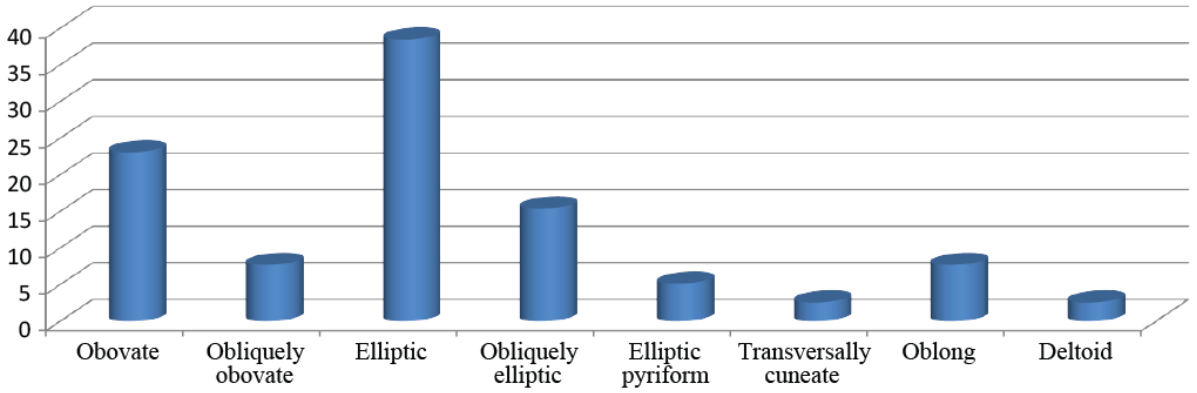

Fig. 9. Bar diagram showing the variation in the seed shapes of the family Orobanchaceae.

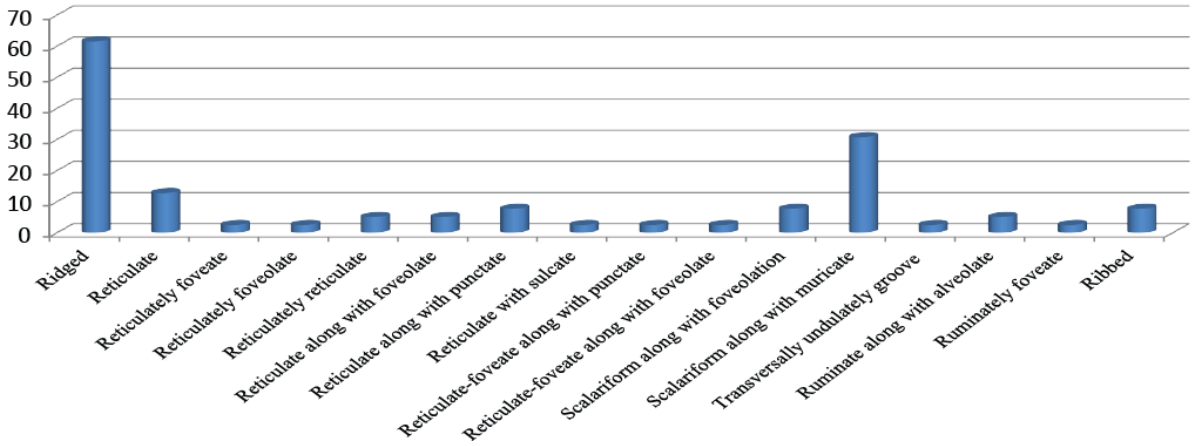

Fig. 10. Bar diagram showing the variation in the surface patterns of the seeds of the family Orobanchaceae.

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