



Pollen morphology of the genus *Silene* (Sileneae-Caryophyllaceae) from Pakistan

Sumaira SAHREEN^{*1}, Mir Ajab KHAN², Akbar Ali MEO³, Asma JABEEN⁴

¹ Botanical Science Division, Pakistan Museum of Natural History Islamabad/Pakistan

² Department of Plant Sciences, Quaid-i-Azam University, Islamabad / Pakistan

³ Book Bank/ Book Shop & Publication cell, Quaid-i-Azam University, Islamabad / Pakistan

⁴ Lecturer, Fatima Jinnah Women University, Rawalpindi / Pakistan

Abstract

Pollen morphological studies of 16 species of the genus *Silene* have been carried out from Pakistan. Pollen grains are polyaperturate or periporate at polar view. There is a great variation in pollen morphology in the species of *Silene*. In *Silene conoidea* 22 – 24 number of pores is a species specific character. *Silene brahuica*, *S. conoidea*, *S. moorcroftiana*, *S. ovalifolia*, *S. villosa*, and *S. vulgaris* have thin exine with 20 and more than 20 number of pori. In *S. apetala*, *S. citrina*, *S. longisepala* and *S. kunawerensis* exine thickness is 5-7.25µm, then number will be 4-10. The palynological features of exine thickness, size of pollen and pori, distance between two pores and pori surface are found useful criteria for species delimitation while sculpturing at LM turned out to be a poor criterion. A dichotomous key is constructed for the species identification using the characters that has been studied.

Key words: Pollen, *Silene*, Caryophyllaceae, Pori, Exine

1. Introduction

Palynology is the science of pollen and spore morphology. It can be used as an instrument of multiple research for systematic botany, paleobotany, paleoecology, pollen analysis, aeropalynology, criminology, allergy stratigraphic correlation of oil bearing rocks and coal fields, and improvement of honey.

Caryophyllaceae is a large and extremely variable family of dicotyledones with 80 genera and 2100 species, which are of wide geographic distribution mostly, includes the ornamental plants and weeds. Arora and Panday (1996) and Bakshi (1984) reported that the family Caryophyllaceae is important due to medicinal as well as ornamental properties. In Pakistan Caryophyllaceae family is represented by 26 genera and about 110 species. (Nasir and Ali, 1986). Eighteen species of Caryophyllaceae are endemic in Pakistan (Chaudhary & Qureshi, 1991). Although palynological evidence has played an important role in discerning natural groups and understanding phylogenetic

* Corresponding author / Haberleşmeden sorumlu yazar: sumairasahreen@gmail.com

relationships (Erdtman, 1969; Walker and Doyle, 1975), however there is a great need for knowledge of pollen morphology in many plant families including Caryophyllaceae especially from Pakistan. Palynological aspects in Pakistan is deficient and the earlier reference dates back to sixties. Few workers Bhutta (1968), Malik (1964), Zahur *et al.*, (1978), Meo *et al.*, (1999) Nasreen and Khan (1998) , Perveen and Qaiser (2003), Dawar *et al.*, (2002) and Ozdemire *et al.*, (2004) have provided commendable quantity of basic and applied information on palynology by providing different pollen data.

Fægri and Iversen (1975) reported that pollens of Caryophyllaceae are periporate. Number of pores is a diagnostic character for delimitation of genera. Yıldız (2001) studied pollen morphology of 45 species belonging to 15 genera of Caryophyllaceae by (LM) and (SEM). On the basis of exine structure, ornamentation and morphological data, 10 distinct types viz., 1. Arenaria type, 2. Stellaria holoste type 3. Cerastium type, 4. Dianthus type, 5. Gypsophila repens type, 6. Lychnis viscaria type, 7. Silene vulgaris type, 8. Silene caryophylloides type 9. Silene conica type and 10. Agrostemma githago type were recognized.

Skvarla (1975), Skvarla and Nowicke (1976), Nowicke and Skvarla (1977), Ghazanfar (1984), Fægri and Iversen (1975), Arkan and Inceoglu (1992), Moore *et al.*, (1997) and Yildiz (1996a, 1996b, 2001a, 2001b, 2005) studied the pollen morphology of different genera of Caryophyllaceae at worldwide level. In this paper for the first time pollen morphology of the genus *Silene* from Pakistan has been presented.

2. Materials and methods

The palynological investigations are based on the herbarium specimen obtained from Quaid-i-Azam University, Islamabad (ISL) by the acetolysis method of Erdtman (1969). A list of specimens investigated is given in the Appendix 1. The pollen grains were first kept in acetic acid for softening and then were used to prepare slides by using glycerine-jelly mixed with 1% Safranin. The prepared slides were studied under the light microscope.

Permanent slides for pollen reference collection has been deposited in the Plant Taxonomy Lab, Department of Plant Sciences, Quaid-i-Azam University, Islamabad. Terminology used is after Erdtman (1969), Fægri and Iversen (1975) and Punt *et al.*, (1994).

3. Results

Table 1 summarizes the measurements of pollen grains from the taxa examined. Light micrographs of *Silene* species are presented in Figure.1.

Size: The size of pollen grains of the species of *Silene* ranges from 25-30µm to 50-55µm. There is great variation in size of the pollen grains. Five species viz. *S. apetala*, *S. brahuica*, *S. citrina*, *S. longisepala*, *S. viscosa* have same pollen diameter (30-35 µm). Similarly three species viz. *S. indica*, *S. moorcroftiana*, *S. tenuis* appear to be same in pollen diameter (40-45µm). Pollen diameter appears to be the largest (50-55 µm) in *S. vulgaris* and the smallest in *S. kunawerensis*.

Symmetry and Shape: The pollen grains are radially symmetrical, isopolar, periporate in all species. Pollen surface is granulate in most of the species while granulate and spinulate in *S. apetala*, *S. indica*, *S. longisepala*, *S. moorcroftiana*, *S. tenuis* and spinulate in *S. arenosa*.

Pore diameter: Pore diameter ranges from 2.5-3.75 μ m to 8-10 μ m. Pore diameter is similar (4-5 μ m) in eight species viz. *S. apetala*, *S. arenosa*, *S. citrina*, *S. falconariana*, *S. indica*, *S. longisepala*, *S. pseudoverticillata* and *S. tenuis*. The distance between pores ranges from 3-4 μ m to 10-12 μ m. *S. brahuica*, *S. moorcroftiana*, *S. ovalifolia* appear the same distance to have between pores (3-4 μ m). Similarly, *S. arenosa*, *S. villosa* have same distance between pores (5-6 μ m) while *S. apetala*, *S. pseudoverticillata*, shows same distance between pores (7-8 μ m). Number of pores varies 4-5 to 22-24 among the taxa. Species viz. *S. arenosa*, *S. pseudoverticillata* and *S. tenuis* appear to have same number of pores (10-12) and *S. indica*, *S. viscosa* show same pore number (13-14). Similarly, *S. brahuica*, *S. ovalifolia* represents same pore number (19-20).

Exine: Exine thickness varies between 1.25-2.0 μ m to 5.0-7.5 μ m among the species (Table 1). Exine thickness emerged to be the highest (5.0-7.5 μ m) in *S. apetala* and the lowest (1.25-2.0 μ m) in *S. brahuica*. Same type of exine thickness (2.5-3.75 μ m) is recorded in *S. arenosa*, *S. ovalifolia*, *S. villosa*, *S. viscosa* and *S. vulgaris*.

Key to *Silene* species

- | | |
|--|------------------------------|
| 1. Diameter of pores less than 4 μ m | <i>S. kunawerensis</i> |
| Diameter of pores more than 4 μ m | 2 |
| 2. Number of pores 22-24 | <i>S. conoidea</i> |
| Number of pores less than 22 | 3 |
| 3. Thin exine with large number of pores | <i>S. brahuica</i> |
| Thick exine with small number of pores | 4 |
| 4. Exine thickness 5-7.5 μ m | <i>S. apetala</i> |
| Exine thickness 1.25-2.5 μ m | 5 |
| 5. Diameter of pores 8-10 μ m | <i>S. vulgaris</i> |
| Diameter of pores less than 8 μ m | 6 |
| 6. Distance between pores 9-11 μ m | <i>S. citrina</i> |
| Distance between pores less than 9 μ m | 7 |
| 7. Pollen size 30-35 μ m | <i>S. viscosa</i> |
| Pollen size more than 35 μ m | 8 |
| 8. Pollen size 40-45 μ m | <i>S. indica</i> |
| Pollen size more than 45 μ | 9 |
| 9. Distance between pores 3-4 μ m with more number of pores 19-20 | <i>S. ovalifolia</i> |
| Distance between pores more than 5 μ m with less number of pores 10-11 | 10 |
| 10. Pollen size 45-50 μ m | <i>S. arenosa</i> |
| Pollen size less than 45 μ m | 11 |
| 11. Distance between pores 5-6 μ m with 16-20 number of pores | <i>S. villosa</i> |
| Distance between pores 7-8 μ m with less number of pores | 12 |
| 12. 10-12 number of pores with thin exine i.e 1.25-2.5 μ m | <i>S. tenuis</i> |
| 12-14 number of pores with exine thickness of 3.75-4.5 μ m | 13 |
| 13. Thick exine 5-6.25 μ m with 5-7 μ m distance between pores | <i>S. longisepala</i> |
| Thin exine 2.5-3 μ m with less distance between pores | 14 |
| 14. 3-4 μ m distance between pores, 5-6 μ m diameter of pores | <i>S. moorcroftiana</i> |
| More than 4 μ m distance between pores, 4-5 μ m diameter of pores | 15 |
| 15. 7-8 μ m distance between pores, 10-12 number of pores | <i>S. pseudoverticillata</i> |
| 8-9 μ m distance between pores, 12-14 number of pores | <i>S. falconariana</i> |

Table 1. Summary of pollen measurements, shape and sculpturing features in *Silene* (*Sileneae*–*Caryophyllaceae*)
(Measurements are in μm , \pm = more or less)

Taxon	Diameter Of pollen	Diameter Of pores	Distance between pores	Number Of pores	Exine thickness	Pore surface	Pollen surface	Pollen type
<i>Silene apetala</i>	30- 35 μm	4- 5 μm	7- 8 μm	4- 5	5- 7.5 μm	Crustate	Granulate, Spinulate	Periporate
<i>S. arenosa</i>	45- 50 μm	4- 5 μm	5- 6 μm	10- 12	2.5-3.75 μm	\pm Crustate	Spinulate	Periporate
<i>S. brahuica</i>	30- 35 μm	5- 6 μm	3- 4 μm	19- 20	1.25- 2 μm	Crustate	Granulate	Periporate
<i>S. citrina</i>	30- 35 μm	4- 5 μm	9- 11 μm	6- 10	5- 6.25 μm	Crustate	Granulate	Periporate
<i>S. conoidea</i>	45- 55 μm	6.5- 7.5 μm	8- 10 μm	22- 24	2- 3 μm	\pm Crustate	Granulate	Periporate
<i>S. falconariana</i>	35- 40 μm	4- 5 μm	8- 9 μm	12- 14	3.75- 4.5 μm	\pm Crustate	Granulate	Periporate
<i>S. indica</i>	40- 45 μm	4- 5 μm	6- 9 μm	13-14	4.5- 5 μm	Crustate	Granulate, Spinulate	Periporate
<i>S. kunawerensis</i>	25- 30 μm	2.5- 3.75 μm	10- 12 μm	4- 6	5- 7.5 μm	Crustate	Granulate	Periporate
<i>S. longisepala</i>	30- 50 μm	4- 5 μm	5- 7 μm	10-11	5- 6.25 μm	\pm Crustate	Granulate, Spinulate	Periporate
<i>S. moorcroftiana</i>	40- 45 μm	5- 6 μm	3- 4 μm	17- 20	2.5- 3 μm	Crustate	Granulate, Spinulate	Periporate
<i>S. Ovalifolia</i>	35- 40 μm	3.75- 5 μm	3- 4 μm	19- 20	2.5- 3.75 μm	Crustate	Granulate	Periporate
<i>S. pseudoverticellata</i>	35- 40 μm	4- 5 μm	7- 8 μm	10- 12	3.75- 4.5 μm	\pm Crustate	Granulate	Periporate
<i>S. tenuis</i>	40- 45 μm	4- 5 μm	6- 7 μm	10- 12	1.25- 2.5 μm	Crustate	Granulate, Spinulate	Periporate
<i>S. villosa</i>	35- 40 μm	7- 7.5 μm	5- 6 μm	16- 22	2.5- 3.75 μm	\pm Crustate	Granulate	Periporate
<i>S. viscosa</i>	30- 35 μm	6- 6.5 μm	6- 8 μm	13- 14	2.5- 3.75 μm	Crustate	Granulate	Periporate
<i>S. vulgaris</i>	50- 55 μm	8- 10 μm	10- 11 μm	18- 20	2.5- 3.75 μm	Crustate	Granulate	Periporate

Figures:

Figure 1 Showing polar view of selected species of *Silene*



Figure 1a: showing pores and exine in polar view (at 100X) of *Siene apetala*



Figure 1c: showing pores in polar view (at 100X) of *Silene citrine*

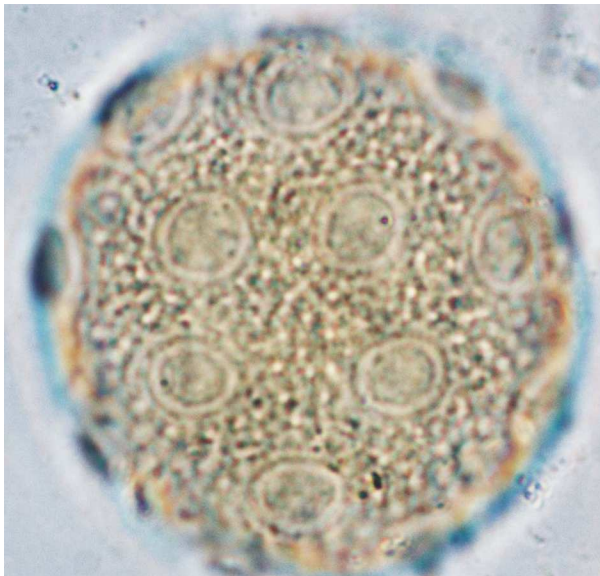


Figure 1b: showing pores in polar view (at 100X) of *Siene arenosa*



Figure 1d: showing pores in polar view (at 100X) of *Siene conoidea*



Figure 1e: showing pores in polar view (at 100X) of *Silene kunawerensis*



Figure 1g: showing pores in polar view (at 100X) of *Silene tenuis*



Figure 1f: showing pores in polar view (at 100 X) of *Silene longisepala*



Figure 1h: showing pores and exine in polar view (at 100X) of *Silene vulgaris*



Figure 1i : showing pores in polar view (at 100X) of *Silene villosa*

4. Discussion

Pollen morphology can be useful in supporting taxonomic suggestions (Clark et al., 1980). It provides useful taxonomic characters for the identification and classification of taxa of the family Caryophyllaceae. Pollen morphological characters are of significance in species delimitation. These are considered supplementary to the general plant morphology and play a critical role in taxonomic and evolutionary debate. Tomsovie (1997) utilized pollen characters as an additional information for systematic studies. Huang (1972) also used pollen characters for systematic purposes.

Silene can be distinguished on the bases of number of pori. In *Silene* number of pori is more than and less than 12. Faegri and Iversen (1975) reported that *Dianthus* and *Silene* have less than 20 number of pori. More than 20 pori is also the character of *Silene*. So there is collaboration between the two studies and both studies are compatible to each other. It indicates that pori is a diagnostic taxonomic character of *Silene*.

All the pollens are polyaperturate or periporate. Thin exine with reduced columellae is a characteristic of primitive Angiosperms. In *Silene* the number of pori and exine thickness is related in such a way that if the exine is thin the number of pori will be greater. The range of exine thickness in *Silene brahuica*, *S. conoidea*, *S. moorcroftiana*, *S. ovalifolia*, *S. villosa*, and *S. vulgaris* is 1.25- 2.5 -3.75 μ m and number of pori is 20 and more than 20. If there is thick exine, then number of pori will be less. In *S. apetala*, *S. citrine*, *S. longisepala* and *S. kunawerensis* exine thickness is 5- 7.25 μ m, then number will be 4-10.

In *S. arenosa*, *S. tenuis* and *S. viscosa* 12 and more pori are found with thin exine i.e. 1.25- 2.5 μ m. In *S. indica*, *S. falconariana* and *S. pseudoverticellata* exine thickness is of medium size i.e. 3.75 – 4.5 μ m with 12 – 20 number of Pori. In *S. arenosa*, *S. conoidea* and *S. vulgaris* pollen size is greater i.e. 50 – 55 μ m.

In studies on the pollen morphology of Caryophyllaceae, Skvarla (1975), Skvarla and Nowicke (1976), Nowicke and Skvarla (1977), Ghazanfar (1984), Arkan and Inceoglu (1992) and Yildiz (1996a, 1996b, 2001a, 2001b, 2005) demonstrated that the pollen are of usually of medium size ranging from 25 – 50µm. The present studies are also in agreement with their contention.

Moore et. al., (1997) and Yildiz (2001a, 2001b) examined exine by SEM of different genera including *S. vulgaris*. Their findings are in accordance with the present studies. Moore et. al., (1997) included Caryophyllaceae in polyantoporate group. In this group some of the species have maximum number of Pori i.e. up to 40. In present studies maximum number of pori is found in *S. conoidea*, *S. vulgaris* and *S. villosa* i.e. 22 – 24.

Yildiz (2001b) also reported maximum number of pori in *S. conoidea* and *S. vulgaris* i.e. 30 – 40. In *S. arenosa*, *S. viscosa* and *S. citrina* exine morphology is quite similar to *Dianthus* with the exception that the tectum is discontinuous and reticulate. These results are in conformity with that of Heslop-Harrison (1968) and Yildiz (2001b).

Silene species are characterized by thick tectum and sharp spines (Skvarla and Nowicke, 1976). Pori number 7-50 has been reported in many studies like Skvarla and Nowicke (1976), Nowicke and Skvarla (1977), Ghazanfar (1984), Arkan and Inceoglu (1992) and Yildiz (1996a, 1996b; 2001a). The variation in number of pori in *Silene* is helpful at specific level.

Yildiz (2001b) reported that number of pori is from 9 – 42 and many of the species have 10 – 20 pori while present studies shows 4 – 24 pori in *Silene* species.

Parent and Richard (1993) and Yildiz (2001b) in their studies on 12 genera and 38 species of Caryophyllaceae distributed in Canada indicated that *Silene* species are polyantoporate that is similar to the present report.

The presence of semi reticulate and reticulate sexine in *S. conoidea* suggests it phylogenetically-advanced species. Taxonomically on the basis of inflorescence *S. conoidea* is higher than other species.

Imperforate exine (Walker, 1974a,b) and fewer number of pori (Van Campo, 1966) are generally accepted as primitive. Pori number is low in genera *Cerastium* and *Stellaria* of the family Caryophyllaceae that is accepted primitive phylogenetically in the Flora of Turkey (Davis, 1967). But the pori number of *Silene* is more than other species. So it is most advanced genus. The phylogeny of Flora of Turkey (Davis, 1967) seems true according to this result.

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Appendix 1. Specimens of *Silene* investigated from various areas of Pakistan

Sr. No.	Taxon	Locality	Collector's Name	Vocher No.
1	<i>Silene apetala</i> Willd.	Quetta	Muqarrab Shah & Wali-ur-Rehman	119323
2	<i>Silene arenosa</i> C. Koch	Waziristan	M. Zubair & Saeed	113483
		Peshawar	Dilawar & Tanveer	107999
		Khyber Agency	Dilawar & Tanveer	109785
		Hazara	M. N. Chaudary & M. A. Siddiqui	14036
		Chitral	Wali-ur-Rehman & Subhan	115102
		Chitral	Wali-ur-Rehman & Subhan	115130
		Bannu	Dilawar & Shahzad	47455
		Bannu	Dilawar & Shahzad	50354
		Dir	Ghulam Farooq	23690
3	<i>Silene brahuica</i> Boiss.	Quetta	Iqbal Dar & M. Arif	23673
		Quetta	Manzoor & Maqsood	50475
		Quetta	Manzoor & Maqsood	52634
		Quetta	Muqarrab Shah & Wali-ur-Rehman	117036
		Quetta	Muqarrab Shah & Wali-ur-Rehman	110032
		Quetta	Muqarrab Shah & Wali-ur-Rehman	111863
		Sibi	Manzoor & Maqsood	88581
		Kalat	Muqarrab Shah & Wali-ur-Rehman	114542
4	<i>Silene citrina</i> Boiss.	Chitral	Muqarrab Shah & Dilawar	60060
		Chitral	Muqarrab Shah & Dilawar	60035
		Chitral	Muqarrab Shah & Dilawar	60038
		Chitral	Muqarrab Shah & Dilawar	59754
		Chitral	Muqarrab Shah & Dilawar	56827
5	<i>Silene conoidea</i> L.	Attock	M. N. Chaudhry & Muqarrab Shah	17215
		Bannu	Shahzad & Dilawar	45301
		Chitral	Muqarrab Shah & Dilawar	60097
		Hazara	Muqarrab Shah, Dilawar	79250
		Hazara	Muqarrab Shah & Dilawar	78244
		Mianwali	Mir Ajab Khan & Maqsood	81535
		Mirpur	Shahzad & Arif	47457
		Muzaffarabad	Shahzad & Wali-ur-Rehman	84407
		Malakand Agency	Mir Ajab Khan & Zawar	107035
		Pishin	Manzoor & Arif	84391
		Quetta	Manzoor & Maqsood	52615
		Quetta	Manzoor & Maqsood	52605

Appendix 1. (Continue)

		Quetta	Manzoor, Maqsood	52613
		Rawalpindi	Manzoor & Javed	1003
		Skardu	Mir Ajab Khan & Nisar	97420
		Swat	Muqarrab Shah & Dilawar	19537
		Kurram Agency	Hafizullah & Ayaz	52645
6	<i>Silene falconariana</i> Benth.	Swat	Muqarrab Shah & Manzoor	14133
		Swat	Muqarrab Shah & Manzoor	14075
7	<i>Silene indica</i> var. <i>indica</i> (Roxb. ex Otth) Benth.	Chitral	M. N. Chaudhry & Mir Ajab Khan	123694
		Chitral	M. N. Chaudhry & Mir Ajab Khan,	123695
8	<i>Silene kunawerensis</i> Benth.	Chitral	Muqarrab Shah & Dilawar	39291
		Chitral	Muqarrab Shah & Dilawar	39292
9	<i>Silene longisepala</i> ** E.	Chitral	Wali-ur-Rehman & Subhan	121185
		Chitral	Wali-ur-Rehman & Subhan	115109
		Chitral	Wali-ur-Rehman & Subhan	115079
		Chitral	Wali-ur-Rehman & Subhan	117017
10	<i>Silene moorcraftiana</i> Wall.	Chitral	Muqarrab Shah & Dilawar	30612
		Skardu	Mir Ajab Khan & Nisar	95318
		Skardu	Mir Ajab Khan & Nisar	100225
		Skardu	Mir Ajab Khan & Nisar	97485
		Skardu	Mir Ajab Khan & Afzal	65018
		Gilgit	Mir Ajab Khan & Afzal	56799
		Ganche	Mir Ajab Khan & Afzal	66559
11	<i>Silene ovalifolia</i> * (Regel & Schmalh) Melzh.	Peshawar	Muqarrab Shah & Ayaz	49329
		Peshawar	Muqarrab Shah & Ayaz	49330
12	<i>Silene pseudo-verticellata</i> E.	Chitral	Muqarrab Shah & Dilawar	30590
		Chitral	Muqarrab Shah & Dilawar	30592
		Chitral	Muqarrab Shah & Dilawar	116714
		Chitral	Muqarrab Shah & Dilawar	117034
		Chitral	Muqarrab Shah & Dilawar	116895
13	<i>Silene tenuis</i> Willd.	Chitral	Muqarrab Shah & Dilawar	64988
		Chitral	Muqarrab Shah, Dilawar	64996
		Gilgit	Mir Ajab Khan & Afzal	62466
		Gilgit	Mir Ajab Khan & Afzal	62465
		Skardu	Mir Ajab Khan & Naeem	100234
		Muzaffarabad	Jan Muhammad	30659
14	<i>Silene villosa</i> Forssk.	Bannu	Hafizullah & Dilawar	50357
		Bannu	Hafizullah, Dilawar	50361
	<i>S. villosa</i>	Bannu	Shahzad & Dilawar	45311
		Bannu	Shahzad, Dilawar	45312
		Mianwali	Mir Ajab Khan & Maqsood	81534
		Mianwali	Mir Ajab Khan & Maqsood	81532
		Mianwali	Mir Ajab Khan & Maqsood	81533
		Mianwali	Mir Ajab Khan & Maqsood	83855

Appendix 1. (Continue)

15	<i>Silene viscosa</i> (L.) Pers.	Chitral	Muqarrab Shah & Dilawar	59733
		Chitral	Muqarrab Shah & Dilawar	56810
		Chitral	Muqarrab Shah & Dilawar	60071
		Chitral	Muqarrab Shah, Dilawar	59732
		Dir	Mir Ajab Khan & Zawar	108724
		Swat	Muqarrab Shah & Dilawar	18820
		Swat	Muqarrab Shah & Dilawar	18546
16	<i>Silene vulgaris</i> (Moench)Garcke	Baltistan	Mir Ajab Khan & Zawar	122318
		Chitral	Muqarrab Shah & Dilawar	62456
		Dir	G. Farooq & Ayaz	23382
		Diamer	M. Zubair & Khalid	117257
		Gilgit	M. N. Chaudhry & Muqarrab Shah	117396
		Hazara	Mir Ajab Khan & Nisar	121184
		Kurram Agency	Hafizullah & Nisar	66834
		Muzaffarabad	Shahzad & Ejaz	101038
		Poonch	Jan Muhammad	16301
		Skardu	Mir Ajab Khan & Nisar	161836
		Swat	Muqarrab Shah & Dilawar	23358

(*= Endangered, **= Vulnerable)

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