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Istituto di Botanica dell'Università di Milano
Istituto Sperimentale di Viticoltura di Conegliano
Istituto di Botanica dell'Università di Messina, Italia

Pollen ultrastructure in different vine cultivars with low productivity [1])

by

Giuliana Lombardo, G. Cargnello, Maria Bassi, F. M. Gerola and Luisa Carraro

Die Feinstruktur der Pollenkörner bei verschiedenen ertragsschwachen Rebensorten

Zusammenfassung. — Um einen möglichen Zusammenhang zwischen niedriger Ertragsleistung und dem Vorkommen acolporater, d. h. ungefurchter und porenloser Pollenkörner aufzudecken, wurde bei einer Reihe von Rebensorten mit Hilfe der Raster- und Transmissionselektronenmikroskopie die Morphologie der Pollenkörner untersucht. Es konnte tatsächlich festgestellt werden, daß die Pollenkörner der untersuchten ertragsschwachen Sorten kugelig, ohne Keimfurchen und ohne Keimporen sind und daß Bacula und Tegmen eine geschlossene Schicht bilden. Ein hoher Ertrag ist jedoch nicht immer mit colporaten und keimfähigen Pollenkörnern korreliert; eine solche Ausnahme bilden z. B. die Blüten an den Geiztrieben der Rebensorte Picolit.

Introduction

In a previous paper (Lombardo *et al.* 1976), we demonstrated that one of the causes of the low production of normal fruits in the main branches of the examined Picolit plants is the incapability of the pollen grains to germinate, most likely because their wall is deprived of furrows and germinative pores. However, in plants with low productivity, yield is always low in the main branches, but fairly normal in the feathers.

Besides, the productivity of Picolit can be different in plants grown in different environmental conditions. It seemed therefore important to investigate if the low productivity was always correlated with the presence of acolporated pollen (without furrows and germinative pores) and to ascertain if the morphology of the pollen grains could be modified by the environmental conditions or by the position of the flowers. For these reasons we extended our studies to the morphology of the pollen grains of other cultivars of *Vitis vinifera* with either high or low productivity.

Materials and methods

We examined the following materials:

1. Pollen grains taken from the flowers of the feathers of plants of Picolit, characterized by a low productivity of the main branches and a high productivity of the feathers. These plants of Picolit derive from clone 31 A.A. and are cultivated

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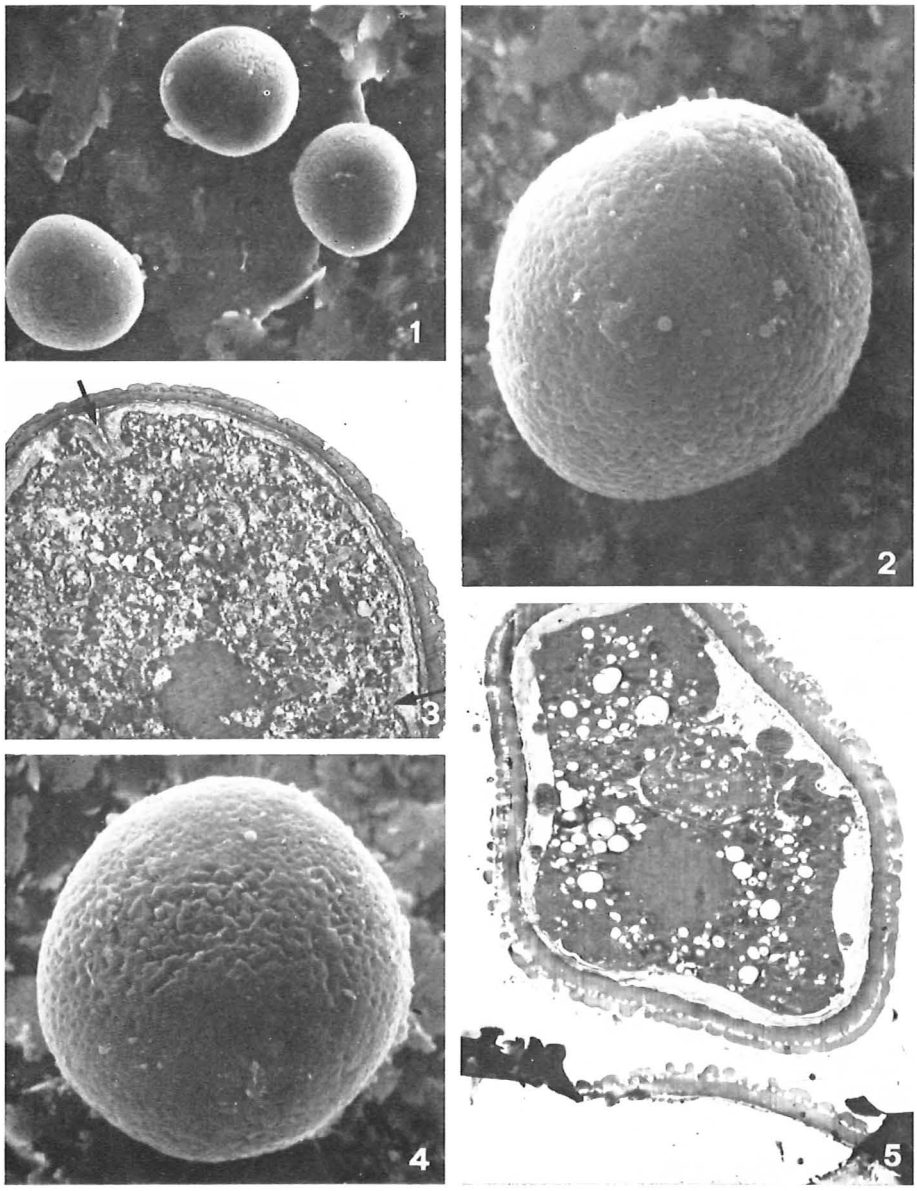

Fig. 1: SEM micrograph of pollen grains of Picolit feathers 31 A.A.G. They appear spherical without furrows and germinative pores. × 2.200.

Fig. 2: SEM micrograph of a pollen grain of a main branch of Picolit 31 A.A.U. It appears spherical, deprived of furrows and germinative pores. × 6.000.

Fig. 3: TEM micrograph of a pollen grain of a main branch of Picolit 31 A.A.U. The intine forms three peculiar invaginations (arrows). × 4.500.

near Gorizia in the wine farm Villa Russiz; they will be indicated in the text as Picolit 31 A.A.G.

2. Pollen grains taken from the flowers of the main branches of the same clone 31 A.A. of Picolit, grown in a different environment, near Cividale del Friuli (Udine), in the wine farm G.B. Cragnolini and characterized by a high productivity also of the main branches. These plants will be indicated as Picolit 31 A.A.U.

3. Pollen grains taken from the flowers of both the feathers and main branches of plants of Razaki rosso (cultivated by the Istituto Sperimentale per la Viticoltura di Conegliano), characterized by a low productivity of the main branches and a high productivity of the feathers. This cultivar will be referred to as Razaki rosso LP (= low productivity).

4. Pollen grains taken from the flowers of both the feathers and main branches of plants of Razaki rosso cultivated in the same place, but imported from a different locality. These plants are characterized by a high productivity also of the main branches and will be referred to as Razaki rosso HP (= high productivity).

5. Pollen grains taken from the flowers of both the feathers and main branches of other cultivars (Bicane, Ceresa and Moscato rosa), characterized by a low productivity of the main branches.

Transmission electron microscopy (TEM). — All the pollen grains described above were fixed in cacodilate-buffered 3 % glutaraldehyde, pH 6.9, at 4 °C for 24 h. After washing in 0.1 M cacodilate buffer, the specimens were postfixed in cacodilate-buffered 1 % osmium tetroxide for 2 h, at 4 °C, dehydrated in ethanol, and embedded in araldite. Being in 75 % ethanol the samples were impregnated with uranyl acetate in semisaturated solution. Ultrathin sections were cut with an LKB Ultrotome III, stained with lead citrate and examined in a Hitachi H11B electron microscope.

Scanning electron microscopy (SEM). — The pollen grains were fixed in phosphate-buffered 3 % glutaraldehyde, pH 6.9, for 24 h. They were then rinsed and exposed to vapours of 1 % osmium tetroxide for further 24 h, dehydrated in ethanol and critical-point dried with CO_2. The samples were coated with carbon and gold in a Balzers BAE 121 coating unit provided with a tilting-rotating stage, and examined with a Jeol JSM U3.

Fig. 4: SEM micrograph of a pollen grain of a feather of Razaki rosso LP. The pollen is spherical, without furrows. × 6.000.

Fig. 5: TEM micrograph of a pollen grain of a main branch of Razaki rosso LP. The sporopollenin wall is continuous, with evident bacula and tegmen. The cytoplasm is dense and full of organelles. × 4.500.

Abb. 1: SEM-Aufnahme einiger Pollenkörner von Geiztrieben der Sorte Picolit 31 A.A.G. Sie sind kugelig, ohne Keimfurchen und -poren. 2.200 ×.

Abb. 2: SEM-Aufnahme eines Pollenkorns von einem Haupttrieb der Sorte Picolit 31 A.A.U. Es ist kugelig, ohne Keimfurchen und -poren. 6.000 ×.

Abb. 3: TEM-Aufnahme eines Pollenkorns von einem Haupttrieb der Sorte Picolit 31 A.A.U. Die Intine ist an drei Stellen nach innen gestülpt (Pfeile). 4.500. ×.

Abb. 4: SEM-Aufnahme eines Pollenkorns von einem Geiztrieb der Sorte Razaki rasso LP. Es ist kugelig und ohne Keimfurchen. 6.000 ×.

Abb. 5: TEM-Aufnahme eines Pollenkorns von einem Haupttrieb der Sorte Razaki rosso LP. Die Sporopollenin-Wand ist ohne Unterbrechungen; Bacula und Tegmen sind deutlich zu erkennen. Das Cytoplasma ist dicht und enthält zahlreiche Organellen. 4.500 ×.

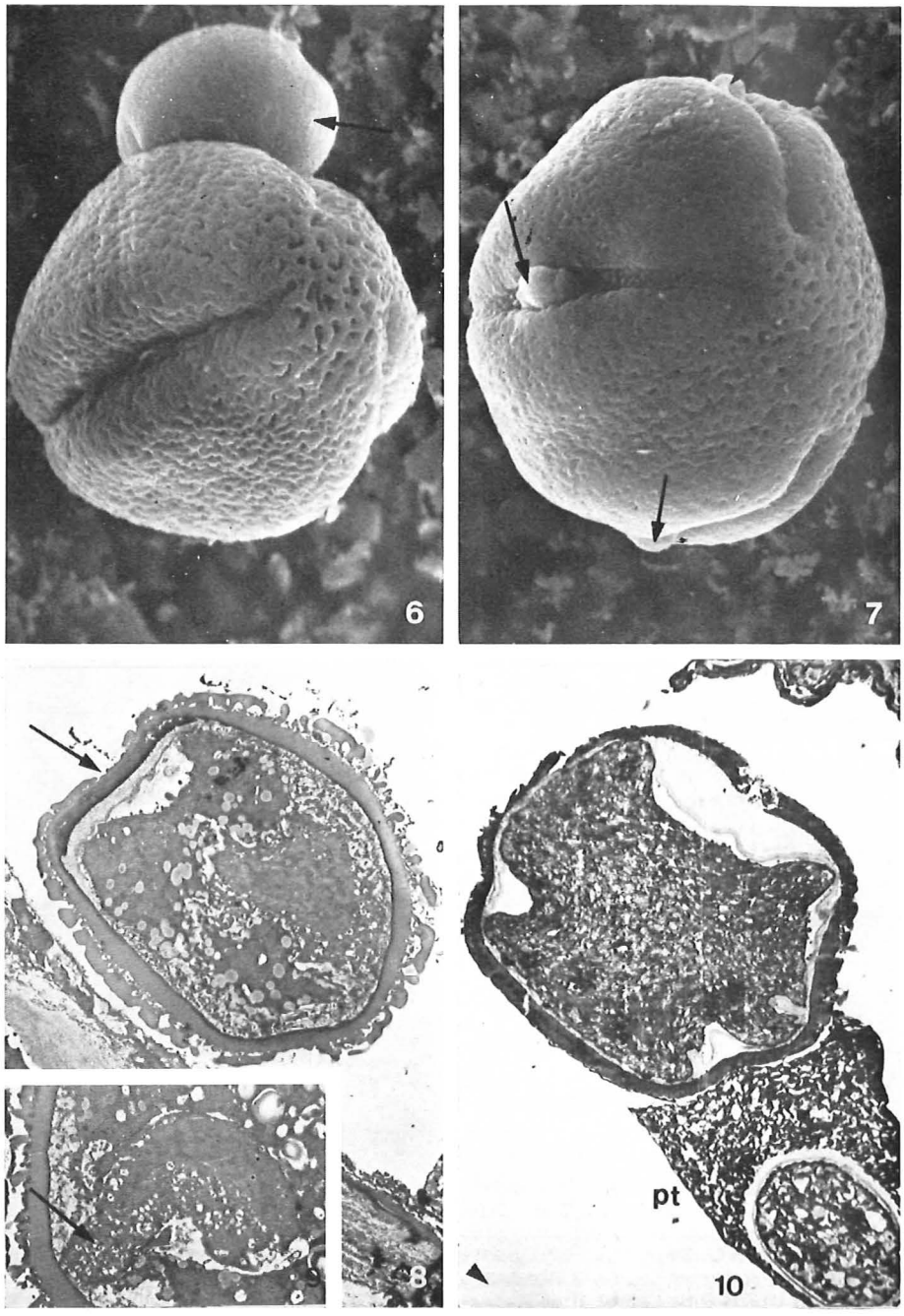

Fig. 6: SEM micrograph of a pollen grain of a main branch of Razaki rosso HP. The grain shows the three furrows typical of vine pollen. A pollen tube is clearly visible (arrow). × 4.500.

Results

Feathers of Picolit 31 A.A.G. — The pollen grains have the same aspect as those of the flowers of the main branches. They are spherical and do not present the three furrows generally typical of vine pollen (Fig. 1).

Main branches of Picolit 31 A.A.U. — The pollen grains, like those of Picolit 31 A.A.G., are deprived of furrows (Fig. 2) and have a continuous layer of bacula and tegmen. The intine is more electron-dense than normal, has a fibrillar aspect and forms three peculiar invaginations regularly spaced along the pollen grain periphery, like the three furrows of normally colporated pollen (Fig. 3).

Razaki rosso LP. — The pollen grains of flowers of the main branches and feathers are always spherical and deprived of furrows and germinative pores (Fig. 4), and, therefore, they are quite similar to those of Picolit. In transversal section, the thickness of the pollen wall appears uniform all round, and bacula and tegmen form a continuous layer (Fig. 5). The cytoplasm has a normal aspect, dense and rich of organelles.

Razaki rosso HP. — The pollen grains of the flowers of both the main branches and feathers show the three furrows generally typical of vine pollen and the emission of the pollen tube is frequently visible (Figs. 6 and 7). In transversal section, the layer of bacula and tegmen is not continuous, but shows interruptions at the level of the furrows (Fig. 8), and formation of the pollen tube is sometimes visible (Figs. 9 and 10). The generative and vegetative cells are well recognizable (Fig. 9).

Bicane, Ceresa and Moscato rosa. — Also in these cultivars the pollen grains of the flowers of the main branches and feathers are spherical, without furrows and germinative pores (Figs. 11, 13 and 15). In transversal section, the wall appears continuous, without interruptions in the layer of bacula and

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Fig. 7: SEM micrograph of a pollen grain of a feather of Razaki rosso HP. The three furrows and the emerging pollen tubes are clearly visible (arrows). × 5.400.

Fig. 8: TEM micrograph of a pollen grain of a main branch of Razaki rosso HP. The tegmen of the sporopollenin wall is interrupted at the level of a furrow (arrow). × 4.500.

Fig. 9: TEM micrograph of the generative cell of a pollen grain of a main branch of Razaki rosso HP. The emission of a pollen tube is clearly visible (arrow). × 7.000.

Fig. 10: TEM micrograph of a pollen grain of a feather of Razaki rosso HP. The cytoplasm shows three invaginations in correspondence with the three furrows. A pollen tube (pt) emerges from one of the furrows. × 4.300.

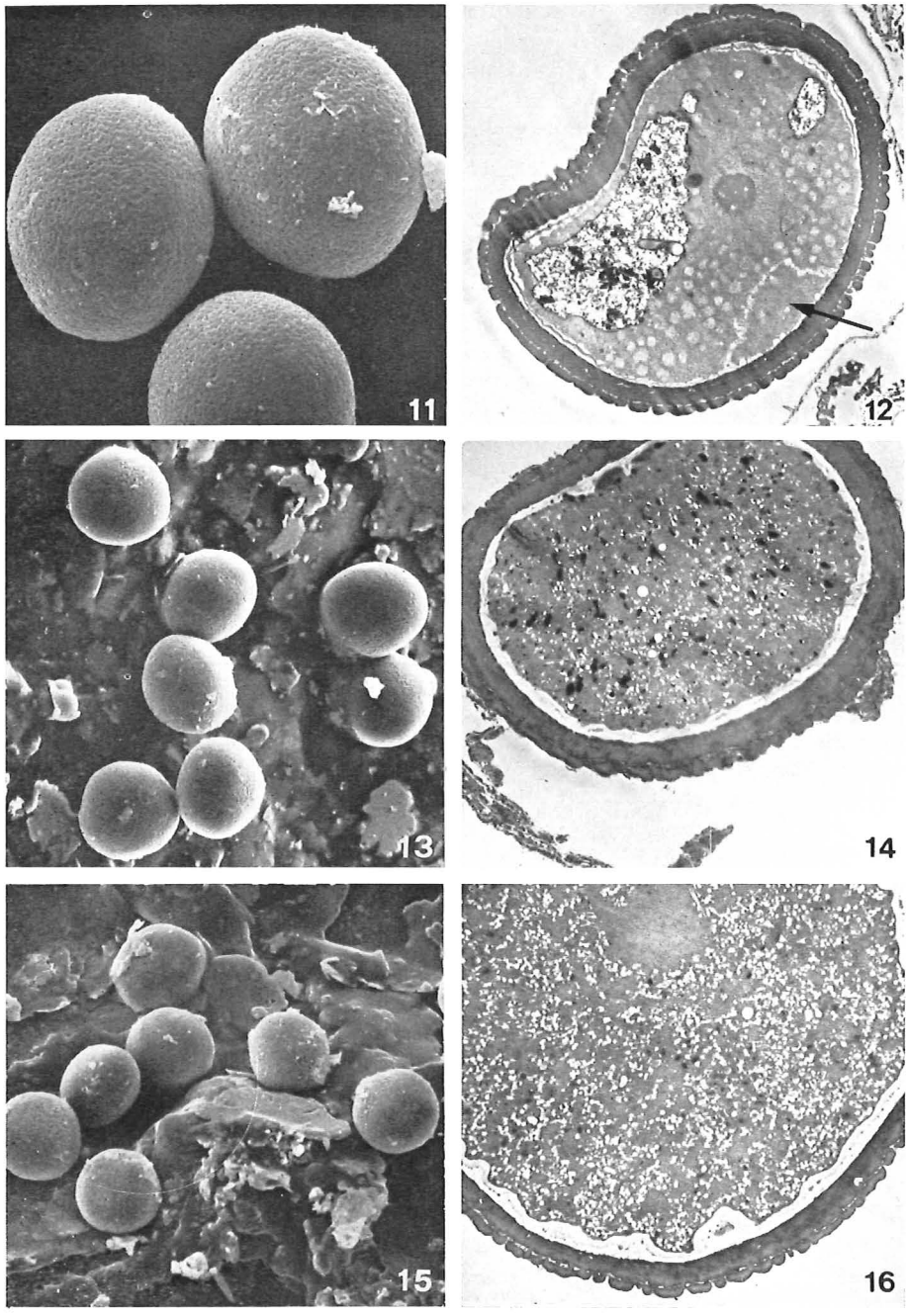
Abb. 6: SEM-Aufnahme eines Pollenkorns von einem Haupttrieb der Sorte Razaki rosso HP. Es zeigt die drei für Rebenpollenkörner typischen Keimfurchen. Ein Pollenschlauch ist deutlich sichtbar (Pfeil). 4.500 ×.

Abb. 7: SEM-Aufnahme eines Pollenkorns von einem Geiztrieb der Sorte Razaki rosso HP. Die drei Keimfurchen sowie der Austritt der Pollenschläuche sind deutlich sichtbar (Pfeile). 5.400 ×.

Abb. 8: TEM-Aufnahme eines Pollenkorns von einem Haupttrieb der Sorte Razaki rosso HP. Das Tegmen der Sporopollenin-Wand ist im Bereich einer Keimpore unterbrochen (Pfeil). 4.500 ×.

Abb. 9: TEM-Aufnahme der generativen Zelle eines Pollenkorns von einem Haupttrieb der Sorte Razaki rosso HP. Der Austritt eines Pollenschlauches ist deutlich sichtbar (Pfeil). 7.000 ×.

Abb. 10: TEM-Aufnahme eines Pollenkorns von einem Geiztrieb der Sorte Razaki rosso HP. Das Cytoplasma zeigt den drei Keimfurchen entsprechende Einstülpungen. Aus einer der Furchen tritt ein Pollenschlauch (pt) aus. 4.300 ×.



Figs. 11, 13 and 15: SEM micrographs of pollen grains of Moscato rosa, Bicane and Ceresa. The grains are all spherical, without furrows and germinative pores. × 4.200, × 1.500, × 1.500.

tegmen (Figs. 12, 14 and 16). The generative and vegetative cells have a normal aspect.

Discussion

It is apparent that the presence of colporated pollen is always concomitant with high productivity, as in Verduzzo (Lombardo *et al.* 1976) and in Razaki HP, while a low productivity is often concomitant with acolporated pollen, as in Picolit 31 A.A.G., Moscato rosa, Ceresa, Bicane and Razaki LP. In fact, in the latter the pollen grains are acolporated and present a continuous layer of bacula and tegmen all along their surface. Though these pollen grains have normally structured generative and vegetative cells, and stain normally with Alexander method (Cargnello and Candussi, in press), they always fail to germinate *in vitro* (Cargnello *et al.*, unpublished data) and were never seen germinating on the stigmas. It can, therefore, be suggested that at least one of the causes of the low productivity of these cultivars may be the impossibility of their pollen to emit the pollen tube, so that the plants are generally self-sterile. However, a low productivity cannot always be ascribed only to the presence of acolporated pollen, because this can be found also in plants with high productivity, as is the case with Picolit 31 A.A.U. and the feathers of Razaki rosso LP. It can, therefore, be stated that in plants where the pollen is colporated and consequently self-fertilization is possible, there are no obstacles to pollination, so that productivity is generally high. Instead, in plants with acolporated pollen, productivity seems to depend also on other factors and not only on the pollen type. In fact, in plants of Picolit artificial pollination of the main branches is much more difficult than in plants with colporated pollen, and field experiments have demonstrated that to increase the productivity of such plants the distance between their flowers and the flowers with colporated pollen must be drastically reduced.

In Picolit, the external morphology of the pollen grains is not modified by the environment, but the internal ultrastructure of the pollen wall of plants with high productivity differs from that of the pollen wall of plants with low productivity. Since the plants belong to the same clone, it seems reasonable to hypothesize a phenotypic variability of the intine structure. Further investigations are required to assess if this hypothesis is true, or if a mutation has instead occurred in Picolit with high productivity.

The fact that the pollen of plants of Razaki cultivated in the same environment but coming from two different places has a different morphology (colporated in Razaki HP, acolporated in Razaki LP) suggests that the presence or absence of fur-

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Figs. 12, 14 and 16: TEM micropraphs of pollen grains of Moscato rosa, Bicane and Ceresa. The sporopollenin wall is continuous, with evident bacula and tegmen. The cytoplasm has a normal aspect and the generative cell is sometimes visible (arrow). × 4.200, × 4.200, × 5.600.

Abb. 11, 13 und 15: SEM-Aufnahmen von Pollenkörnern der Sorten Moscato rosa, Bicane und Ceresa. Sie sind kugelig und zeigen keine Keimfurchen und -poren. 4.200 ×, 1.500 ×, 1.500 ×.

Abb. 12, 14 und 16: TEM-Aufnahmen von Pollenkörnern der Sorten Moscato rosa, Bicane und Ceresa. Die Sporopollenin-Wand ist nicht unterbrochen; Bacula und Tegmina sind deutlich sichtbar. Das Cytoplasma wirkt normal; gelegentlich ist die generative Zelle zu erkennen (Pfeil). 4.200 ×, 4.200 ×, 5.600 ×.

rows might be a genotypic character. For this reason it seems likely that the examined plants of Razaki belong to two different cultivars easily recognizable by scanning electron microscopy.

Summary

The morphology of pollen grains of different vine cultivars has been examined by transmission and scanning electron microscopy to see if a correlation could be found between low productivity and the presence of acolporated pollen. Actually, it was found that in the examined cultivars with low productivity the pollen is round, deprived of furrows and germinative pores, and surrounded by a continuous layer of bacula and tegmen. However, high productivity is not always correlated with the presence of colporated pollen, as is the case with the flowers of the feathers of Picolit.

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Dr. GIULIANA LOMBARDO
Istituto di Scienze Botaniche
Università degli Studi
Via G. Colombo, 60
20133 Milano
Italia