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 microsporeocyte undergoes meiosis in order to form a tetrad of microspores stuck 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 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 aluminum stubs, coated with gold and then examined with a Philips 501B SEM.

For transmission electron microscopy (TEM), the material was fixed in 2 % glutaraldehyde (GA) in 0.05 M Cadodylate buffer, pH 7.4, for 1 h at room temperature, rinsed in the same buffer and post-fixed in 1 % osmium tetroxide, dehydrated in ethanol and embedded in low viscosity resin. Sections were cut with a diamond knife using a LKB ultraome 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 Toluidine 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 Axioskop 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 simultaneous cytokinesis 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
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). Tetrads 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 electron-dense cytoplasm with active dictyosomes (D); x 38000 (4).

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 electron-dense; 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 vacuoles (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 IVENSEN 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 electron-dense 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 electron-dense 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.
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 destined 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-MARTIR et al. 1970; SANGER and JACKSON 1971 c; CHAREZNSKA et al. 1989; KUANG and MGURAVE 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 death 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 antiserum 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 (LEDGER 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 LEDBERGER and PORTER 1970). In *Vitis* the function of GC tannin deposits, in addition to the above mentioned functions could be storage of nutrients...
References


RECEIVED May 18, 1999.