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in

Oliveira M.M. (ed.), Cordeiro V. (ed.).
XIII GREMPA Meeting on Almonds and Pistachios

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 63

2005
pages 123-127

Pollen morphology and analysis of Iranian wild pistachio

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SUMMARY – There are three species of wild pistachio: *P. atlantica, P. khinjuk* and *P. vera* in the forests of Iran. Rechinger described in Flora Iranica three sub-species of *P. atlantica*: *mutica, kurdica,* and *cabulica*. In order to solve the taxonomical problems between these three sub-species we have compared the morphology of their pollen. Light and scanning electron microscopes were used with conventional methods. All subspecies are perforate and foveolate on the surface. The average diameter of pollen grains was analysed using the SPSS software. According to the analysis based on polar length, the pollen from sub-species *cabulica* is different from that of other sub-species.

Key words: Wild pistachios, Iran, pollen analysis, SEM, taxonomy.

RESUME – *"Morphologie et analyse du pollen chez des pistachiers sauvages d'Iran". Il y a trois espèces de pistaches dans les forêts d'Iran :* P. atlantica*,* P. khinjuk *et* P. vera*. Rechinger dans Flora Iranica a distingué trois sous-espèces pour* P. atlantica *:* mutica, kurdica*, et* cabulica*. Pour résoudre les problèmes de taxonomie qui existent entre ces trois sous-espèces, nous avons comparé la morphologie de leur pollen. Nous avons utilisé le microscope photonique et electronique à balayage avec les techniques classiques. Le type de pollen de toutes les sous-espèces est perforé et fovéolé en surface. Le diamètre moyen des différénts pollens est analysé avec le logiciel SPSS. Le pollen de la sous-espèce* cabulica *est différent des autres par l'analyse de diamètre polaire.*

Mots-clés : *Pistaches sauvages, Iran, analyses de pollens, SEM, taxonomie*.

Introduction

Iran is not only the main origin of pistachio, but it also contains the most genetic diversity among wild and cultivated pistachio of the world. The only place in Iran where the pistachio forest is not observed is a small area near the Caspian sea in the north of country (Abrishami, 1995). Three species of pistachio are native of Iran, namely: *P. atlantica, P. khijuk* and *P. vera. P. atlantica* contains three sub-species, namely *mutica, kurdica*, and *cabulica* (Rechinger, 1969). Although pistachios have such high diversity in Iran, no noticeable research has been done on its genetic diversity. There are also taxonomical problems for distinction between these three sub-species that are together in some forests, and they compose the population in the same places. The sub-species of *P. atlantica* can also fertilize each other. In a previous cytogenetic research, we have determined the chromosome number of these pistachios: for *P. khinjuk* 2n = 24, for *P. atlantica* 2n = 28, for *P. vera* 2n = 30 (Fasihi Harandi *et al.* 1996). Thus there is no difference in chromosome number between sub-species of *P. atlantica*. In the other research we have analysed the isozymes of these pistachios. Two results were concluded from these researches: (i) *P. atlantica, P. khijuk* and *P. vera* belong to three distinct groups; and (ii) similarities between *P. khijuk* and *P. vera* is more than *P. atlantica* (Fasihi Harandi *et al.,* 2000). So we decided to compare for the first time their pollen morphology to have more characters for resolving the taxonomical problems.

Materials and methods

Polliniferous materials were taken from Yassuj, Bandar Abas, and Taleghan for *P. khinjuk;* for *P. vera* from Sarakhs; for subsp. *mutica* and *kurdica* from Kohkilouye va boerahmad, Kerman and Yassuj; and for subsp. *cabulica* from Balouchetan and Kohkilouye.

The pollen grains were prepared for light and scanning electron microscope (SEM) by the standard methods described by Erdtman (1952) and Moore *et al.* (1991). For light microscopy, the pollen grains were mounted on unstained glycerine jelly and observed on a Zeiss III RS microscope. For SEM studies pollen grains were fixed in 3% glutaraldehyde in 0.1 M Sorensen phosphate buffer and dehydrated in ethanol series (50%, 70%, 100%). Then pollens were mounted on a double cellotape coated with gold in a sputter coater apparatus in 150 A for 3 min. The same examination was carried out on a Zeiss electron microscope.

The terminology used is in accordance with Walker and Doyle (1976) and Moore *et al.* (1991). Analysis of variance (ANOVA) is used as a statistical method by SPSS 10.0.5 Software. For each sample, the diameter of 100 pollen grains was calculated randomly.

Results and discussions

Results of morphological studies show that all pollens of different species are of the same type. They are circular in equatorials, perforate (pentaforate) and foveolate on the surface (Figs 1 to 12).

The averages of diameter of pollen for each specimen are presented in Fig. 13: *P. khinjuk* 28 µm, *P. atlantica* subsp*. mutica* 25 µm, subsp. *kurdica* 29.7 µm, subsp*. cabulica* 33.75 µm and *P. vera* 31.8 µm. Analysis of variance (ANOVA) show that subsp. *cabulica* is different from subsp. *kurdica* and subsp. *mutica* (Tables 1a and 1b). We can reason that all pollens of different sub-species resemble in size and morphology as they can fertilize each other in nature. The genetic variation of different populations within each species and subspecies may result from inter-fertilization among them. Also maybe *P. atlantica* subsp. *cabulica* with different morphology of leaves, grains, pollen size and climate will be considered as a new species: *P. cabulica*. The results of morphology and analyses of pollens are not according to the results of chromosome numbers and isozyme analysis. Thus we suggest that the use of molecular markers is the only remained way to resolve taxonomical problems and the problems of genetic distance of this genus. To achieve this aim we propose macrosatelite DNA analysis as a suitable technique.

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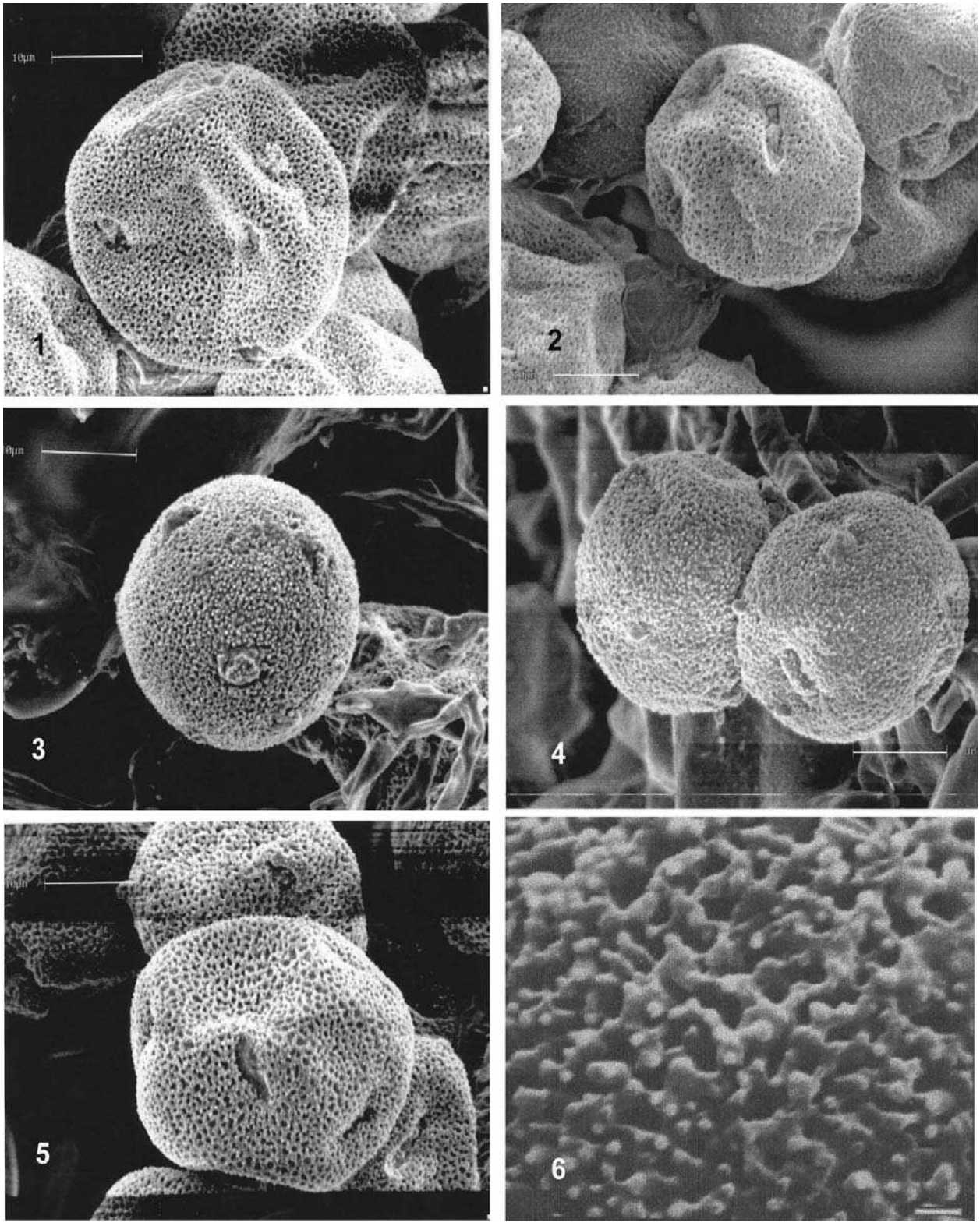
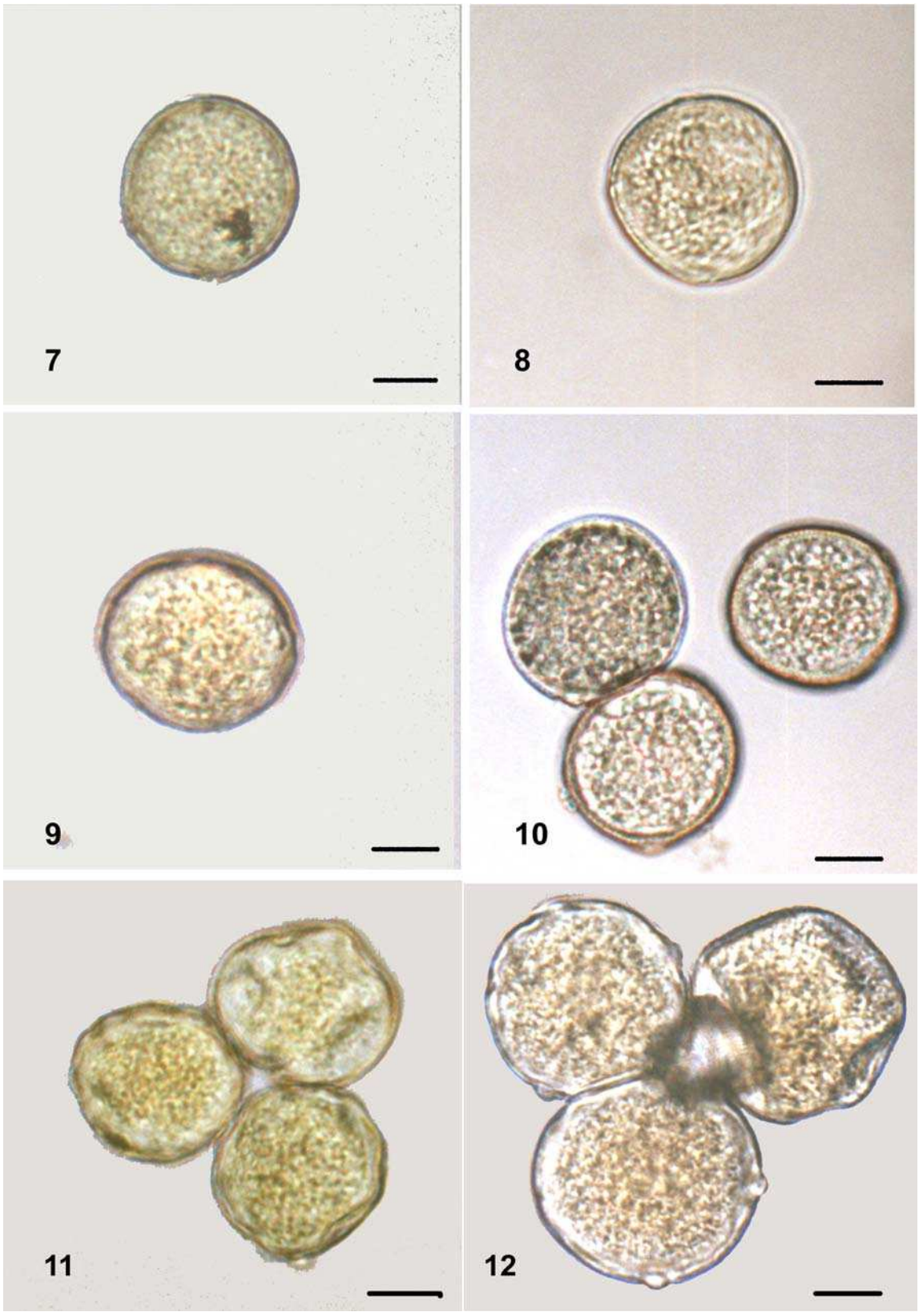


Figs 1-6. (1) *Pistachia vera;* (2) *Pistachia khinjuk;* (3) *Pistachia atlantica* subsp. *mutica;* (4) *Pistachia atlantica* subsp. *kurdica;* (5) *Pistachia atlantica* subsp. *cabulica;* (6) Details of perforation on the surface of *Pistachia atlantica* subsp. *mutica* pollens (scale: 10 µm).



Figs 7-12. Images of pollen grains of pistachios in light microscopy. (7) *Pistachia atlantica* subsp. *mutica;* (8) *Pistachia vera;* (9) *Pistachia atlantica* subsp. *kurdica;* (10) *Pistachia khinjuk;* (11) *Pistachia atlantica* subsp. *kurdica;* (12) *Pistachia atlantica* subsp. *cabulica.* Sometimes 3 pollen grains attach each other. In Figs 10, 11 and 12 the germinating pores are evident.

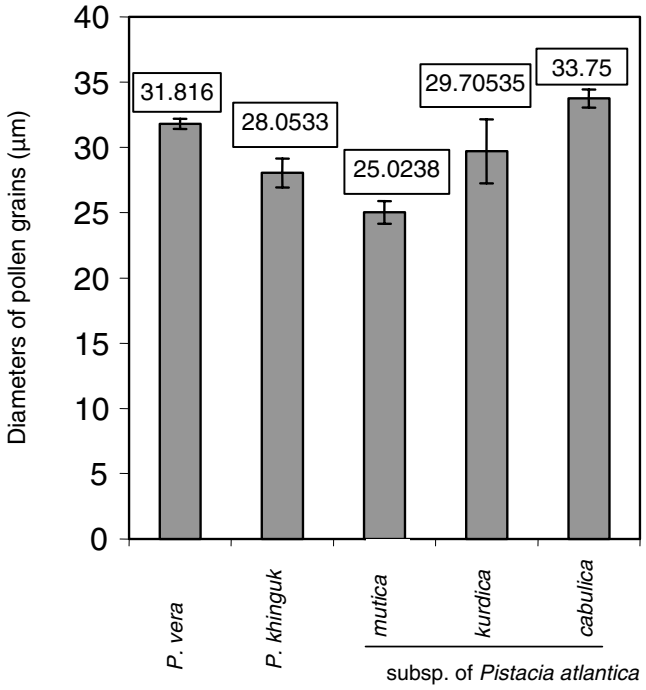


Fig. 13. The averages of diameters of pollen grains in pistachios species and sub-species (µm).

Table 1a. Statistical description of pollen grain diameters. 1: subsp. *mutica*, 2: subsp. *kurdica*, 3: subsp *cabulica*, 4: *P. khinjuk*, 5: *P. vera*

No.	N	Mean	Std. deviation	Std. error	95% Confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
1	10	12.70200	0.39276	0.12420	12.42104	12.98296	12.070	13.290
2	17	15.24406	1.11077	0.26940	14.67296	15.81516	13.661	17.679
3	16	16.87497	0.86433	0.21608	16.41440	17.33554	15.385	18.077
4	16	13.40625	2.45641	0.61410	12.09732	14.71518	4.400	15.000
5	16	15.90797	0.69914	0.17479	15.53542	16.28051	15.000	17.188
Total	75	15.00261	1.99349	0.23019	14.54395	15.46127	4.400	18.077

Table 1b. Analysis of variance (ANOVA) of pollen grain diameters. This table shows that there are significant variation in pollen grain diameters between each population of every species and subspecies

	Sum of squares	df	Mean squares	F	Significance
Between groups	163.899	4	40.975	22.033	0.000
Within groups	130.176	70	1.860		
Total	294.076	74			