



## Pollen Morphology of Iranian *Celtis* (Celtidaceae -Ulmaceae)

Mehrdad ZARAFSHAR<sup>1</sup>, Moslem AKBARINIA<sup>1\*</sup>, Ali SATTARIAN<sup>2</sup> and Laurentius Josephus Gerardus van der MAESEN<sup>3</sup>

<sup>1</sup> Department of Natural Resource, Tarbiat Modares University, Noor, Mazandaran, Iran

<sup>2</sup> Gonbad Kavoss University, Department of Forestry, Iran

<sup>3</sup> Wageningen University, Biosystematics Group, Wageningen, the Netherlands.

**ABSTRACT:** Pollen morphology of all Iranian *Celtis* species was examined and compared with some African *Celtis*, using light and scanning electron microscopy. Pollen grains are triporate or tetraporate and the main shapes are prolate and subprolate. Most of the Iranian *Celtis* grains are bigger than those of African species. Sculptural density of pollen is lower than in African *Celtis*.

**KEY WORDS:** Pollen, *Celtis*, Iran, Africa.

Received 10 January 2010

Revision accepted 22 October 2010

UDK 581.33:582.711(55)

### INTRODUCTION

Celtidaceae (formerly Ulmaceae - Celtididae) (*sensu* ELIAS 1970; GRUDZINSKAYA 1967; JUDD *et al.* 1994; OMORI & TERABAYASHI 1993; UEDA *et al.* 1997) comprise ca. 150 species classified in 9 genera distributed in the Northern Hemisphere and Southern Africa (SATTARIAN 2006). Some species of the family are ornamental; some are used for timber and reforestation (SATTARIAN 2006) and some attract wildlife because of their sweet fruits (WHITTEMORE & TOWNSEND 2007). In Iran, the plantations are established in the semi-arid ecological setting especially in the Alborz and Zagros mountain ranges (KHATAMSAZ 1990).

The subfamilies Celtidoideae and Ulmoideae possess different types of pollen grains: the *Celtis*-type and the *Ulmus*-type, an exception is *Zelkova* (Celtidoideae) which has pollen grains of *Ulmus*-type (ERDTMAN 1943, 1946, 1972; FAEGRI & IVERSEN 1950; FISCHER 1890; FRITZSCHE 1832; GRIEBEL 1930; JIMBO 1933; SELLING 1947; SHATTUCK 1905; WODEHOUSE 1935; ZANDER 1935, 1941).

Based on exine sculpturing and structure, pollen grains of the Ulmaceae can be placed into six different types (TAKAHASHI 1989). Members of the Ulmoideae all share

tetra- or pentaporate, oblate to spheroidal pollen grains varying in size from 23µm to 42µm and distinguished by wholly granular ectexine and regulate sculpturing with spinules (ZAVADA 1983). Within the Celtidoideae five types of pollen grains have been described. *Ampelocera* pollen is distinguished by being triporate or tetra- or pentaporate and having a palisade rather than granular ectexine (TAKAHASHI 1989). *Chaetachme* grains are similar to those of *Ampelocera* but they have a very thin endexine and a perforated exine with spinules. A densely warty exine and a middle granular layer distinguish the triporate pollen grains of *Girronniera*. Densely spaced warts and microechinules and the absence of a granular layer in the exine characterize *Lozanella*, *Parasponia*, and *Trema*. These grains are radially or bilaterally symmetrical and either diporate or triporate. *Aphananthe*, *Celtis*, and *Pteroceltis* are tri- to pentaporate and all have middle granular layer in the exine and spinules with a rod-like substructure on surface. Pollen grain size in the Celtidoideae ranges from 15µm to 29µm.

Previously, pollen morphology of Iranian *Celtis* has not been studied in detail for comparison with species from Asia and other regions. The aim of the present research is

\*correspondence: akbarim@modares.ac.ir

to verify the pollen morphological characters, to study the diversity and range of variation in Iranian *Celtis* species and to use these data in taxonomic revision of Iranian *Celtis*.

## MATERIALS AND METHODS

The botanical material was taken from the Iranian Herbarium in Tehran (TARI) (Table 1). Fertile anthers were processed according to the standard methodology of acetolysis (ERDTMAN 1943). The measurements of the pollen grains were taken soon after acetolysis, in a maximum within a week. The arithmetic averages of polar and equatorial axes were examined by light microscopy (LM). These parameters were based on measurements of 10-20 pollen grains. The polar axis/equatorial axis (P/E) proportion was used to define the shape and symmetry.

Other details of pollen morphology were examined by scanning electron microscopy (SEM). For scanning electron microscopy (SEM) observation (Fig. 1), dried flowers or buds were taken. Pollen grains from mature anthers were mounted using a fine needle on aluminum stubs with double-sticky tape. Prepared stubs were sputter-coated with gold for 4-6 minutes (Bal-Tec, Swiss). After coating, the specimens were observed with a XL30 Philips Scanning Electron Microscope, at 15-25 KV. All

photomicrographs are taken at the laboratory of SEM, Tarbiat Modares University (Tehran, Iran).

## RESULTS

**Size.** The following size classes, based on the length of longest pollen or spore axis have been suggested (ERDTMAN 1946): very small spore/pollen ( $\leq 10\mu$ ), small (10-25 $\mu$ ), medium size (25-50 $\mu$ ), large (50-100 $\mu$ ), very large (100-200 $\mu$ ), gigantic ( $\geq 200\mu$ ).

The size of pollen grain has been shown in Table 2. The smallest pollen grains are found in *C. tournefortii* Lam., the length of polar axis is 25 $\mu$ m and equatorial diameter 17 $\mu$ m. The largest pollen is from *C. glabrata* Steven ex Planch., the length of polar axis being 26  $\mu$ m and equatorial diameter 16  $\mu$ m.

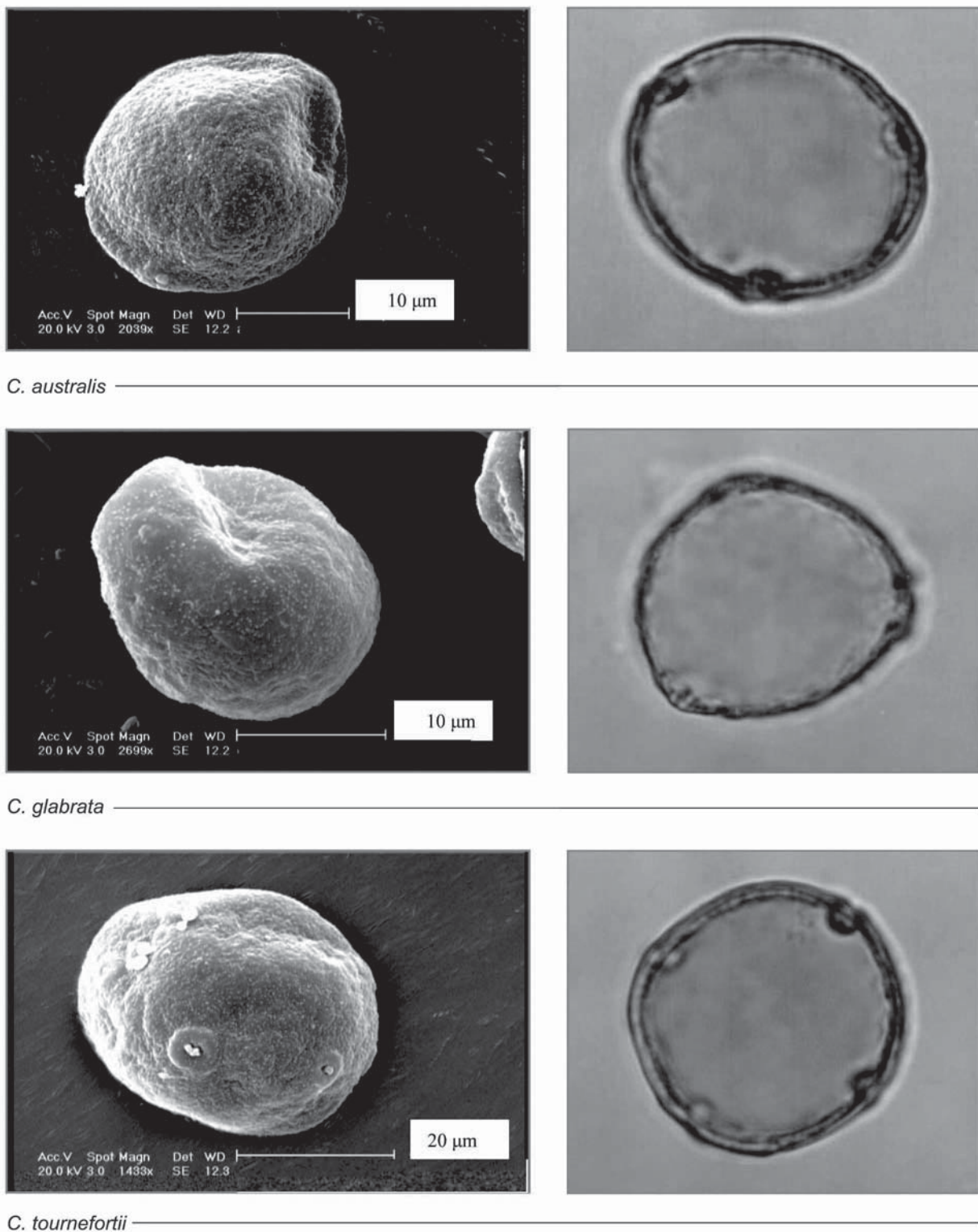
**Shape.** The ratio between the mean polar axis (P) and the mean equatorial diameter (E) is used to assign the pollen grain to shape classes as follows (ERDTMAN 1943): peroblate ( $\leq 0.5$ ), oblate (0.5-0.75), subspheroidal (0.75-1.33), suboblate (0.75-0.88), oblate spheroidal (0.88-1), prolate spheroidal (1-1.14), subprolate (1.14-1.33), prolate (1.33-2), perprolate ( $\geq 2$ ). According to classification of shape and ratio of P/E, the shape of Iranian *Celtis* pollen grains is mainly prolate and subprolate.

**Table 1.** List of specimens used in the study of pollen morphology

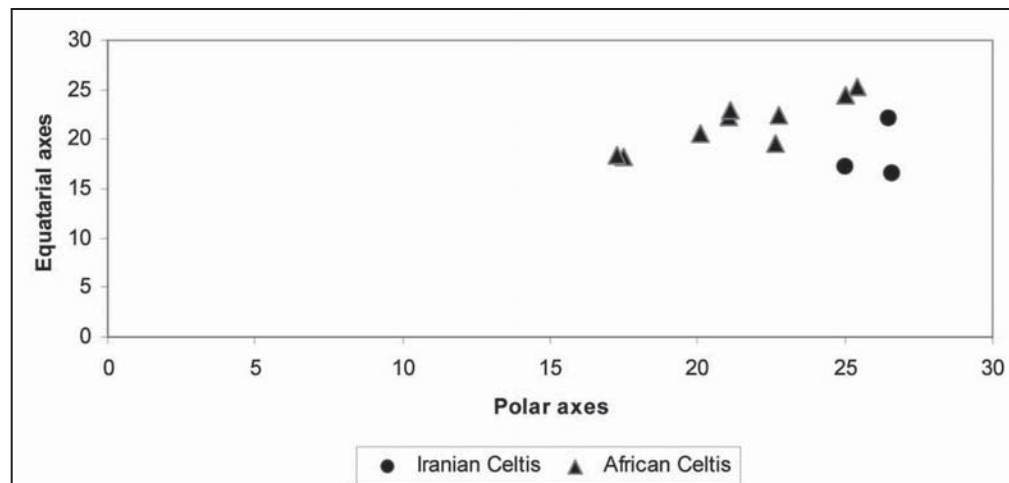
Taxon	Collection place	Collector	Collector/ no	Date
<i>C. australis</i> L	Amol. Mazandaran	Zarafshar. M	s.n	2008
<i>C. glabrata</i> Steven ex Planch	Semirum. Esfahan. Dena mountain	V. Mozafarian	62166	1987
<i>C. tournefortii</i> Lam	Khan baneh. Kordestan	Fatahi	1907	1985

**Table 2.** Pollen morphological data of *Celtis* species. Polar axis (P), equatorial axis (E), ratio P/E, shape, density of sculptural elements (S), all measurements in  $\mu$ m, density of sculptural elements: number per 100  $\mu$ m<sup>2</sup>.

Taxon	P (Mean $\pm$ SD) (Max-Min)	E (Mean $\pm$ SD) (Max-Min)	P/E	Shape	S (Mean)
<i>C. australis</i>	26.5 $\pm$ 2.1 (20-30)	22 $\pm$ 2 (20-30)	1.204	Sub-Prolate	250
<i>C. glabrata</i>	26.6 $\pm$ 2.2 (20-30)	16.6 $\pm$ 2.1 (15-25)	1.602	Prolate	115
<i>C. tournefortii</i>	25 $\pm$ 1.9 (25- 30)	17.22 $\pm$ 1.22 (15- 20)	1.451	Prolate	250



**Fig 1:** Pollen grains of Iranian *Celtis*  
(Left: electron microscopy, Right: light microscopy)



**Fig 2.** The mean size of pollen grains of Iranian and African *Celtis* species.

**Notice:** African *Celtis* (SATTARIAN *et al.* 2005)

**Apertures.** The pores are round shape to ovary shape, of different sizes.

**Surface ornamentation.** Surface sculpture of exine is irregularly cone-shaped and the apex is pointed, prominent and sculptural density varies between 100-300 elements per  $100\mu\text{m}^2$ .

#### GENERAL DESCRIPTION

***Celtis australis* L.** Grains semi-triangular or polygonal in shape, subprolate in shape, triporate. Area of the exine surrounding a pore is sharply differentiated from remainder of exine. Irregular cone-shaped elements on the surface of pollen and apex are pointed. Sculptural density is  $250/100\mu\text{m}^2$  and very prominent.

***Celtis glabrata* Steven ex Planch.** Grains polygonal, prolate in shape, triporate. Sunken area in the aperture and area of the exine surrounding a pore are differentiated from the remainder of the exine. There are irregular cone-shaped elements on the surface of pollen. Sculptural density is  $100-130/100\mu\text{m}^2$ .

***Celtis tournefortii* Lam.** Grains polygonal, prolate in shape, tetraporate. Sunken area in the aperture surrounding a pore is differentiated from the remainder of the exine. There are irregular cone-shaped elements on the surface of the pollen and the sculptural density is  $200-300/100\mu\text{m}^2$ .

**Classification.** On the basis of apertures, number of the pores, surface ornamentation, sculpture elements and pollen shape, we distinguished two different types. The first type is present in *C. tournefortii* with 4 pores and is exceptional in Africa and Asian species of *Celtis*. The second type, present in *C. australis* and *C. glabrata*, has prolate and subprolate grains with 3 pores. This type is generally seen in the majority of species of *Celtis*.

#### DISCUSSION

This work confirms some differences in pollen grains of Iranian *Celtis* based on the number of the pores. It also confirms results from the other research that African *Celtis* is generally triporate (SATTARIAN *et al.* 2005) and shows some new objectives for researchers.

The main shapes are prolate as can be seen in the majority of Iranian *Celtis* (*C. glabrata* and *C. tournefortii*). A majority are triporate. But some differences also are seen in comparison with the African *Celtis* e.g., the sunken area in Iranian *Celtis* is not the same as in African *Celtis* (double-sunken). Most of the Iranian *Celtis* grains are bigger than those of African species (Fig 2). The density of sculptural elements is lower than in African *Celtis*. Among Iranian *Celtis* species two types are recognizable, but in African *Celtis* 3 types were distinguished. In the ratio of P/E Iranian *Celtis* (1.204-1.602) differences are higher than in African *Celtis* (0.79-1.116) (SATTARIAN *et al.* 2005).

#### CONCLUSIONS

Pollen grains of Iranian *Celtis* species (Celtidaceae-Ulmaceae) are prolate and subprolate. According to the number and position of apertures, pollen grains are triporate or tetra-porate. Apertures are simple with a sunken area. Annulus is much more differentiated especially in *C. australis*. The size of pollen is not more different than that in the African *Celtis*. The ornamentation is variable and irregularly arranged. The density of sculptural elements is variable ( $100-300$  elements per  $100\mu\text{m}^2$ ). These results are a kind of pollen-grain description in Iranian *Celtis* species and also indicated that main characters for pollen grains are pores, apertures and ornamentations. This current work created some new objectives e.g. some research for *C. tournefortii* should be done because of some ambiguity for the number of pores in that species.

**Acknowledgements** – We appreciate Steven. R Manchester (University of Florida) to improve the manuscript linguistically.

## REFERENCES

- ELIAS TS. 1970. The Genera of *Ulmaceae* in the Southern United States. *J. Arnold. Arbor.* **51**: 18-40.
- ERDTMAN G. 1943. An introduction to pollen analysis. Chronica Botanica, Waltham, Mass. 238 pp.
- ERDTMAN G. 1946. Pollen morphology and plant taxonomy: VI. On pollen and spore formulae. *Sven. Bot. Tidskr.* **40**: 70-76.
- ERDTMAN G. 1972. Pollen Morphology and Plant Taxonomy: Angiosperms. Hafner Publishing Company, New York.
- FRITZSCHE J. 1832. Beiträge zur Kenntniss dds Pollen 1. Berlin: Stellin and Elbing
- FÆGRI K. & IVERSEN J. 1950. Textbook of Modern Pollen Analysis. Munksgaard. Copenhagen, 169 pp.
- FISCHER HJ. 1890. Beiträge zur vergleichenden Morphologie der Pollenkörner. PhD Thesis.
- GRIEBEL C. 1930. Zur Polleanalyse des Honigs. *Z. Unters. Lebensmittel* **59**: 79 – 441.
- GRUDZINSKAYA IA. 1967. *Ulmaceae* and Reasons for Distinguishing *Celtidaceae* as a Separate Family *Celtidaceae* Link. *Botanicheskii Zhurnal.* **52**: 1723-1748. (In Russian with English summary).
- JIMBO T. 1933. The diagnosis of the pollen forest trees. *J. Sci. Rep. Tohoku. Imp. univ. ser.* **4**(8), 289.
- JUDD WS, SANDER RW & DONOGHUE MJ. 1994. Angiosperm Family Pairs, Preliminary Cladistic Analyses. Harvard Papers in Botany **5**: 1-51.
- KHATAMSAZ M. 1990. Iran Flora (*Ulmaceae*) Number 4. Agriculture Ministry. Press, Research. 25 pp.
- OMORI Y & TERABAYASHI S. 1993. Gynoecial Vascular Anatomy and its Systematic Implications in *Celtidaceae* and *Ulmaceae* (*Urticales*). *J. Plant. Res.* **106**: 249-258.
- SATTARIAN A, van den BERG, R.G. & van der MAESEN, L.J.G. 2005. Pollen morphology of *Celtis*( *Celtidaceae*). *Feddes Repertorium* **117**(1-2): 34-40.
- SATTARIAN A. 2006. Contribution to the Biosystematics of *Celtis* L. (*Celtidaceae*) with Special Emphasis on the African Species. PhD Thesis Wageningen University. 142 pp. Wageningen.
- SELLING O. 1947. Studies in Hawaiian pollen statistics, Bernice P. Bishop Mus. Spec. Publ., **38**: 1-430.
- SHATTUCK CH. 1905. A morphological study on *Ulmus americana*. *Bot. Gaz.* **40**: 209-223.
- TAKAHASHI M. 1989. Pollen morphology of Celtidaceae and Ulmaceae, A Reinvestigation. In: Crane PR & Blackmore S (eds.) Evolution, Systematics, and Fossil history of the Hamamelidae, Vol. 2. Oxford, Clarendon Press. Pp. 253-265.
- UEDA K, KOSUGE K & TOBE H. 1997. A Molecular Phylogeny of *Celtidaceae* and *Ulmaceae* (*Urticales*) Based on rbcL. *J. Plant. Res.* **110**: 171-178.
- WHITTEMORE AT & TOWNSEND M. 2007. Hybridization and self-compatibility in *Celtis*: AFLP analysis of controlled crosses. *J. Am. Soc. Hort. Sci.* **132**(3): 368-373.
- WODEHOUSE R. 1935. Pollen grains, their structure, identification and significance in science and medicine. McGraw-Hill Book Company, Inc., New York and London. pp 574.
- ZANDER E. 1935. Beiträge zur Herkunftsbestimmung bei Honig, I (Berlin)
- ZANDER E. 1941. Beiträge zur Herkunftsbestimmung bei Honig, III (Leipzig).
- ZAVADA M. 1983. Pollen Morphology of *Ulmaceae*. *Grana* **22**: 23-30.

## REZIME

# Morfologija polena kod iranskih predstavnika roda *Celtis* (Celtidaceae -Ulmaceae)

Mehrdad ZARAFSHAR, Moslem AKBARINIA, Ali SATTARIAN, Laurentius Josephus Gerardus van der Maesen

Izučavana je morfologija polena svih vrsta roda *Celtis* iz Irana i upoređivana sa afričkim predstavnicima roda, uz pomoć svetlosnog i elektronskog mikroskopa. Zrna polena su triporna ili četriporna a oblike uglavnom prolatan ili subprolatan. Polen iranskih vrsta je uglavnom veći od afričkih predstavnika, dok je kod iranskih predstavnika skulpturalna gustina manja.

**Ključne reči:** Polen, *Celtis*, Iran, Afrika.

