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Assessment of pollen dimorphism in populations of *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi in Spain

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Summary

A comparative scanning electron microscopy study and biometric analysis of pollen of 14 Spanish *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi populations was performed. In all the investigated populations pollen grains show a marked degree of dimorphism. Pollen grains from male flowers are prolate spheroidal and tricolporate in shape, while the pollen grains from female flowers are spheroidal to oval, unaperturate, with no colpi.

K e y w o r d s : palynology, electron microscopy, wild grapevine.

Introduction

Palynological studies in grapevines have mainly focused on cultivars. In the description of the pollen, qualitative characters, such as exine microrelief, separate elements, and quantitative characters, such as polar axis, equatorial axis, mesocolpium, apocolpium, and length and width of the colps are used (Roytchev 1995). In addition, palynobiometric investigations of the aperture complex elements in grapevine were carried out by Roytchev (1995). Pollen grains of grapevine are normally 3-zonocolporate, spheroidal to prolate, with very long, narrow, slit-like, slightly, but distinctly sunken ectoaperture (colpus). The endoaperture is a circular pore, with narrow, but distinct costae. Estimations of polar and equatorial dimensions vary, depending on the mounting medium used (glycerine jelly or silicone oil) (Punt *et al.* 2003). However, some hybrids and mutants present a high proportion (up to 80 %) of anomalous pollen (Lombardo *et al.* 1976, Vallania *et al.* 1996). For example in some plants of 'Loureiro', Abreu *et al.* (2006) found spherical and inaperturate (acolporated) pollen grains. Spherical pollen with no furrows is typical in the "abnormal" (not hermaphrodite) group of grapevine cultivars according to Ahmedullah (1983).

In most grapevine pollen grains the exine is thin to fairly thin, while the sexine is as thick as the nexine or even slightly thicker (at poles) (Punt *et al.* 2003). Ornamentation is scabrate-verrucate to foveolate; however, classification of grapevine cvs on the sole basis of exine features was shown to be arbitrary by Pandeliev and Roytchev (1996) and Roytchev (1997). Pollen dimorphism has been reported from dioecious species of *Vitis* (*V. aestivalis, V. coignetiae, V. riparia*), where female pollen is inaperturate (acolporated) and lacks germination structures. Male pollen is tricolporate and averages over 2000 grains per flower (average 3000 grains per flower in hermaphrodite cvs) (Kevan *et al.* 1985, 1988, Kimura *et al.* 1997). Pollen grains in *Vitis vinifera* cvs with functionally female flowers are spheroidal or deformed, with neither furrows nor pores (acolporated), regardless of rootstock and provenance (Cargnello *et al.* 1980, Roytchev 1998). The acolporated pollen grain is viable, although no germination has been recorded (Lombardo *et al.* 1978, Abreu *et al.* 2006,). Cultivars with acolporated pollen grains produce fewer fruits than those with tricolporate pollen (Abreu *et al.* 2004). As regards the shape, size and surface ultrastructure of the aperture complex, the pollen grains of dioecious Eastern European wild grapevine do not differ substantially from the pollen grains of *Vitis vinifera* cvs (Roytchev 1998). Male pollen in *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi is tricolporate (Inceoglu *et al.* 2000). In the present work, scanning electron microscopy (SEM) was used to investigate dimorphism in the pollen of *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi populations in Western Europe.

Material and Methods

Pollen samples. The investigations were carried with pollen from 14 different populations of *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi from Spain (see Appendix) and four samples of cvs (accessions are located at the Viticultural Research Station of Rancho La Merced, Jerez de la Frontera (Cádiz)). The pollen was sampled from 2000 to 2003 by cutting flowers and brushing the anthers and pollen into an Eppendorf tube using a soft brush. Optical Microscopy. For microscopy (Olympus BH2) observation, the mature anthers were fixed immediately after sampling in phosphate-buffered 3 % glutaraldehyde, pH 6.3. Scanning Electron Microscopy (SEM). Fresh mature pollen grains were analyzed by SEM. Critical Point Drying was used since this technique brings about the phase change from liquid to dry gas without affecting surface tension and is therefore suitable for delicate biological specimens. The pollen grains were covered with carbon in a vacuum evaporator before being observed in SEM (JEOL Jsm 6300). Pollen grains were measured directly on the screen of the electron microscope and pictures of pollen grains were taken for each of the tested populations.

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Results and Discussion

Number of pollen grains in male and female flowers. To assess the number of pollen grains we used SEM images of the open anthers. As expected, the anthers of the wild female flowers produce much fewer pollen grains than the male equivalent (10-100 compared with 1500-3000 grains respectively) (Figs 1 and 2). Dimorphism in pollen. Typically two aperture types were observed. Tricolporate pollen grains were observed in male pollen, and unaperturate grains were observed in "female" pollen. In Andalusian cvs ('Muscatel of Alexandria', 'Palomino Fino', 'Pedro Ximénez', 'Zalema') as well as in 'Tempranillo' (the famous Spanish cultivar) and wild male individuals, the pollen was observed to be globular and tricolporate (Fig. 3). It was also observed that in structure the pollen of female individuals of the wild grapevines showed neither furrows nor pores. The female pollen of *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi, was an ovoid to spherical sac (Fig. 4), very different from the functional tricolporate pollen grains

Fig. 1: Anther from a female flower of *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi.

Fig. 2: Anther from a male flower of *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi.

Fig 3: Pollen grain from male flower of *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi.

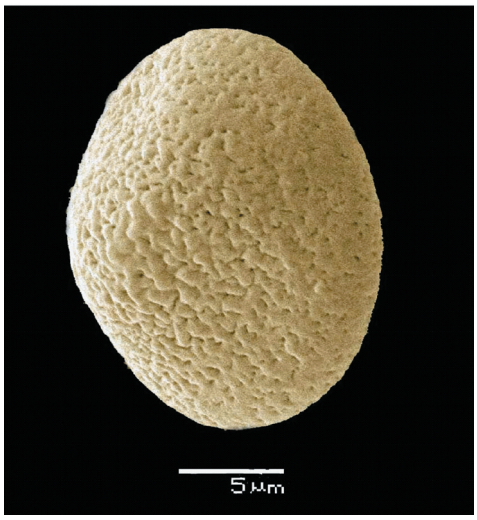

Fig. 4:Pollen grain from female flower of *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi.

observed in male flowers. These unaperturate grains do not germinate, as Caporali *et al.* (2003) indicated. The size of the pollen grains (polar axis x equatorial axis) of the analyzed populations ranged from 17 x 18 to 23 x 22 µm for male flowers and from 15 x 16 to 21 x 20 µm for female flowers (Table). The pollen, therefore, from female flowers of wild grapevines is smaller, and also smaller than those from female cultivars (Table).

Conclusion

The current study contributes to the characterization of pollen grain dimorphism in *Vitis sylvestris* in SW Europe and confirms evidence from Eastern Europe. Dioecious *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi presents pollen dimorphism, as do other dioecious *Vitis* species from Asia and North America. Pollen grains from female flowers of wild grapevines are smaller than those from male flowers, hermaphrodite flowers and those from female cultivars. Pollen dimorphism is therefore more marked in wild populations which suggests female cultivars originate from hermaphrodite individuals.

T a b l e

Biometric parameters in grapevine pollen

Axis	*Vitis vinifera*	*Vitis vinifera* subsp. *sylvestris*
	Grape cvs (hermaphr.)*	Male**
	Average (µm)	Average(µm)
Polar	22.95 ± 1.88	20,5 ± 3,12
Equatorial	15.81 ± 2.74	19.9 ± 1.67
Colpa		
Mesocolpium	11.08 ± 1.15	9.8 ± 2.3
Apocolpium	4.7 ± 0,62	3.8 ± 0.74
Colpus length	19.42 ± 2.3	16.8 ± 2.8
Colpus width	1.17 ± 0.43	1.5 ± 0.3
	Abnormal cvs (female)*	Female**
Polar	22.47 ± 1.75	18.2 ± 2.79
Equatorial	23.27 ± 3.09	18.3 ± 2.01

* Values calculated from Roytchev *et. al.* (1994), Roytchev (1995, 1997), Marasali *et al.* (2005).

** Average values for the populations analyzed (SEM images).

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Appendix: Populations analyzed

V11/Alcalá de los Gazules/6, ALCALA DE LOS GAZULES (Cádiz) (6), Long. 264466.9512 - 263242.3179, Lat. 4025570.7163 - 4024586.1879, Habitat: Alluvial, Female 32, Male 11, Total 43.
V11/Grazalema/1, GRAZALEMA (Cádiz) (1), Long. 277183.0464 - 278776.4879, Lat. 4072300.2454 - 4072505.5557, Habitat: Alluvial, Female 15, Male 42, Total 57.
V11/Ubrique/1, UBRIQUE (Cádiz) (1), Long. 278905.3979 - 278634.9496, Lat. 4059239.7443 - 4057427.0074, Habitat: Alluvial, Female 17, Male 8, Total 25.
V11/Zahara de la sierra/1, ZAHARA (Cádiz) (1), Long. 277139.345 - 277630.1291, Lat. 4078223.4194 - 4078025.5341, Habitat: Alluvial, Female 10, Male 27, Total 37.
V14/Córdoba/1, CORDOBA (Córdoba) (1), Long. 354383.4954 - 354641.0519, Lat. 4201262.3728 - 4200641.1258, Habitat: Alluvial, Female - , Male - , Total 44.
V14/Montoro/1, MONTORO (Córdoba) (1), Long. 386797.7545 - 387040.6586, Lat. 4213019.7143 - 4211197.4394, Habitat: Alluvial, Female 10, Male 20, Total 30.
V14/Posadas/4, POSADAS (Córdoba) (4), Long. 309464.7639 - 309980.0769, Lat. 4193610.6575 - 4193691.2352, Habitat: Alluvial, Female 30, Male 14, Total 44.
V18/Loja/1, LOJA (Granada) (1), Long. 402926.53 - 403254.42, Lat. 4118615.21 - 4118175.84, Habitat: Alluvial, Female 4, Male 16, Total 20.
V21/Almonte/5, ALMONTE (Huelva) (5), Long. 185000.655 - Lat. 4116606.9619, Habitat: Sands, Female 13, Male 39, Total 52.
V21/Aroche/3, AROCHE (Huelva) (3), Long. 148087.1113 - Lat. 4209977.022, Habitat: Alluvial, Female 4, Male 23, Total 27.
V23/Guarromán/2, GUARROMAN (Jaén) (2), Long. 426012.5744 - 425543.4913, Lat. 4215405.4225 - 4214731.5537, Habitat: Alluvial, Female 5, Male 8, Total 13.
V23/Santa Elena/1, SANTA ELENA (Jaén) (1), Long. 455973.2352 - 452064.7599, Lat. 4250080.4007 - 4245509.8958, Habitat: Alluvial, Female 12, Male 48, Total 60.
V29/Antequera/1, ANTEQUERA (Málaga) (1), Long. 371176.2213 - 371152.5348, Lat. 4087996.6694 - 4091326.1464, Habitat: Alluvial, Female - , Male - , Total 12.
V41/Cazalla de la Sierra/1, CAZALLA DE LA SIERRA (Sevilla) (1), Long. 262280.9331 - 265309.1922, Lat. 4201784.3239 - 4206818.7439, Habitat: Alluvial, Female 14, Male 41, Total 55.
V41/Guillena/1, GUILLENA (Sevilla) (1), Long. 221503.395 - 220459.3272, Lat. 4172789.8104 - 4173843.6511, Habitat: Alluvial, Female 4, Male 7, Total 11.