

The micromorphology investigation of some allergenic¹ pollens of the Fabaceae (Leguminosae) in Kermanshah² province (West of Iran)

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Abstract

One of the most important allergens is pollen and a wide range of allergenic plants are in Leguminosae family. In this research, four fresh allergen pollen of this family including *Melilotus officinalis* (L.) Desr. (Yellow Sweet-Clover), *Spartium junceum* L. (Spanish broom), *Robinia pseudoacacia* L. (Black Locust) and *Trifolium repens* L. (White clover) were taken from nature of Kermanshah. The pollen grains were studied by Light Microscopy (LM) for all studied species. In addition, the pollen grains of *S. junceum* were studied by Scanning Electron Microscopy (SEM). Results showed that the pollen grains of these genera were isopolar, spheroidal or subprolate and prolate, medium-size, tricolporate, triangular polar view, oval equatorial view and with microtuberculate or microreticulate ornamentation. Therefore, the genera of Fabaceae had as very heterogeneous allergenic pollen grains.

Keywords: Allergenic plants, exine Surface ornamentation, Fabaceae, Pollen grain, Tricolporate.

1. INTRODUCTION

Several palynological works had done and included: Five genera of *Vicieae* tribe studied by using scanning electron microscopy and compared this data with the other tribes of Fabaceae [1]. The pollen grains of *Melilotus indica* (Linn.) L. were investigated as allergen pollens [2]. An illustrated collection of Iranian plants and allergen pollens presented [3]. The pollen micromorphology of *Spartium junceum* L. and *Lagerstroemia indica* L. were investigated as Allergenic species [4]. The Identification of allergen pollens were done in Kermanshah province [5, 6]. Pollen morphology of four species of *Lathylus sativus* L. were studied and showed that the pollen grains were medium to large size, oval and elongated oval with reticulate exine ornamentation [7]. Pollen analyses of nineteen Chinese honeys were studied [8]. They identified 61 pollen types from thirty three plant families in Natural Honeys. Among the studied species, the pollen of *Robinia pseudoacacia* L., *Sophora japonica* L. were Fabaceae type [8]. The pollen morphology of sixteen *Trifolium* taxa in Istanbul was investigated by using the obtained data from LM observations which of nine taxa were taken by scanning electron microscopy [9]. The pollen grain *Melilotus bicolor* L. as an endemic species from Turkey were investigated [10]. In this research, the pollen grains

are generally trizonocolporate, radially symmetrical, isopolar and subprolate. Sculpturing is usually microreticulate or rarely rugulate and microreticulate in the polar optical section [10]. Also, the pollen micromorphology of *Melilotus bicolor* Boiss. & Balansa. belongs to the tribe *Trifolieae* was studied [10]. According to this study, the pollen grains were generally trizonocolporate and microreticulate ornamentation [10]. Based on the allergenic plant list, the pollen of *Melilotus officinalis* (L.) Desr. and *Trifolium repens* L. had a weak allergen, and black locust (*Robinia pseudoacacia* L.) had a moderate allergen effect [11].

The aim of our research has been provided to identify a micromorphological survey for four allergen species from Fabaceae family.

2. MATERIAL AND METHODS

Fresh pollen grains were taken from available cultivable plants in Kermanshah nature. Kermanshah Province, situated in western Iran, spreads over an area of 25,000 km². It lies between lat. 45.5° and 48° E, long. 33.7° and 35.3° N. The province is bounded on the north by Kurdistan province, on the south by Ilam province, on the southeast by Lorestan province, on the east by Hamedan province and on the west by Iraq country [12].

The pollen grains were acetolyzed according to [13] and mounted in glycerine jelly. The colpi features and exine surface ornamentation studied using Dino capture camera mounted on a Leitz light microscope (HM-LUX3) with a magnification of 400, also at least 25 pollen grains measured in polar and equatorial view. Measurements were recorded using 40x objective of light microscopy, and crossed micrometer eyepiece graduation. The pollen data for all species examined are summarized in Table 1.

Spanish broom (*Spartium junceum*) samples were used to study of pollen grain by scanning electron microscopy. Then, the pollen grains located on sampling leg and gold sprayed on pollens. Finally, Spanish broom pollen grains were studied by using Philips scanning electron microscope (XL30). Micrographs with a magnification of 10000 prepared from Spanish broom pollen grains. Descriptive terminology follows [14-16].

3. RESULTS AND DISCUSSION

Pollen features of studied species as follows:

Yellow Sweet-Clover (*Melilotus officinalis*): Pollen grains, isopolar, prolate, medium (23.7 ± 2 μ m), tricolporate (Fig. 1A), the general outline is elliptical spheroidal or triangular, equatorial view elongated oval, polar axis dimensions 30.3-23.6-20.5 μ m, equatorial axis dimensions 27.7-18.4-14.5 μ m, P/E (polar axis/equatorial axis) 1.28, microtuberculate or psilate ornamentation

68 Black Locust (*Robinia pseudoacacia*): Pollen grains isopolar, spheroidal, elongated oval
69 (Fig. 1B), medium ($27.8 \pm 1.9 \mu\text{m}$), tricolporate, the general outline; polar view triangular (Fig.
70 1C), equatorial view elongated oval, polar axis dimensions $31.9-27.8-23.0 \mu\text{m}$, equatorial
71 axis dimensions $29.7-26.3-22.9 \mu\text{m}$, P/E 1.05, with tubercular or microechinate surface
72 ornamentation.

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74 Spanish broom (*Spartium junceum*): Pollen grains isopolar, subprolate, medium (35.2 ± 2.1
75 μm), tricolporate, the general outline; prolate spheroidal or triangular (Fig. 1D), equatorial
76 view oval (Fig. 1E), polar axis dimensions $30.6-35.2-40.2 \mu\text{m}$, equatorial axis dimensions
77 $24.4-30.8-36.8 \mu\text{m}$, P/E 1.14, with microreticulate surface ornamentation (Fig. 2A). Muri was
78 seen solid with $0.2 - 1.0 \mu\text{m}$ wide. The Lumina are $0.1 - 0.9 \mu\text{m}$ in diameter (Fig. 2B).

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80 White clover (*Trifolium repens*): Pollen grains, isopolar, subprolate, medium ($21.7 \pm 1.7 \mu\text{m}$),
81 tricolporate, the general outline; prolate spheroidal or triangular (Fig. 1G), equatorial view
82 broad oval (Fig. 1F), polar axis dimensions $17.7-21.7-22.0 \mu\text{m}$, equatorial axis dimensions
83 $16.2-18.4-22.0 \mu\text{m}$, P/E 1.17 with microtuberculate or psilate ornamentation.

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85 Faboideae as the subfamily in Fabaceae and following features were noted for studied
86 pollen grains (450 genera of Europe): pollen grains tricolporate, poro-colporate and porate.
87 Also they studied another species of *Melilotus* pollen grain in which is similar to *M. officinalis*
88 in aspect of size and colpi type (tricolporate) [17]. The pollen grains on some studied genera
89 in Fabaceae are like as follow: *Arachis*, *Onobrychis*, *Hedysarum* (tricolporate pollens), *Galega*,
90 *Sophora*, *Thermopsis*, *Lupinus*, *Genista*, *Laburnum* (tricolporate pollens); *Ononis*,
91 *Medicago*, *Trifolium*, *Melilotus*, *Colutedea*, *Chesneya*, *Oxytropis*, *Glycyrrhiza* (triporate
92 pollens). In majority of genera have reticulate ornamentation and perforate ornamentation
93 observed in *Lathyrus*, *Medicago*, *Colutea* and striate ornamentation in *Coronilla*, *Glycyrrhiza*
94 [1]. The exine surface ornamentation in studied genera is similar to the investigation [1]. The
95 pollen grains of *Trifolium*, *Ononis*, *Melilotus* in Trifolieae tribe are similar to each other; in the
96 other hand, the pollen grain of *Medicago* is almost similar to *Lotus* in Loteae tribe. However,
97 *M. officinalis* has microtuberculate and psilate ornamentation; in spite of previous research
98 who introduced it as reticulated one [1]. In addition, the results obtained pollen grain of
99 *Spartium junceum* using SEM showed that pollen grain is spheroid, tricolporate and
100 microreticulate ornamentation. *M. bicolor* as an endemic species from Turkey with
101 microreticulate or, rarely, regulate ornamentation were seen [10]. In our research,
102 microtuberculate or psilate ornamentation was observed in *M. officinalis*. Thus, this character
103 in this genus is varied and specified in species level.

104 Both *Robinia pseudoacacia* and *Trifolium repens* had a moderate allergen effect [11]. In our
105 research *R. pseudoacacia* had specific pollen micromorphological characters include:

spheroidal shape and with tubercular or microechinate surface ornamentation. Also, prolate spheroidal or triangular shapes with microtubercular or psilate ornamentation were seen for *T. repens*.

Spartium junceum L. had identified as major allergen pollen type in west of Iran [4]. The microreticulate surface ornamentation and subprolate shape as qualitative characters were distinct for this species.

4. CONCLUSION

The pollen grain features are observed different on the studied species and have variability. So that, The pollen micromorphology features can more useful on identifying types of allergen species.

COMPETING INTERESTS

The authors of this manuscript declare that they have no conflict of interests.

AUTHORS' CONTRIBUTIONS

The authors designed this research, conducted the laboratory work, and wrote the manuscript. All of the authors read and approved the final manuscript.

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203 **Table. 1.** Palynomorphological data of the investigated species by Light Microscopy.

No.	Specific name	Pollen size (μm)	polar axis (P) (μm)	equatorial diameter (E) (μm)	P/E	Form
1	<i>Melilotus officinalis</i> (L.) Desr.	23.6 \pm 2	20.5-23.6-30.3	14.5-18.4-27.7	1.28	Prolate
2	<i>Robinia pseudoacacia</i> L.	27.8 \pm 1.9	23.0-27.8-32.0	23.0-26.3-29.7	1.05	Spheroidal
3	<i>Spartium junceum</i> L.	35.2 \pm 2.1	30.6-35.2-40.2	24.4-30.8-36.8	1.14	Subprolate
4	<i>Trifolium repens</i> L.	21.7 \pm 1.7	17.7-21.7-22.0	16.2-18.4-22.0	1.17	Subprolate

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UNDER PEER REVIEW

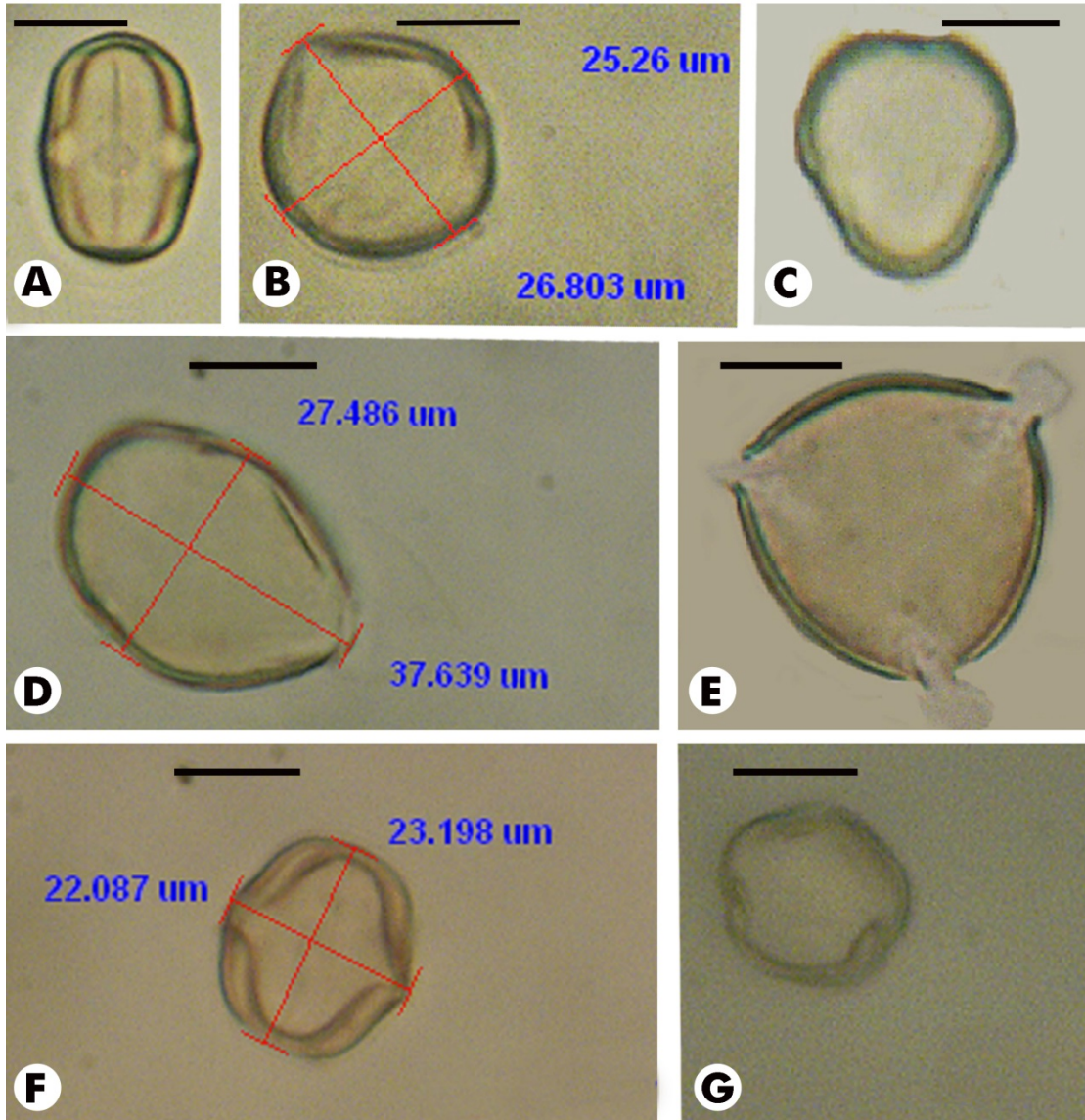


Fig. 1: Light microscopic pictures of pollen grains: A) Equatorial view in *Melilotus officinalis*; B) Equatorial view of *Robinia pseudoacacia*, C) Polar view in *R. pseudoacacia*, D) Equatorial view in *Spartium junceum*, E) Polar view in *S. junceum*, F) Equatorial view in *Trifolium repens*, G) Polar view in *T. repens* (Scale bar=10 μ m).

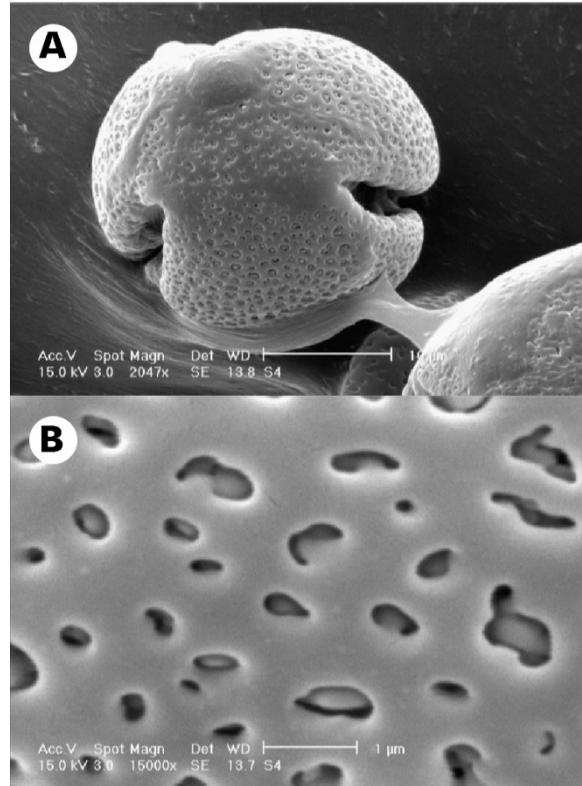


Fig. 2. Scanning electron micrographs of pollen grain in *Spartium junceum*. A) Polar view, B) microreticulate ornamentation exine in detail.