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Ultrastructural characteristics of pollen development in *Vitis vinifera* L. (cv. Sangiovese)

by

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Summary: Ultrastructural studies on the development of microspores to ripe pollen (cv. Sangiovese) showed that the diploid microsporocyte undergoes meiosis in order to form a tetrad of microspores sticked together by a callose wall. After callose digestion, 4 independent microspores were released. With successive growth, the single nucleus of each microspore undergoes an asymmetrical division forming the bicellular pollen grain (vegetative cells containing the generative cells). Only after pollination and pollen tube growth the two sperms will be formed. The mature pollen of Sangiovese is tricolpate; the exine is scabrate-verrucate in the equatorial zone and foveolate in the polar region.

Key words: Vitis vinifera, Sangiovese, pollen grain, microsporogenesis.

Introduction

The pollen grain is the male gametophyte of the angiosperms with two kinds of structural and functional cells. The male reproductive cells, with little cytoplasm, are either single generative cells (bicellular type) or pairs of sperm cells (tricellular type). The second type of cell is a larger, non-reproductive vegetative cell, whose cytoplasm encloses the generative cell or the sperm cells in a cell-within-a-cell arrangement (see Cresti *et al.* 1999).

The mature pollen grains can also be considered as storage cells, which must supply nutrients to the rapidly growing pollen tube (Cresti *et al.* 1975).

The present paper is directed to study the pollen development and mature pollen of *Vitis vinifera*, cv. Sangiovese, to better understand the biology of the sexual reproduction of these species. The cv. Sangiovese has its origin in Tuscany and is widely distributed in this region, quantitatively it is one of the most important components of Chianti, Brunello, Vernaccia and Vino Nobile di Montepulciano wines.

A previous account of certain features on pollen, biology of reproduction and genetic biodiversity of Sangiovese was given by Lombardo *et al.* 1978; Castelli *et al.* 1986; Ciampolini *et al.* 1996; Sensi *et al.* 1996; Vignani *et al.* 1996.

Material and Methods

Anthers of *Vitis vinifera* L. (cv. Sangiovese) were collected at different stages of development from plants cultivated in the experimental fields of the Siena Agricultural High School in Italy. For scanning electron microscopy (SEM), fresh mature pollen grains were mounted with tape on aluminium stubs, coated with gold and then examind with a Philips 501B SEM.

For transmission electron microscopy (TEM), the material was fixed in 2 % glutaraldehyde (GA) in 0.05 M Cacodylate buffer, pH 7.4, for 1 h at room temperature, rinsed in the same buffer and post-fixed in 1 % osmium tetroxyde, dehydrated in ethanol and embedded in low viscosity resin. Sections were cut with a diamond knife using a LKB ultratome III and stained with uranyl acetate and lead citrate; observations were made using a JEOL Jem 100B electron microscope at 80 kV.

For cytochemical examination, the samples were embedded in Historesin (LKB) after fixation in GA. Semithin sections were then stained with Toloudine blue and Periodic acid-schiff (PAS) reaction (O'Brien and McCully 1981), Sudan Black B (Russell *et al.* 1976) and Vanillin-HCl (Heslop-Harrison and Heslop-Harrison 1985) and examined with a Zeiss Axiophot light microscope.

Results

The microspore mother cells (MMC) at the prophase-leptotene stage (Fig. 1) show a polyedric form, a roundish nucleus with only one nucleolus and numerous organelles, e.g. mitochondria, plastids, dictyosomes and rough endoplasmic reticulum (RER) profiles. Tapetum and microspore mother cell walls are pectocellulosic in nature. At this stage the plasmodesms between tapetum and MMC are closed, meanwhile some plasmodesms between MMC and MMC give origin to cytomictic channels (Fig. 2).

The deposition of callose between the primary cellulose wall and the plasmalemma of each individual microspore occurs at the end of the simultaneus cytokinese leading to tetrad formation. Each tetrad is enclosed in the callosic wall of the MMC from which it is derived and the single microspores are also separated from the callosic walls. The

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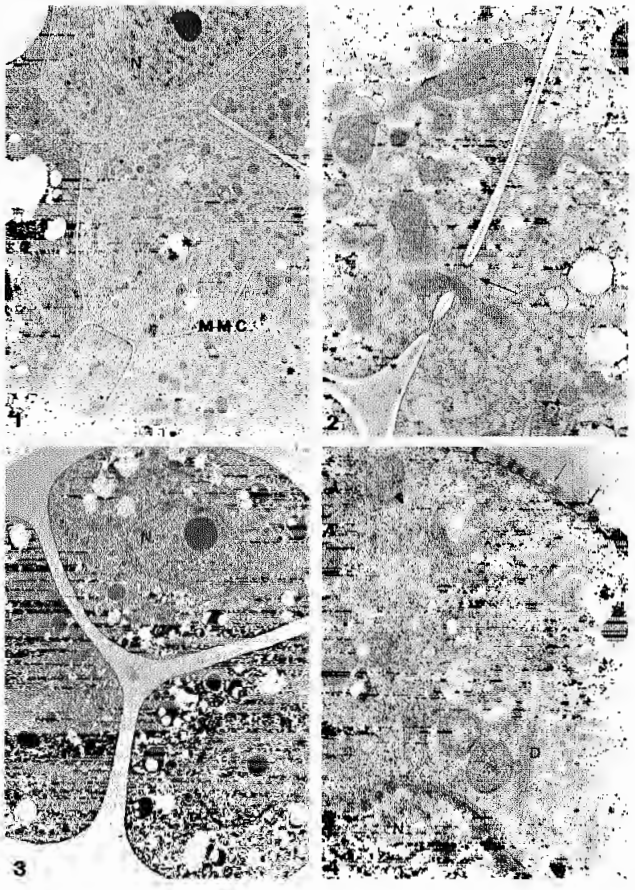
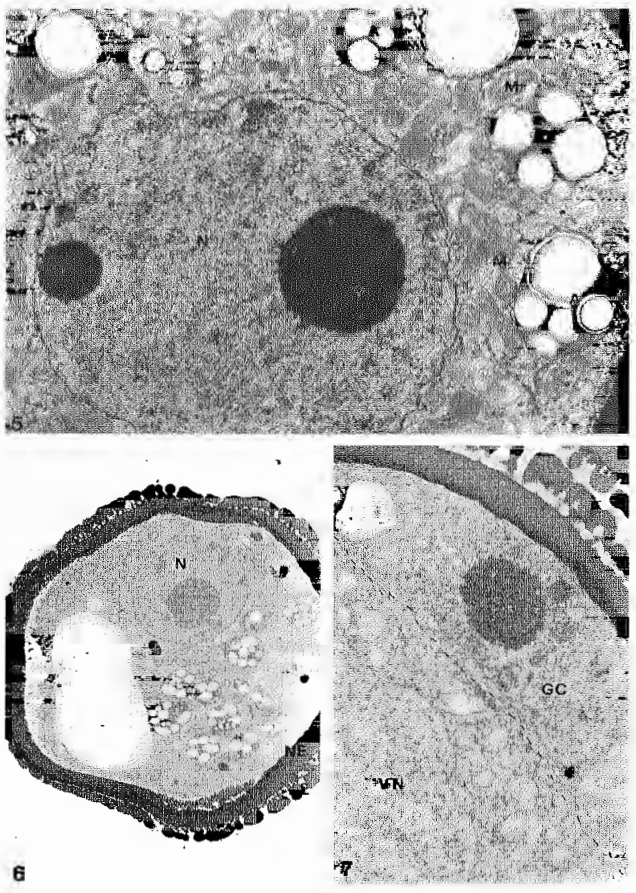


Figs. 1-4: Microspore mother cells (MMC) at the prophase-leptotene stage; tapetum (T); x 4200 (**1**). Cytomictic channel between two microspores (arrows); x 19200 (**2**). Tetrad showing rounded nucleus (N) and plastids (P) containing starch grains; x 6000 (**3**). Portion of a young microspore at the tetrad stage showing primexine deposition (arrow). Note the electrondense cytoplasm with active dictyosomes (D); x 38000 (**4**).



Figs. 5-7: Portion of a free microspore showing amyloplasts (A) with numerous starch grains; mitochondria (M); x 16000 (**5**). Free microspore with the exine (E) and nexine 1 (Ne) increased in thickness; x 5600 (**6**). Bicelled microspore showing a portion of the central vegetative nucleus (VN) and generative cell (GC-dotted line) appressed to the plasma membrane; x 16000 (**7**).

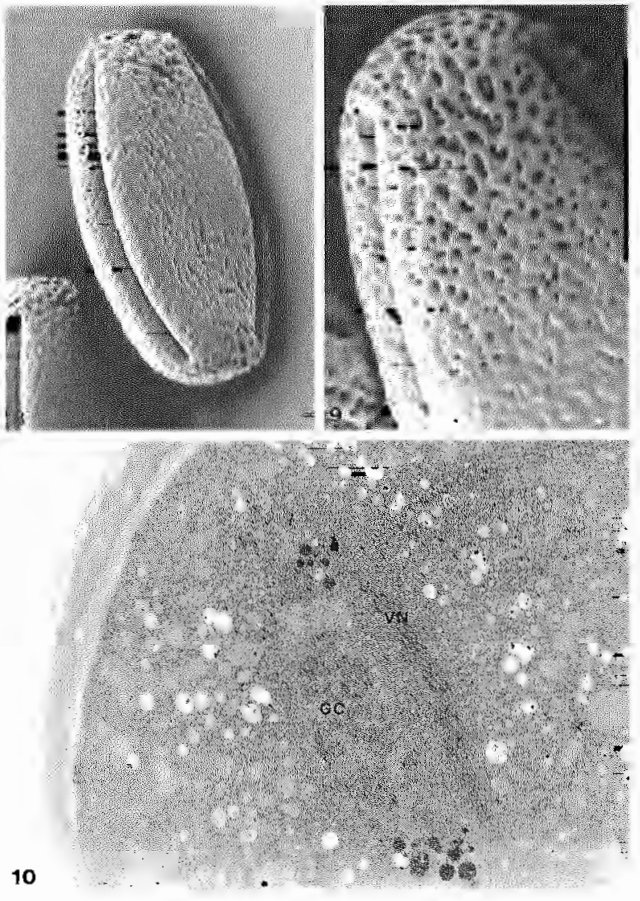
tetrads, which are roundish in shape, contain a single nucleolus which is also roundish. The nuclear membrane contains numerous pores and the plastids reveal one or more starch grains (Fig. 3). During the deposition of the primexine (Fig. 4) the cytoplasm continues to be electrondense; the dictyosomes, formed by elongated cisternae, produce numerous vesicles, and the ribosomes appear to be aggregated in polysomes. After the dissolution of the callosic wall, the microspores are released; they grow in volume and become roundish. The roundish shaped nucleus contains one or two large nucleoli. In the cytoplasm there are amyloplasts containing numerous starch grains (Fig. 5). During this period there is a concomitant rise in width and complexity of the exine (sexine and nexine 1 increase: nexine 2 is laid down) and the cytoplasm begins to fill with vacuols (Fig. 6).

The microspore nucleus, which remains in a central position during the tetrad stage, migrates to the cell periphery and is positioned near the plasma membrane (Fig. 6). At the cortical position, the spore nucleus undergoes a mitotic division resulting in a bicellular pollen grain with a smaller generative cell (GC) within the cytoplasm of a larger vegetative cell (VC) (Fig. 7). Vacuoles formed during the free microspore period are reabsorbed and the vegetative nucleus (VN) migrates to the center of the cell. Initially the GC is appressed to the plasma membrane (Fig. 7). Before shedding the GC is located in the central portion of the VC closely connected to the VN.

Mature grain

External morphology: Sangiovese pollen is tricolpate (Fig. 8), the exine is scabrate-verrucate in the equatorial zone and foveolate in the polar zone (Fig. 9) (FAEGRI and IVERSEN 1964; CIAMPOLINI and CRESTI 1981).

Internal morphology: The GC migrates into the VC assuming a spindle shape (Fig. 10). The GC is separated from the cytoplasm of VC by an indented wall-like structure; in cross sections it is roundish whilst in longitudinal sections it has the shape of a crescent moon; it is localized in the middle of the grain near or closely associated with the VN forming the Male Germ Unit (MGU). The nucleus fills most of the cell and has electrondense masses of chromatin. The cytoplasm of the GC contains many ribosomes, dictyosomes, mitochondria and some small cisternae. A remarkable feature of the GC is the large quantity of small electrondense roundish bodies localized at the two extremities of the cell (Fig. 10). It is on the basis of Sudan B black and Vanillin-HCl staining and its ultrastructural appearance that this substance is considered as tannin.



Figs. 8-10: Mature pollen grain at scanning electron microscope; x 4000 (**8**). Portion of an enlarged pollen grain in the polar and equatorial zone; x 8000 (**9**). Mature pollen grain showing the generative cell and the vegetative nucleus: Note the electrondense rounded bodies localized at the extremity of the generative cell; x 8000 (**10**).

The nucleus of the VC is large, irregularly shaped, the chromatin is less electrondense than the generative nucleus. Many stacked RER cisternae can be seen in the cytoplasm, especially between GC and VN. The cytoplasm contains numerous mitochondria usually located in the peripheral zone while the ribosomes are distributed all over the cytoplasm or gathered in irregular masses. In the cytoplasm there are roundish weakly electrondense bodies (Fig. 8) that could represent the proplastids that are seen later on in the pollen tube forming lamellae and starch grains. Many inclusions, the nature of which is rather difficult to interpret, are frequently encountered inside vacuoles; they should be considered small autophagic vacuoles containing residual products.

Discussion

The ultrastructural features of Sangiovese pollen during its development does not seem to have any particularities in comparison with other bicellular pollen grains that have been described to date (reviews: MASCARENHAS 1989, CRESTI and TIEZZI 1992).

The process of microgametogenesis in *Vitis vinifera* can be briefly summarized in two different steps. First, within the anther locule, a diploid microsporocyte undergoes meiosis in order to originate a tetrad of microspores which are held together by a thick callose wall. Successively the callose is digested by appropriate enzymes and 4 independent microspores are released. With the further growth of each microspore, the single nucleus migrates to the peripheral portion of the cell where it undergoes an asymmetrical division (first haploid pollen mitosis). The resulting bicellular pollen grain consists of a small GC and a large VC. Inside the anthers the *Vitis* pollen development stops at this stage. Secondly, after anthesis and pollen shedding, the GC is destinated to divide once more (second haploid pollen division) in order to produce the two sperm cells involved in double fertilization.

In general, in the last few decades particular attention has been paid to the main steps: meiosis, first and second haploid pollen division. These stages have been extensively investigated in different species focussing the attention on the composition of the organelles, and cytoskeletal elements and their association with the VN forming the MGU (ANGOLD 1968; HESLOP-HARRISON 1968; SANGER and JACKSON 1971 a, b; RUSSELL and CASS 1981; CRESTI *et al.* 1984; VAN WENT 1984; DUMAS *et al.* 1985; RUSSELL *et al.* 1990; see also CRESTI *et al.* 1999). On the contrary only a few reports have focussed on the differentiation and development of the VC within the pollen (GIMENEZ-MARTIN *et al.* 1970; SANGER and JACKSON 1971 c; CHARZYNSKA *et al.* 1989; KUANG and MUSGRAVE 1996) even though there have been many reports of pollen germination and tube growth (review: CAI *et al.* 1997).

The organelles of the VC generally provide evidence of high-level metabolism of sugar, lipids or proteins (CRESTI *et al.* 1991) used after pollen activation for the tube elongation and transport of male gametes along the style and ovary. In *Vitis* the vegetative storage substances are mainly proteins and lipids, the presence of sugar seems rather limited. In any case the VN and VC play a more active role in the development of pollen than the GC. However, the VC is a cell with a programmed dead because its role ends after fertilization and it does not undergo further mitotic division. The molecular basis of the VC fate has not yet been clarified but recently TANAKA *et al.* 1998, with the immunofluorescence method using specific antisera raised against two histones of *Lilium longiflorum*, gave an interesting explanation. They suggested that a preferential decrease in the level of histone (H1) in pollen nuclei is essential for the development of the male gametophyte cell through large-scale expression of genes that include pollen-specific genes which results in pollen germination and growth of the pollen tube.

One interesting feature present in the GC cytoplasm of *Vitis* are dark deposits (tannins) located at the two poles of the cell. Tannin deposits occur very widely among the higher plants (LEDBETTER and PORTER 1970). No general rule seems to govern the distribution of tannins: extracellularly deposited along the primary walls, dispersed in the cytoplasm or concentrated in the vacuoles. The functions of these deposits are different. Some hypotheses have been based on their antioxidant activity and still others on their capacity to discourage predators (see LEDBETTER and PORTER 1970). In *Vitis* the function of GC tannin deposits, in addition to the above mentioned functions could be storage of nutrients

for the carbohydrate metabolism necessary for GC division and sperm cell formation after pollination.

References

ANGOLD, R. E.; 1968: The formation of generative cell in the pollen grain of *Endymion non-scriptus* (L.). J. Cell Sci. **3,** 573-578.

CAI, G.; MOSCATELLI, A.; CRESTI, M.; 1997: Cytoskeletal organization and pollen tube growth. Trends Plant Sci. **2,** 86-91.

CHARZYNSKA, M.; MURGIA, M.; CRESTI, M.; 1989: Ultrastructure of the vegetative cell of *Brassica napus* pollen with particular reference to microbodies. Protoplasma **152,** 22-28.

CASTELLI, S.; PISANI, P. L.; RINALDELLI, E.; 1986: Research on pollen morphology of some grapevine clones. Riv. Ortoflorofrutticult. Ital. **69,** 287-297.

CIAMPOLINI, F.; CRESTI, M.; 1981: Atlante dei Principali Pollini Allergenici Presenti in Italia. Ed. Univ. Siena.

- -; FALERI, C.; DI PIETRO, D.; CRESTI, M.; 1996: Structural and cytochemical characteristics of the stigma and style in *Vitis vinifera* L. var. Sangiovese (Vitaceae). Ann. Bot. **78,** 759-764.

CRESTI, M.; PACINI, E.; SARFATTI, G.; SIMONCIOLI, C.; 1975: Ultrastructural features and storage function on *Lycopersicum peruvianum* pollen. In: L. MULCAHY (Ed.): Gamete Competition in Plants and Animals. North Holland Publ. Co., Amsterdam.

- -; CIAMPOLINI, F.; KAPIL, R. N.; 1984: Generative cells of some angiosperm with particular emphasis on their microtubules. J. Submicrosc. Cytol. **16,** 317-326.

- -; BLACKMORE, S.; VAN WENT, J. L.; 1991: Atlas of Sexual Reproduction in Flowering Plants. Springer-Verlag, Berlin, Heidelberg, New York.

- -; TIEZZI, A.; 1992: Sexual Plant Reproduction. Springer-Verlag, Berlin, Heidelberg.

- -; CAI, G.; MOSCATELLI, A.; 1999: Fertilization in Higher Plants: Molecular and Cytological Aspect. Springer-Verlag, Berlin, Heidelberg.

DUMAS, C.; KNOX, R. B.; GAUDE, T.; 1985: The spatial association of the sperm cells and vegetative nucleus in the pollen grain of *Brassica*. Protoplasma **124,** 168-174.

FAEGRI, K.; IVERSEN, S.; 1964: Textbook of Pollen Analysis. Blackwell Scientific Publ. Oxford.

GIMÉNEZ-MARTIN, G.; RISUENO, M. C.; SOGO, J. M.; 1970: Development of the vegetative cell in pollen grain. Cytologia **35,** 77-90.

HESLOP-HARRISON, J.; 1968: Synchronous pollen mitosis and the formation of the generative cell in massulate orchids. J. Cell Sci. **3,** 457-466.

- -; HESLOP-HARRISON, Y.; 1985: The secretory system of the stigma of *Oenothera organensis* Munz: Same developmental and quantitative features. Isr. J. Bot. **34,** 187-204.

KUANG, A.; MUSGRAVE, M. E.; 1996: Dynamics of vegetative cytoplasm during generative cell formation and pollen maturation in *Arabidopsis thaliana*. Protoplasma **194,** 81-90.

LEDBETTER, M. C.; PORTER, K. R.; 1970: Introduction to the Fine Structure of Plant Cells. Springer Verlag, Berlin, Heidelberg, New York.

LOMBARDO, G.; CARGNELLO G.; BASSI, M.; GEROLA, F. M.; CARRARO, L.; 1978: Pollen ultrastructure in different vine cultivars with low productivity. Vitis **17,** 221-228.

MASCHARENHAS, J. P.; 1989: The male gametophyte in flowering plants. Plant Cell **1,** 657-664.

O'BRIEN, J. P.; MCCULLY, M. E.; 1981: The Study of Plant Structure. Principles and Selected Methods. Termacharphi-Melbourne.

RUSSELL, S. D.; KAUSTINEN, P.; KAUSTINEN, H. M.; 1976: Differential staining of tannin in section of epoxy-embedded plant cell. Stain Technol. **51,** 237-240.

- -; CASS, D. D.; 1981: Ultrastructure of the sperms of *Plumbago zeylanica* 1. Cytology and association with the vegetative nucleus. Protoplasma **107,** 85-107.

- -; CRESTI, M.; DUMAS, C.; 1990: Recent progress on sperm characterization in flowering plants. Physiol. Plant. **80,** 669-676.

SANGER, J. M.; JACKSON, W. T.; 1971 a: Fine structure study of pollen development in *Haemathus Katherinae* Baker. 1. Formation of vegetative and generative cells. J. Cell Sci. **8,** 289-301.

- -; - -; 1971 b: Fine structure study of pollen development in *Haemathus Katherinae* Baker. 2. Microtubules and elongation of the generative cells. J. Cell Sci. **8,** 303-315.

- -; - -; 1971 c: Fine structure study of pollen development in *Haemathus Katherinae* Baker. 3. Changes in organelles during development of vegetative cells. J. Cell Sci. **8,** 317-329.

SENSI, E.; VIGNANI, R.; RHODE, W.; BIRICOLTI, S.; 1996: Characterization of genetic biodiversity with *Vitis vinifera* L. Sangiovese e Colorino genotypes by AFLP and ISTR DNA marker technology. Vitis **35,** 183-188.

TANAKA, I.; ONO, K.; FUKUDA, T.; 1998: The developmental fate of angiosperm pollen is associated with preferential decrease in the level of histone H1 in the vegetative nucleus. Planta **206,** 561-569.

VAN WENT, J. L.; 1984: Unequal distribution of plastids during generative cell formation in *Impatiens*. Theor. Appl. Genet. **68,** 305-309.

VIGNANI, R.; BOWERS, J. E.; MEREDITH, C.; 1996: Microsatellite DNA polymorphism analysis of clones of *Vitis vinifera* Sangiovese. Sci. Hort. **65,** 163-169.

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