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Palynological and seed micromorphological studies on *Plantago* L. species in North-Eastern of Iran

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Key words: Palynology, Micromorphology, Iran, *Plantago*, pollen.

Abstract

This study has examined palynological and seed micromorphological characteristics of eight *Plantago* species growing in NE Iran. There are a few reports about *Plantago* pollen and seed. So above studies was done on *Plantago* species for assessing the variation of seed and pollen features. For this, the pollen and seed were noticed by SEM. Some differences such as Polar axis length/Equatorial length axis, shape and ornamentation were perceived. According to the results, seed ornamentation showed significant variation among the specimens and lead to prepare identification key for studied species.

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Introduction

Plantago L. belongs to Plantaginaceae family has 22 species in Iran which eight species distributed in NE Iran (Patzak 1965) Due to phenyl propanoid, glycosides and verbascosides this genus, has antimicrobial, antifungal, antitumor characters and antioxidant composition (Toda *et al.*, 1985, Galvez *et al.*, 2005). Leaves and seeds of *Plantago major* have been used for centuries to treat diseases relating to skin, digestive organs and blood circulation like wounds, inflammation and hypertension (Zubair, 2010). The seeds used in stomach ulcer disease and rhino conjunctivitis, compress for snakebites (Chmielewska *et al.*, 2012) and the cholesterol lowering effect (König 2012). Despite many studies of medicinal characteristics, there is a few reports about *Plantago* pollen and seed. The previous reports of palynological and seed micromorphological studies on *Plantago* followed the Northwest European pollen flora (Clarke and Jones 1980), Pollen morphology and chromosome numbers of the family Plantaginaceae in North America (Basset and Crompton 1968), Biosystematic study on *Plantago* species (sections *Coronopus* and *Oreades*) (Cartier 1970), pidermal and seed coat surface characters of plants: Systematics applicability and some evolutionary aspects (Barthlott 1981), Palynological study of the genus *Plantago* in the Iberian peninsula (Ubera *et al.*, 1988), On the Taxonomy of Plantaginaceae Juss. Sensu Lato: evidence from SEM of the Seed Coat (Shehata 2006). In the present

research, palynological and seed micromorphological characteristics of *Plantago* species in NE Iran were evaluated. The aim of this study was to observe the variation of pollen and seed features and distinguishing their role in identification of *Plantago* species in NE Iran.

Materials and methods

For palynological and seed micromorphological studies, the extracted seven pollen and eight seeds species of *Plantago* in NE Iran were coated with sputter and studied by SEM respectively. Then features such as the size, shape, polar axis length/ equatorial axis length and ornamentation were evaluated in magnification 120, 5000, 10000 and 20000. Their localities were presented in Table 1. Pollen and seed terminology was based on Punt *et al.*, 2007 and Hesse *et al.*, 2009.

Results and discussion

Pollen characteristics in seven studied species of *Plantago* displayed, pantaporate pollen with suboblate, prolatep-spheroidal shape and verrucate to granulate ornamentation with andwithout annulus. There was no significant characteristics in studied *Plantago* The detail of pollen characteristics was presented in Table 2 and Fig. 1A-G. The seed micromorphological study results showed, prolate, subprolate to perprolate shape including areolate and reticulate ornamentation (Table 3 and Fig. 2A-O).

Table 1. The locality of studied *Plantago* species.

Species	Locality
<i>P. major</i>	Shirvan, Firouz Gholjogh, 2600 m, Hoghoughi, 9722 (IAUM); North west of Mashhad, Moghan, 850 m, Aiatollahi and Zangouei, 14952 (FUMH).
<i>p. ovata</i>	88 km Boshrouieh to Tabas, Aiatollahi and Zangouei, 13778 (FUMH).
<i>P. maritima</i>	10 km Quchan to Mashhad, 1317 m, Hoghoughi, 9723 (IAUM); Birjand, between asghouk and khorashad, bagheran mount, Alang Rahizak, 1950 m, Jopharchi & Zangouie, 17716 (FUMH).
<i>P. coronopus</i>	North east of Mashhad, 2 km east of Sanganeh to Chahchahe, 400 m, Joharchi & Zangouie, 17428 (FUMH).
<i>P. psyllium</i>	South east of Tabas, Niaz village, Morteza Ali, 1350 m, Rafei and Zangouei, 26290 (FUMH).
<i>P. evacina</i>	South west of Torbat Jam, Eskandarabad, 1050 m, Joharchi & Zangouie, 19953 (FUMH); South west of Srakhs, between Salehabad and Baghbaghou, Rahmatabad hills, 689 m, Joharchi and Nasseh, 42669 (FUMH).
<i>P. gentanoides</i>	Birjand, between Asghouk and Khorashad, Bagheran mount, Alang Rahizak, 1950 m, Jopharchi and Zangouie, 17718 (FUMH),.
<i>P. lanceolata</i>	Shandiz, Zoshk village, 2000 m, Hogoughi, 9724 (IAUM); South west of Bojnourd, Rein, eastern slope of Shahneshin mount, 1700 m, Zangouei and Memariani, 376/0 (FUMH).

Table 2. The details of studied *Plantago* species pollen characteristics.

species	pollen shape	pollen ornamentation	P/E
<i>P. major</i>	Prolate-spheroidal	Verucate, granulate, without annulus in pore, pore 1 membrane granular	
<i>P. ovata</i>	Oblate spheroidal	Outline infolding, verucate-granulate	0.9
<i>P. maritima</i>	Prolate-spheroidal	Granulate, without annulus in pore, pore membrane granular	1.04
<i>P. coronopus</i>	Oblate spheroidal	Verucate- granulate, with prominent annulus in pore, pore membrane granular	0.9
<i>P. psylimum</i>	Sub oblate	Outline infolding, Verucate- granulate	0.8
<i>P. evacina</i>	Prolate-spheroidal	Outline infolding, verucate-granulate	1.05
<i>P. lanceolata</i>	Oblate spheroidal	Outline infolding, verucate-granulate	0.9

In all previous reports, pollen of *Plantago* was periporate, apolar, spheroidal, with 4-16 pores, faint or no annulus to markedly protruding annulus and pore with an operculum or granular membrane, tectate-perforate, spinulate, psilate to distinctly verrucate, occasionally insulate (Ubera *et al.*, 1988). Gorenflot (1959) and Aubert *et al.* (1962) explained palynological characters did not contribute much in

identification. Also, Blaise (1963) “Blaise (1963) studied *P. coronopurus* and *P. niacrorrhiza* palynologically and observed similar characteristics in both species, although he demonstrated a clear relationship (direct for the former and inverse in the latter) between the number of pores and the diameter of the pollen grains” (Ubera *et al.*, 1988).

Table 3. The details of studied *Plantago* species seed characteristics.

species	seed shape	seed ornamentation	P/E	Muri shape	Lumina shape
<i>P. major</i>	Prolate	Areolate with wrinkled surface	1.4	-	-
<i>P. ovata</i>	Prolate	Reticulate,	2	smooth	Weakly smooth
<i>P. maritima</i>	Prolate	Regulate sometimes areolate	1.7	-	-
<i>P. coronopus</i>	Prolate	Reticulate, free standing collumelae	1.8	Irregular prominent	Wrinkled with prominent columella
<i>P. psylimum</i>	Sub prolate	Bi- reticulate	1.3	Smooth, linear	bi- Irregular reticulate surface
<i>P. gentianoides</i>	Sub prolate	Reticulum cristatum	1.3	Thick, prominent	very Wrinkled surface
<i>P. lanceolata</i>	perprolate	Negative reticulate	2.3	Deep smooth	and Prominent, almost smooth surface

Cartier (1970) and Bassett and Cromptom (1968) and reported a direct relation between the diameter of the pollen grain and the ploidy level in *Plantago* species. Moreover, Cartier (1970) showed differences in some species of the different sections *i.e.* between *P. alpina* Villares and *P. maritima* (subsp. *serpentina*) on the basis of the presence of annulus in the former and its absence in the latter. Rahn (1974) studied the *Plantago* species in North America and described a relationship between the diameter and polyploidy of pollen. Clarke and Jones (1980) studied Plantaginaceae from North- Western Europe by LM

and SEM and proposed a key for the pollen types tectate-perforate, spinulate, psilate to distinctly verrucate, occasionally insulate (Ubera *et al.*, 1988). Furthermore, Ubera *et al.* (1988) mentioned pore membrane granular with supracteal spinules in *Plantago* studied pollen. He reported *P. major* pollen with (-5) 6 (-9) pores, without annulus; *P. coronopus* pollen with (-5) 6 (-8) pores and markedly protruding annulus, *P. maritima* pollen with (-5) 6 (-7) pores and very smooth annulus, *P. lanceolata* pollen with 10-12 pores, an operculum on markedly protruding spinulate annulus, *P. ovata* pollen with 4-5(-6)

pores, without annulus (Ubera *et al.*, 1988). While, in present report *P. maritima* had no annulus and *P. ovata* had annulus.

Since seed characteristics varied among the studied species, consequently, an identification key of species was presented on the basis of seed shape and ornamentation.

The seed results showed, prolate to perprolate class including vesiculate and reticulate ornamentation (Table 3 and Fig. 3).

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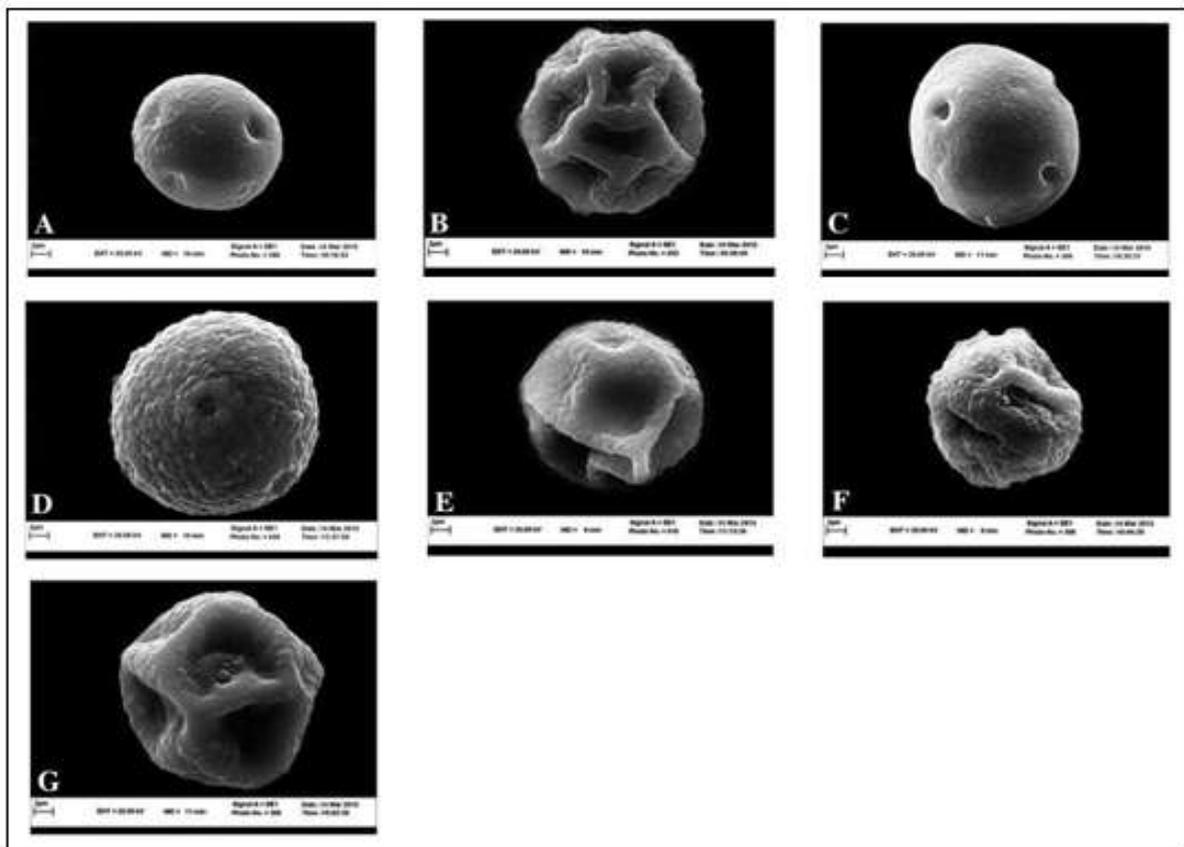


Fig. 1. Electro-micrograph of *Plantago* pollen. (A) *P. major*, (B) *P. ovata*, (C) *P. maritima*, (D) *P. coronopus*, (E) *P. psyllium*, (F) *P. evacina*, (G) *P. lanceolata* × 10000.

Identification key of studied Plantago species based on seed micromorphological data

- 1 No reticulate ornamentation.....2
- Reticulate or negative reticulate ornamentation.....3
- 2 Colliculate ornamentation*P. major*
- Ruminant ornamentation*P. maritima*
- 3 Negative reticulate.....*P. lanceolata*
- Reticulate or bi-reticulate.....4

- 4 Bi-reticulate, smooth bi-linear muri.....*P. psyllium*
- Reticulate with smooth or prominent muri.....5
- 5 Prominent muri.....6
- Smooth and thin muri.....*P. ovata*
- 6 Reticulate with free standing collumalae..... *P. coronopus*
- reticulum cristatum.....*P. gentianoides*

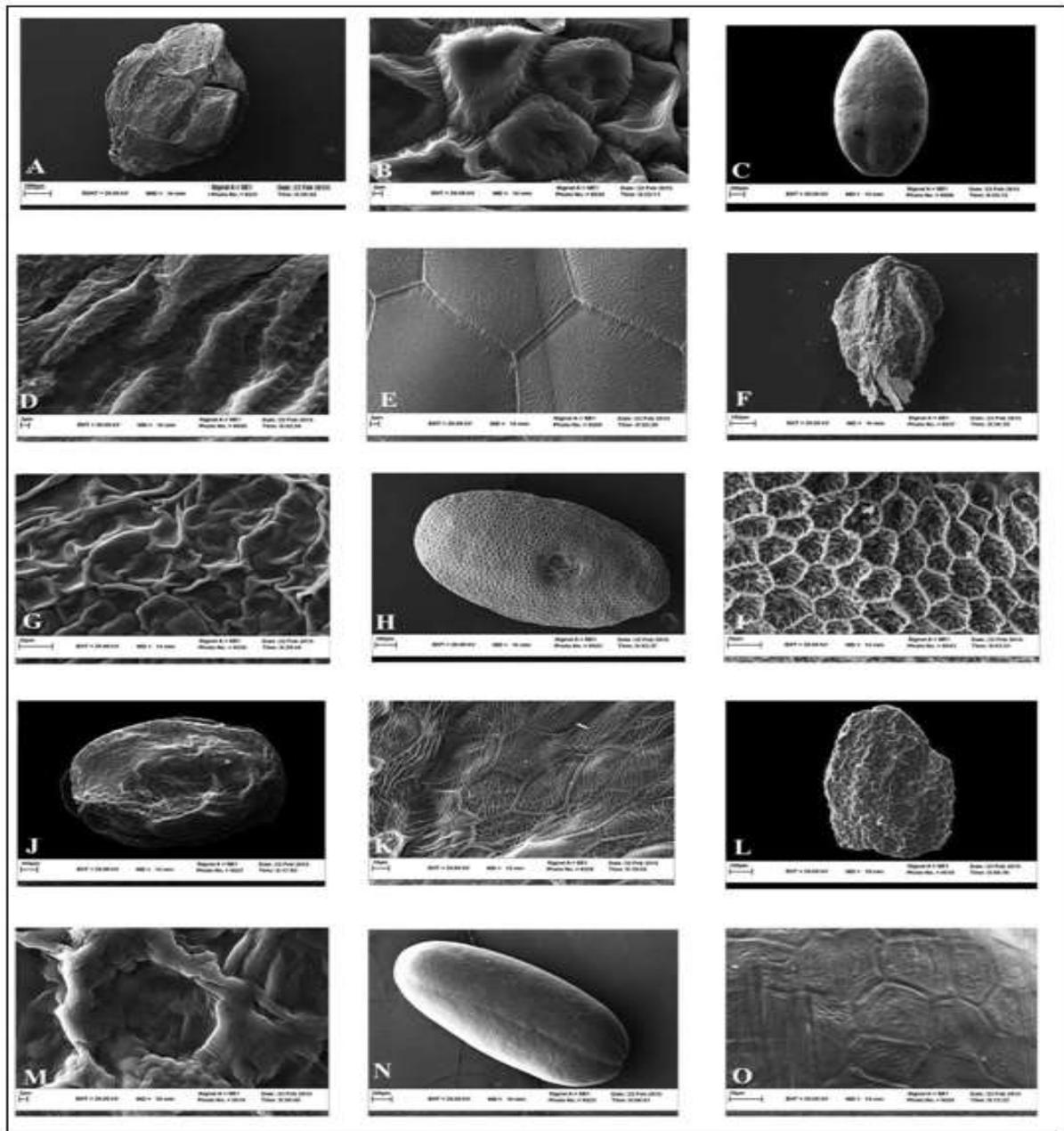


Fig. 2. Electro-micrograph of *Plantago*seed. (A,B) *P. major* × 200,5000. (C,D,E) *P. ovata* × 200,2000,5000. (F,G) *P. maritima* × 200, 2000. (H,I) *P. coronopus* × 200, 2000. (J,K) *P. psyllium* × 200, 2000. (L,M) *P. gentanoides* × 200, 2000. (N,O) *P. lanceolata* × 200, 2000.

Conclusion

Barthlott (1981) stated that SEM of the seed coat can be a good taxonomic and phylogenetic marker at the subgeneric to sub familial level. Shehata (2006) believes seed micromorphology can be a valuable tool in addressing the phylogeny and the relationships in the family and now, it seems, despite similar pollen in studied *Plantago* species, seed characteristics can help to identify studied species.

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