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Studies on the pollen morphology of the genus *Dianthus (*Caryophyllaceae) from Pakistan

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Abstract

Pollen morphological studies of 7 species of the genus *Dianthus* have been carried out from Pakistan by light microscopy. Pollen grains are Polypantoporate or periporate. There is a great variation in pollen morphology in the species of *Dianthus*.In *Dianthus crinitus* very thick exine with much prominent columella is species specific character. The palynological features of exine thickness, size of pollen, pori number, 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: *Dianthus*, 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.

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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. The family Caryophyllaceae is important due to medicinal as well as ornamental properties (Arora and Panday, 1996; Bakshi, 1984). In Pakistan,Caryophyllaceae family is represented by 26 genera and about 110 species (Nasir and Ali, 1986).Chaudhari & Qureshi (1991) reported 18 species of Caryophyllaceae to be endemic in Pakistan. Although palynological evidence has played an important role in discerning natural groups and understanding phylogenetic 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 are 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 various sorts of pollen data. Faegri and Iversen (1975) repoted that pollens of Caryophyllaceae are periporate. Number of pores is a diagnostic character for delimitation of genera.

Yildz (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 holostea L., 3. Cerastium type, 4. Dianthus type, 5. Gypsophila repens type, 6. Lychnis viscaria type, 7. Silene vulgaris type, 8. Silene caryophylloides subsp. Subulata (Poiret) Otth, 9. Silene conica type and 10. Agrostemma githago L., were recognized. Skvarla (1975), Skvarla and Nowicke (1976), Nowicke and Skvarla (1977), Ghazanfar (1984), Faegri 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. The purpose of present investigation is to provide palynological information of 7 species of *Dianthus* (Silenae) of family Caryophyllaceae which would be helpful to establish classification and phylogenetic relationship with Caryophyllaceae.

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 this material was used to prepare slides by using glycerine-jelly mixed with 1% Safranin. The prepared slides were studied under the light microscope using oil immersion.

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), Faegri 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 *Dianthus* species are presented in Figure 1.

Size: The size of pollen grains of the species of *Dianthus* ranges from 30-35 µm to 50-55µm. There is little variation in size of pollen grains. *D. barbatus* and *D. orientalis* have pollen grains similar in size (Table1).

Symmetry and Shape: The pollen grains are radially symmetrical, isopolar, periporate in all species and pollen surface is granulate in most of species except *D. angulatus, D. barbatus* and *D. orientalis* where surface is granulated as well as spinulate.

Pore diameter: Pore diameter ranges from 2.5-3.7µm to 6.0-10.0 µm. Pore diameter is similar in *D. crinitus* and *D. jacquemontii.* Pore surface is crustate in *D.angulatus* , *D. barbatus* and *D. orientalis* while ± crustate in *D.anatolicus, D. caryophyllus, D. crinitus* and *D. jacquemontii*.The distance between pores ranges from 5.0-6.0µm to 12.0- 14.0µm. Pore distance is same in *D. caryophyllus* and *D. orientalis*. Similarly, number of pores varies from 6 to 11 among the taxa. *D. barbatus*, *D. caryophyllus* and *D. orientalis* have same number of pores (8-9).

Exine: Exine thickness varies between 1.25-2.5µm to 5.0-6.25µm among the taxon. : Exine thickness emerges to be highest in *D. crinitus* (5.0-6.25µm) and the lowest (1.25-2.5µm) in *D.angulatus* and *D. barbatus.* Similar type of exine thickness (2.5-3.75µm) is noted in *D.anatolicus, D. caryophyllus* and *D. jacquemontii*.

Key to Dianthus species

1. Thick exine i.e.05-6.25µm ,prominent columella ……..……..*D. crinitus*
   Thin exine i.e.1.25-2.5µm, not prominent columella…….…..….2

2. Distance between pores 5-6µm……..…..…..…*D. anatolicus*
   Distance between pores more than 6µm….……..…….....…..3

3. Diameter of pore less than 5µm ……....….*D. barbatus*
   Diameter of pore less than 5µm…….…….....…4

4. Pollen size smaller i.e. 30-35µm …….……...……*D. jacquemontii*
   Pollen size larger i.e. above 40µm …….…….……5

5. Number of pores 10-11…….…….…*D. angulatus*
   Number of pores 08-09 …….……6

6. Diameter of pores 5-6µm …….……*D. orientalis*
   Diameter of pores 6-10µm …….…...…….*D. caryophyllus*

Table 1. Pollen Morphological Features of *Dianthus* (Silenae- Caryophyllaceae) Species

Taxon	Diameter Of pores	Distance between pores	Number Of pores	Exine thickness	Pore surface	Pollen surface	Pollen type
Dianthus anatolicus	2.5- 3.75µm	5- 6µm	9- 10	2.5- 3.75µm	±Crustate	Granulate	Periporate
D. angulatus	5- 10µm	12.5- 13µm	10- 11	1.25- 2.5µm	Crustate	Granulate, Spinulate	Periporate
D. barbatus	2.5- 5µm	10- 13µm	8- 9	i.25- 2.5µm	Crustate	Granulate, spinulate	Periporate
D. caryophyllus	6- 10µm	10- 12.5µm	8- 9	2.5- 3.75µm	±Crustate	Granulate	Periporate
D. crinitus	5- 6.25µm	12- 14µm	7- 8	5- 6.25µm	±Crustate	Granulate	Periporate
D.jacquemontii	5- 6.25µm	7.5- 10µm	6- 8	2.5-3.75µm	±Crustate	Granulate	Periporate
D. orientalis	5- 6µm	10- 12.5µm	8- 9	2.5- 3 µm	Crustate	Granulate, Spinulate	Periporate

(Measurements are in µm, ±= more or less)

Figures:

Figure 1. Showing poler view of selected species of *Dianthus*

Fig. 1a. Showing pores in polar view (at 100X) of *Dianthus anatolicus*

Fig. 1b. Showing pores in polar view (at 100X) of *Dianthus angulatus*

Fig. 1c: Showing pores in polar view (at 40 X) of *Dianthus barbatus*

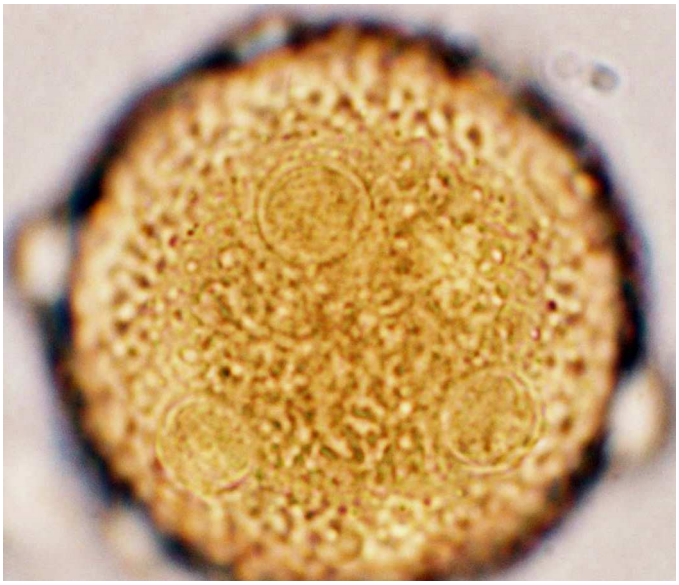

Fig. 1d. Showing pores in polar view (at 100X) of *Dianthus barbatus*

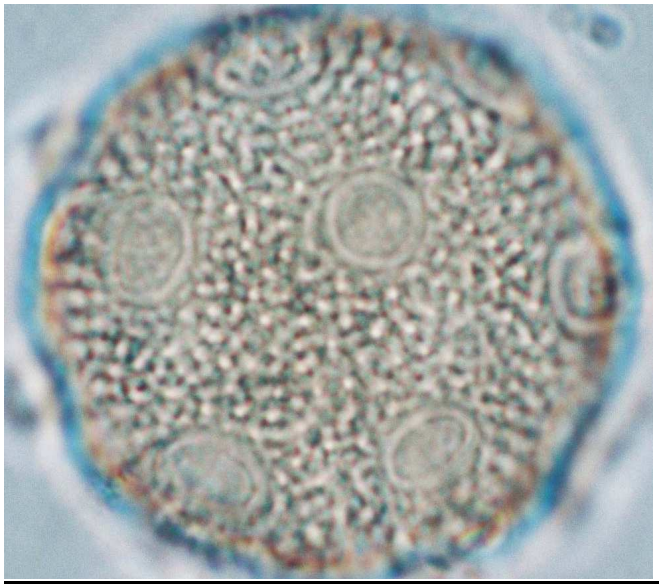

Fig. 1e: Showing pores in polar view (at 100X) of *Dianthus caryophyllus*

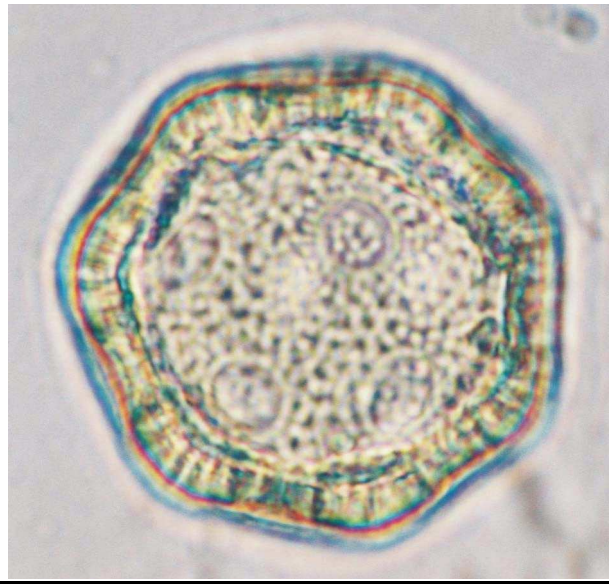

Fig.1f: Showing pores and exine in polar view (at 100X) of *Dianthus crinitis*

4. Conclusions

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 additional information for systematic studies. Huang (1972) also used pollen characters for systematic purposes.

Fig. 1g: Showing pores and exine in polar view (at 100X) of *Dianthus jacquemontii*

Palynological studies of different genera of family Caryophyllaceae have been carried out first time from Pakistan. A lot of work has been carried out in Turkey. During the study of pollen many of the polar views were examined. The pollen characters in some species help to distinguish at species level.

In *Dianthus* number of Pori is less than 12 while in *Silene* they are more than 12. Faegri and Iversen (1989) reported that *Dianthus* and *Silene* have less than 20 number of Pori. It indicates that pori is a constant character which seems to be helpful at generic level. All the pollens are polypantoporate or periporate. Thin exine with reduced columellae is a characteristic of primitive Angiosperms. In *Dianthus* and *Silene* exine and columella is not reduced to the extent to be considered as primitive feature in this group.

In *Dianthus crinitus* exine is very thick i.e. 5 – 6.25µm with much prominent columella, so it is a specie specific character. In *D. jacquemontii* pollen size is smaller than all other species of *Dianthus*. It is noted that when exine is thick, the diameter of Pori is smaller i.e. 2.5 – 3.75µm in *D. anatolicus.* In *D. orientalis* exine is thin with Pori size 5 – 6µm.

Exine thickness, pollen diameter, sculpturing and pori surface are same in *D. angulatus* and *D. barbatus* but there is a difference in diameter and number of pori which is less in *D. barbatus* as compared to *D. angulatus.*

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, 2002) demonstrated that the pollen are 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, 2002) examined exine by SEM of different genera including *Dianthus* species. Their findings are in accordance with the present studies. Moore *et. al.,* (1997) included Caryophyllaceae in

polypantoporate 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 *D. angulatus* i.e.10-11.

Dianthus barbatus and *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, 2000). Yildiz, (2001b) reported that number of pori is from 9 – 42 and many of the species have 10 – 20 pori while present studies shows 8 – 9 pori in *D. barbatus*.

Yildiz (2001b) observed that pollen diameter of *D. orientalis* was between 35 and 45µm. Pori diameter was between 4.5 and 5.5µm. The distance between two Pori ranged from 11 – 12µm. The number of Pori ranged from 14 – 17. The present studied has revealed that pollen diameter of *D. orientalis* is between 40 and 50µm. Pori diameter was between 5 and 6µm. The distance between two Pori ranged from 10 – 12.5µm. The number of Pori ranged from 8 – 9. However, in the present study the number of Pori is less but there is no major difference between two studies, it indicates a range of variation in number of pori from Pakistan.

Since there has not been any palynological data available on *Dianthus* in Pakistan, this investigation is attempted to represent first reference on the subject. Diameter of pollen, and Pori, thickness of exine, distance between two Pori, the number of pori, surface of pori and sculpturing of *Dianthus* and *Silene* would be helpful to understand taxonomic position within Caryophyllaceae.

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Appendix I: Specimens of *Dianthus* investigated from various areas of Pakistan

Sr. No.	**Taxon**	**Locality**	**Collector**	**Vocher No.**
1	*Dianthus anatolicus* Boiss.	Swat	Muqarrab Shah & Dilawar	14074
		Swat	Muqarrab Shah & Dilawar	68515
		Diamer	M. Zubair & Khalid Javed	117253
		Diamer	M. N. Chaudary & Muqarrab Shah	119262
		Gilgit	M. Fayyaz & Ahamad	118073
2	*Dianthus angulatus* Royle	Dir	Mir Ajab Khan & Zawar Hussain	115117
		Gilgit	Mir Ajab Khan & Zawar Hussain	122033
		Gilgit	Shazad & Ashraf	41049
		Chitral	Muqarrab Shah & Dilawar	60075
		Chitral	Muqarrab Shah & Dilawar	62445
3	*Dianthus barbatus* L.	Chitral	Muqarrab Shah & Dilawar	56746
		Muzaffarabad	Jan Muhammad	19529
		Muzaffarabad	Jan Muhammad	19528
4	*Dianthus caryophyllus* L.	Rawalpinidi	Dilawar, Khalid	108728
		Rawalpinidi	Dilawar & Khalid	54418
5	*Dianthus crinitus* Sm.	Chitral	Wali-ur-Rehman & Subhan	115135
		Chitral	Wali-ur-Rehman & Subhan	115080
		Gilgit	Wali-ur-Rehman & Muqarrab Shah,	118916

Appendix I: (Continue)

		Gilgit	M. N. Chaudary & Muqarrab Shah	118915
		Kohat	Hafizullah & Dilawar	47505
		Kohat	Hafizullah & Dilawar	50379
		Kurram Agency	Hafizullah & Ayaz	68502
		Malakand Agency	Mir Ajab Khan & Zawar Hussain	105459
		Waziristan	M. Zubair & Saeed	115127
6	*Dianthus jacqumontii* Edgew.	Skardu	M. N. Chaudary & Muqarrab Shah	117259
		Muzaffarabad	Shazad Iqbal & M.Ayaz	99126
		Chitral	Shazad Iqbal & M.Ayaz	62452
		Hazara	Shazad & Ashraf	30638
		Hazara	Shaukat & Nisar	30558
		Mansehra	M. N. Chaudary & Muqarrab Shah	97414
7	*Dianthus orientalis* Adams.	Skardu	Mir Ajab Khan & Afzal	64990
		Skardu	Mir Ajab Khan & Nisar	65900
		Skardu	Mir Ajab Khan & Nisar	66858

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