www.biodicon.com Biological Diversity and Conservation

ISSN 1308-5301 Print; ISSN 1308-8084 Online BioDiCon 2/3 (2009) 1-6

Pollen Morphology of some *Plantago* species native to Saudi Arabia and their taxononomic implication

Najat Abdul-Wahab BUKHARİ *

Botany and Microbiology department, College Of Science, King Saud University, Medical Studies and Science Section, 11495 Riyadh, Saudi Arabia

Abstract

Pollen grain morphology of 6 *Plantago* species of the family Plantaginaceae collected from Saudi Arabia have been observed under light and scanning electron microscope (SEM). Description of grain sculpture is illustrated by SEM. Plantaginaceae is a stenopalynous family. Pollen grains are free, medium-sized or small, radially symmetrical, apolar, porate, spherical or prolate spheroidal in shape with verrucate granulated sculpturing. Internal aperture has pores scattered on the wall of pollen grains. Identification feature of individual taxa include type of aperture, ornamentation of the pollen grain, annulus structure and pore diameter. The pollen morphology of the family Plantaginaceae is significantly helpful at specific level. On the basis of exine ornamentation 2 distinct pollen types viz., *Plantago – major* and *Plantago* – □*vate*-type are recognized.

Key words: *Plantago*, Pollen grains, Plantaginaceae, SEM

1. Introduction

Plantaginaceae is a small family of c. 3 genera and 275 species, chiefly of North Temperate Zone and south East Africa (Mabberley, 1987). In Saudi Arabia it is represented by a single genus *Plantago* with 14 species (Collenette, 1999). The genus *Plantago* is characterized by herbaceous habit; leaves in a basal rosette with prominent parallel veins; spicate or capitate inflorescences or wiry scapes; flowers 4-merous; corolla membranous; stamens often exerted. Seeds of *Plantago psyllium* are used as a laxative. Major investigations were using pollen grains as palynological evidence in paleobotany (Bernardello and Lujan, 1997; Carine and Scotland, 1998; Dessein *et al.*, 2000; Delaporte *et al*., 2001).

Generative parts of plants, mature pollen grains in particular, are much less variable than vegetative parts. Hence, pollen is a convenient material for investigation, because it is in a steady physiological stage, has a single set of chromosomes and forms large populations. Mature pollen grain size, exine sculpturing, and number of pores are the most distinctive features (Erdtman, 1952; Faegri and Iversen, 1992; Klimko *et al*., 2000). Considerable palynological studies have been done on the pollen morphology of the family Plantaginaceae and pollen grains of the genus *Plantago* have been divided into several types.

The earliest report is that of Erdtman (1952), Kapp (1969), Serbanescu-Jitariu (1971), Solomon *et al*., (1973), Rao & Shukla (1975), Moore & Webb (1978), Kuprianova & Alysoshina (1978). Markgraf & Dantoni (1978) who studied the pollen of Plantaginaceae. In all these studies no attempt has been made to correlate the pollen characters with the taxonomy of the family.

There are scarce reports on pollen morphology of various species of Plantaginaceae found in Saudi Arabia. Despite intensive research, there are still some discrepancies in classification, morphological descriptions of *Plantago* species. In present study pollen morphology has been used as a taxonomic feature of genus *Plantago* for species delimitation. This is an attempt to correlate the pollen characters with the taxonomy of the genus.

* *Corresponding author* / Haberleşmeden sorumlu yazar: najatab@ksu.edu.sa

2. Materials and methods

Pollen samples were obtained from King Saud University Herbarium (KSUH) or collected from the field. The lists of voucher specimens are deposited in KSUH. The pollen grains were prepared for light (LM) and scanning microscopy (SEM) by the standard methods described by Erdtman (1952). For light microscopy, the pollen grains were mounted in unstained glycerin jelly and observations were made with a Nikon Type-2 microscope, under (E40, 0.65) and oil immersion (E100, 1.25), using 10x eye piece. For SEM studies, pollen grains suspended in a drop of water was directly transferred with a fine pipette to a metallic stub using double sided cellotape and coated with gold in a sputtering chamber (Ionsputter JFC-1100). Coating was restricted to 150A. The SEM examination was carried out on a Jeol microscope JSM-T200. Additionally, we analyzed a sample of pollen of *P. alpina* L. for comparison with *P. maritime*, because of its earlier classification (Clark and Jones, 1977). Pollen grain dimensions were measured in the equatorial (E) and Polar (P) plane by means of laboratory imaging software. The measurements were taken for 20 pollen grain from each species. The ratio of length in the P plane to that in the E plane enabled an assessment of microscopic shape, which was classified in accordance with Erdtman (1952); Faegri & Iversen (1964); Kremp (1965) and Walker & Doyle (1976). We also measured pore diameter and annulus width. Moreover qualitative traits concerning the stage of development of the annulus, operculum and aperture membrane were taken into account. Values of those traits are given in Table 1.

Table 1. Values of those traits

Trait	Description
No annulus	0
Annulus with disjunctive verrucae	1
Partly opened	2
Well developed annulus	3
Operculum solid	2
Aperture membrane granulate	1
Verrucae slightly convex	1
Verrucae clearly convex	2
Verrucae well developed	3

3. Results

Pollen grains of *Plantago* species are usually radially symmetrical, medium sized or small, spherical or prolate spheroidal or prolate, always verrucate (verrucae varying in size and shape), with granulation. The annulus in the Plantaginaceae seems to be formed by thickening and aggregation of verrucae around the pores. In some species the annulus is continuous, but in other cases the verrucae are disjunctive and form a partial annulus. The operculum in some species is a solid lid whose structure seems to be identical with the rest of sexine. Other species have a partial operculum, with sexine granules on the aperture membrane. These granules are usually widely spaced and small, but may be larger. Exine is rather thin or moderately thick, sexine undulating, about twice as thick as nexine (Clark and Jones, 1977; Malgorzata *et al*., 2004). Tectum generally aerolate to scabrate.

Key to the pollen types

+ Tectum areolate …..……..…….…… *Plantago major* –type
- Tectum scabrate ……...……..……. *Plantago ovata* – type

3.1. Species: *P. coronopus*

Plantago major-type (Fig 1A-C)
Pollen class: 5-8 pantoporate.
Size: medium, P, 27.35 µm; E, 23.27 µm.
P/E ratio: 1.2: grains mostly prolate spheroidal or spherical.
Aperture: Ectoaperture- pore circular in outline, often sunken, pore diameter 2.06 µm; margin well-defined, regular with a wide, continuous, raised annulus 2.76 µm, membrane covered with irregular sexine granules, forming an ill-defined and poorly developed operculum.
Ornamentation: Verrucate, the verrucae large, coarse, well defined, irregular to circular in outline, channels between verrucae with very few puncta.
Comments: The distinguishing character of the *P. coronopus* type is the very prominent annulus, which is more and better developed than in any other species in Saudi Arabia. These results consistent with other results obtained by Al-Quran, 2004.

3.2. Species: *P. lanceolata*

Plantago major-type (Fig1 D-F)
Pollen class: 9-12 pantoporate.
Size: medium, P, 26.12µm; E, 22.14µm.
P/E ratio: 1.1: grains usually prolate spheroidal or spherical.
Aperture: Ectoaperture- pore circular in outline, pore diameter 2.86 µm; margin well-defined, annulus slightly raised 1.76 µm wide; well-defined solid operculum, truly formed. Endoaperture-pore congruent are highly developed with ectoaperture and considered as distinguishing feature in pollen grain.
Ornamentation: Verrucate, the verrucae large, irregular in outline, often rather diffuse, channels between verrucae with minute puncta.
Comments: The most obvious distinguishing character of the *P. lanceolata* type is the complete operculum. This type is the only of the family that is truly operculate (Clark and Jones, 1977). Our results consistent with the results of Sadowska *et al*., 1986 who reported that operculum in this species was well defined and annulus was rather narrow.

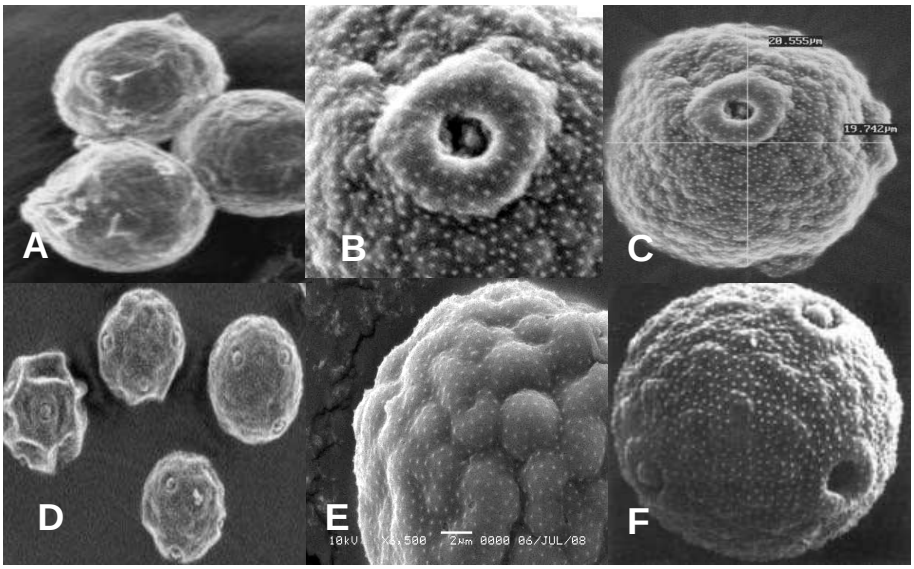

Figure 1. Scanning micrographs: *Plantago coronopus:* A, pollen grain; B and C, Exine pattern. *Plantago lanceolata:* D, pollen grain; E and F, Exine pattern. Scale bar = A, D =10; B, C, E and F = 1 µm

3.3. Species: *P. cylindrica*

Plantago Ovata-type (Fig 2 A-C)
Pollen class: 5-8 pantoporate.
Size: medium, P, 26.35 µm; E, 24.27 µm.
P/E ratio: 1.11: grains mostly prolate spheroidal or spherical.
Aperture: Ectoaperture- pore rather than irregular to circular in outline, often sunken, pore diameter 1.86 µm; margin ill defined, annulus absent; membrane covered with sexine granules forming ill defined operculum.
Ornamentation: Verrucate, the verrucae large, coarse, well defined, irregular in outline, not concentrated or differentiated around pores.
Comment: The verrucae of *P. cylindrica* are large, dense and very coarse. Sculpture more rough and conspicuous than in any other pollen type in the family. Our results confirmed the data on pore diameter given by Clarke and jones (1977) and Faegri and Iversen (1992).

3.4. Species: *P. albicans*

Plantago ovata-type (Fig 2 D-F)
Pollen class: 5-8 pantoporate.
Size: medium, P, 26.52µm; E, 23.67µm.
P/E ratio: 1.12: grains usually subprolate, prolate spheroidal or spherical.
Aperture: Ectoaperture-pore rather irregular to circular or elliptical, sometimes slightly sunken, pore diameter 2.26 µm; margin ill-defined, irregular. Absence of annulus, membrane covered with granules forming an ill-defined operculum. Endoaperture-pore congruent with ectoaperture.

Ornamentation: Verrucate, the verrucae well defined, slightly convex, irregular in outline, variable around pores, channels between verrucae with minute puncta.
Comments: Marked differences were observed in both quantitative and qualitative traits.

3.5. Species: *P. ciliata*

Plantago ovata-type (Fig 2 G-I)
Pollen class: 5-8 pantoporate.
Size: medium, P, 23.25µm; E, 19.56 µm.
P/E ratio: 1.18: grains usually subprolate.
Aperture: Ectoaperture- pore rather irregular to elliptical in outline, often clearly sunken, pore diameter 3.66 µm; margin ill- or well defined. Absence of annulus, membrane covered with sexine granules forming an ill-defined operculum. Endoaperture-pore congruent with ectoaperture.
Ornamentation: Verrucate, the verrucae very well defined, varying in size and shape, small or large, club-shaped. Channels between verrucae with distinct but fine granulation.
Comments: Pollen grains of *P. ciliate* were varied in size to a large extent. Apart from those described above, some grains were small sized. Klimko *et al*., 2004 reported the differences in pollen grain morphology and fully justify the separation of *P. ciliate as a distinct species.*

3.6. Species: *P. ovata*

Plantago ovata-type (Fig 2 J- L)
Pollen class: 5-8 pantoporate.
Size: medium, P, 25.15µm; E, 22.56 µm.
P/E ratio: 1.11: grains usually spheroidal.
Aperture: pori-small, pore diameter 5.56 µm; circular in outline with operculum and annulate membrane covered with sexine granules thicker than nexine.
Ornamentation: Tectum scabrate, scabrae coarse or fine. The outline more or less circular shaped. Channels between verrucae with distinct but fine granulation.
Comments: Distinguished character of this type is the wide pore size reached up to 5.56 µm sunken membranous annulus and circular operculum.

4. Conclusions

Plantaginaceae is stenopalynous family (Erdtman, 1952). Pollen grains of Plantaginaceae are characterized by a polar, spheroidal, operculate to non operculate, annulate to non annulate, pantoporate with scabrate or areolate tectum. Pollen of all the 6 species, belonging to a single genus i.e., *Plantago* are remarkably uniform in their pollen characters. However, species of *Plantago*, show little variation in their tectum type.

On the basis of tectum two distinct pollen types are recognized viz., *Plantogo major* and *Plantago ovata.* Pollen type: *Plantago- major* is readily distinguished by its areolate tectum in which 2 species viz., *P. lanceolata and P. coronopus are* included. On the basis of presence and absence of operculum and numbers of pores these species are further divided into two species and one species group. (See key to the species group). Pollen type: *Plantago ovata* is delimited by its scabrate tectum. In the *Plantogo ovata*-type, 4 species viz., *P. cylindrical, P. albicans, P. ciliate* and *P. ovata.* L., are included. Similar to *Plantago major*- type, this pollen type is also further divided into two groups on the basis of exine thickness. In one group pollen grains have 0.66 µm thick exine, while the remaining species of this pollen type have 1.2-2.47 um thick exine.

Pollen studies clearly indicate that the genus *Plantago* is a homogenous taxon in accordance with the morphology of the genus. Perveen & Qaiser (2004) examined 14 species of plantago from Pakistan by SEM. Their findings are in consistent with the present studies. Using these palynological studies to delimit the different species of *Plantago* (family Plantagoinaceae) is useful, because it is obvious from the results, that some palynological parameters may be useful to distinguish the 6 types of pollen grains. These are; the presence or absence of operculum and their development; annulus surface and margins of ectaperture and costae of endoaperture. From the results of the present study the following taxonomic key has been concluded:

+ Raised annulated
- Well developed solid operculum…….……*P. lanceolata*
- Ill-defined and poorly developed operculum……..…… *P. coronopus*

++ Sunken annulated
- Pore size up to 2.26 µm ……..…….. *P. cylindrica*
- Pore size up to 1.86 µm.……..…….. *P. albicans*
- Pore size up to 3.66 µm.……..…….. *P. ciliate*
- Pore size up to 5.56 µm.……..…….. *P. ovata*

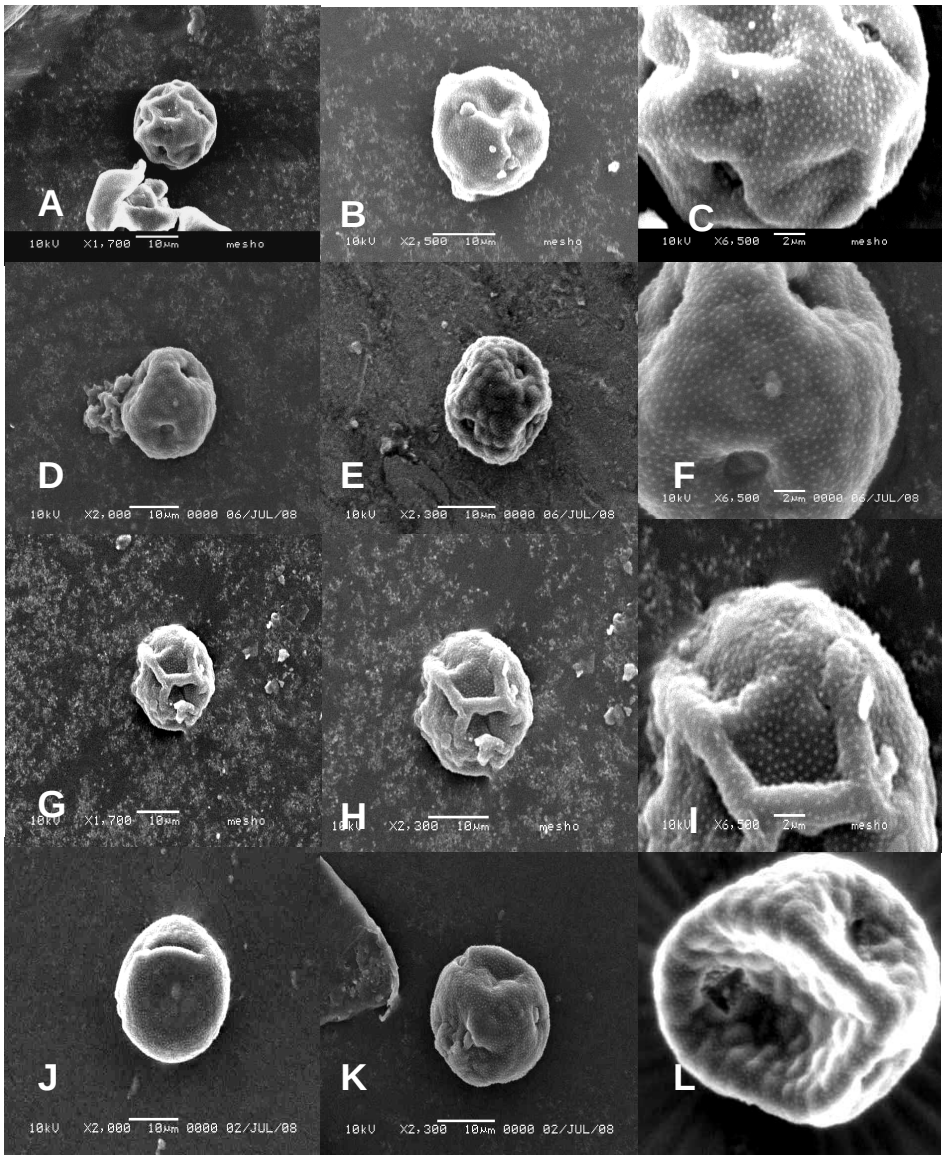

Figure 2. Scanning micrographs: *Plantago cylindrica:* A, pollen grain; B and C, Exine pattern. *Plantago albicans:* D, pollen grain; E and F, Exine pattern. *Plantago ciliata:* G, pollen grain; H and I, Exine pattern. *Plantago ovata:* J, pollen grain; K and L, Exine pattern. Scale bar = A, D, G, J =10; B, C, E and F, H, I, K, L = 1 µm.

Acknowledgements

I would also like to thank Dr. Mohamed Ibrahim, associate professor in botany department, Faculty of science, Alexandria University and king Saud university for his suggestions and revision the manuscript.

References

Al-Quran, S. A. 2004. Pollen morphology of Plantagoniaceae in Jordan. Pak. J. Biol. Sci. 7 (9): 159

Bernardello, L., Lujan, M.C. 1997. Pollen morphology of tribe *Lycieae*: *Crabowskia*, *Lycium*, *Phrodus* (Solanaceae). Review of palaeobotany and palynol., 96:255-280.

Carine, M. A., Scotland, R. W.1998. Pollen morphology of *Strobilanthes blume* (Acanthaceae) from Southern India. Review of Palaeobotany and Palynology, 103:143-165.

Clark, G.C.S., Jones, M.R. 1977. Plantagoniceae. In: The North west European Pollen flora. Rev. palynol. 24(4): 129-154.

Collenettes, S. 1999. Wild flowers of Saudi Arabia, N.C.W.C.D. Riyadh: 81 – 84.

Delaporte, K. 2001. Morphological analysis to identify the pollen parent of an ornamental interspecific *Eucalyptus*. Scientia Horticulturae, 89:57-74.

Dessein, S. 2000. Pollen morphological survey of Pentas (Rubiaceae-Rubioideae). Review of Paleobotany and Palynology, 112:189-205.

Banks, H., Gasson, P. 2000. Pollen morphology and wood anatomy of Crudia group (Leguminosae, Caesalpiniodeae, Detarieae). Botanical J. Linnean Soc., 134: 19-59.

Erdtman, G. 1952. Pollen Morphology and Plant Taxonomy. Angiosperms. Chronica Botanica Co., Waltham, Massachusettes. 1952.

Faegri, K., Iversen, J. 1964. Testbook of Pollen Analysis. Munksgaard, Copenhagen.

Grant, M., Blackmore, S., Morton, C. 2000. Pollen morphology of the subfamily Aurantioideae (Rutaceae). Grana 39:8–20

Kapp, R.O. 1969. How to know pollen and spores. Pictured key. Nature series, M.C. Brown. Company publishers Dubuque, Iowa, X + 249, 299fig.

Kazmi, M.A. 1974. Plantaginaceae. In: Flora of Pakistan. (Eds.): E. Nasir & S.I. Ali. No. 62 pp. 1- 21, Islamabad.

Kremp, G.O.W. 1965. Encyclopaedia of Pollen Morphology, Univ. Arizona Press, Tuscon, U.S.A.

Klimko, M., Kluza, M., Kreft, A. 2000. Morphology of pollen grains in three varieties of *Helianthus annuus* L. Rocz. Akad. Rol. W Pozn. S. Botanika 3: 135- 142.

Klimko, M., Idzikowska, K., Truchan, M., Kreft, A. 2004. Seed sculpture of polish species of the genus Plantago L. Acta Soc. Bot. Pol. 73(2) 103-111.

Kuprianova, L.A., Alyoshina, L. A. 1978. Pollen dicotyledoneaerum Florae Partis Europareae. URSS. Lamiaceae-Zygophyllaceae. (In Russian). Nauka 184 p. Akad. Sci. USSR .L. Komarov. Inst. Bot.

Mabberley, D.I. 1987. The plant book. Camb. Univ. Press, Cambridge, New York.

Markgraf, V., Dantoni, H. L. .1978. Pollen Flora of Argentina. Univ., Arizona Press, Tuczon.

Moore, P. D., Webb, J. A. 1978. An illustrated guide to pollen analysis. Hodder and Stoughton, London.

Perveen, A., Qaiser, M. 2004.Pollen flora of Pakistan: XXXVIII Plantaginaceae. Pak. J. Bot., 36(1):19-24.

Rao, A.R., Shukla, P. 1975. Pollen flora of upper Gangetic plane. Today and Tomorrow‘s printers &publishers, New Delhi 30 p.

Sadowaska, A., Kuszell, T., Loren, K. 1986. *Plantago lanceolata* L. In: Kartoteka palinologiczna roslin polskish. Zeszyty Przyrod. Opol. Tow. Przyj. Nauk. 24: tab.294.

Serbanescu – Jitariu, G. 1971. Ceretari palinologica asupra reprezentantilor familiei Plantaginaceae din flora romana. Anal Univ. Buc., Biol. Veg., 20: 69-73.

Solomon, A.M., King, J.E., Martin P.S., Thomas, J. 1973. Further scanning electron photomicrographs of Southeastern pollen grains. Arizona. Acad. Sci., 135-157.

Victor, J. E., Van Wyk, A. E. 2000. Pollen morphology of Phyllosma and Sheilanthera (Diosminae: Rutaceae) and its taxonomic implications. Grana 39:103–107

Victor, J. E., Van Wyk, A. E. 2001. Pollen morphology of Euchaetis and Macrostylis (Diosminae-Rutaceae) and its taxonomic implications. Grana 40:105–110

Walker, J.W., Doyle, J.A. 1976. The basis of Angiosperm phylogeny: Palynology. Ann. Mo. Bot. Gard., 62: 666-723.

Willis, J.C. 1973. A dictionary of the flowering Plants & Ferns. VII ed. University press, Cambridge.

(Received for publication 19 April 2009; The date of publication 01 December 2009)