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Development of Anther Wall Throughout Microsporogenesis in *Vitis vinifera* L. Cv. Çavuş

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ABSTRACT

At the early prophase stage of meiosis in microspore mother cells, five rows of cells were identified in *Vitis vinifera* L. Cv. Çavuş anthers consisting of a single layer epidermis in the anther wall surrounding the sporagenous tissue, one row of endothecium, one to two rows of middle layer and one row of tapetum layer. It was found that tapetum layer in anther cross sections comprises cells with lrgae nucleus and dense cytoplasm. It was observed that, during further development, epidermis was split into piece**,** tapetum cells developed abnormally and pollen mother cells grew up to larger sizes in some samples. Those cells were identified to consist of often two, occasionally one and rarely three to six nuclei. Moreover, differences were observed in nucleus size as well. It was reported that normal mitosis division and sticky division in tapetum cells occurred at the same time.

Key Words: Microsporogenesis; Anther wall; *Vitis vinifera* L.

INTRODUCTION

As it transmits food to sporagenous cells during pollen development in anthers, tapetum, the most inner part of anther wall, has a physiological role in pollen development. During pollen development tapetum layer contributes to basic events like transmittal of food to microspore mother cells, formation of callose in microspore mother cells during meiosis, differentiation of microspores after tetrad phase, formation of pollen wall, synthesis and secretion of substances such as triphin and polenkitt (Pacini *et al*., 1985; Chapman, 1987; Ünal, 1988; Pacini, 1990).

The cytological and genetic studies on anther development in male fertile and male sterile samples show that dead pollen formation in male sterile samples stems from the defective development of tapetum that would disable its functioning (Graybosch & Palmer, 1988; Kaul, 1988; Goldberg *et al*., 1993; Gorman & Mccormick, 1997; Chaubal *et al*., 2000; Fei & Sawhney, 2001; Smith *et al*., 2002; Nonomura *et al*., 2003; Steiner-Lange, 2003).

An ultrastructural study of the microsporogenous tissue of other plants, from premeiotic stages to anthesis, were reported recently (Polowick & Sawhney, 1993; El-Ghazaly & Rowley, 1999; Hermann & Palser, 2000; Suzuki *et al.,* 2001; Teıxeıra *et al.,* 2002).

Though constituing a small proportion in species *Vitis vinifera*, it is known that pollens are sterile in functional female flower types. Microsporogenesis was investigated in a very small group of defective types, and defects during meiosis were examined (Hilpert, 1958, 1959; Staudt & Kassrawi, 1972; Me *et al*., 1984; Silva *et al*., 2001). Besides, it was seen that no observations and evaluations regarding anther wall and tapetum development were emphasized in these studies.

For exploring the relation between pollen defects and tapetum cells, this study examines the cells forming the anther wall and particularly the tapetum layer in Çavuş type grape, which has been characterized by its functional female flower structure and sterile pollens (Fidan & Çelik, 1980).

MATERIALS AND METHODS

In this study, Çavus type obtained from the experimental vineyards of Ankara University Faculty of Agriculture Department of Horticulture was employed. This type with functional female flowers has a well-developed female organ with flowers curled downwards (Fig. 1).

Samples were taken from various flower stages everyday between 9:00-10:30 AM, during the period 9th April-4 May in years 2001-2002. The anthers in flower buds, after its leaves having been taken out from their perianth, were fixed in formalin-acetic acid-alcohol (FAA) solution and preserved under a binocular microscope. After the fixation process, the samples were dehydrated during alcohol series, and embedded in paraffin after the process of paraffin saturation in xylol. With Reichert microtome, sections of 10-12 µm thickness were taken from the materials embedded in paraffin. Getting stained with hematoxylin with Heidenhain Fe (Johansen, 1940) and Safranin-Fast green (Algan, 1981), they were mounted in Canada balsam, examined with light microscope and their microphotos were taken.

Fig. 1. Morphological appearance of female and male organs in Çavuş type flower

Fig. 2. A: Section in breadth from a young anther. Bar = 50 µm. B: Wall layers in a young anther Bar = 20 µm. (E:. Epiderma, ML: Middle layer, PMC: Pollen mother cell, F: Filament, En: Endothecium, T: Tapetum, L: Loculus)

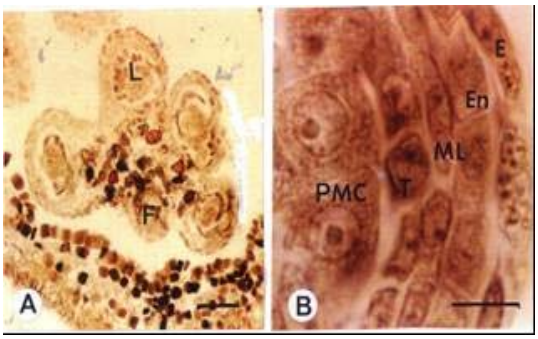


Fig. 3. Number of nuclei in tapetum cells. A: Three nuclei, B: Four nuclei, C: Five nuclei. Bars = 10 µm.

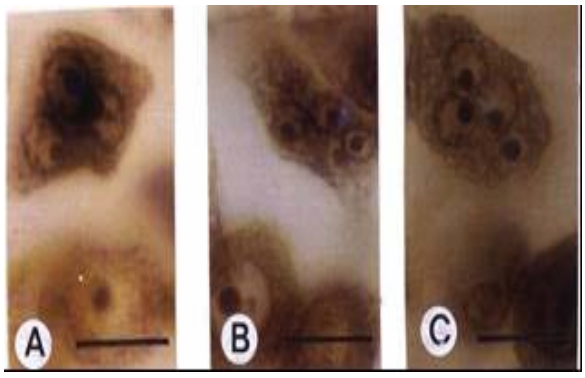


Fig. 4. Normal mitosis division in tapetum cells. A: Metabolic phase, B: Prophase, C: Metaphase, D: Anaphase, E: Telophase, F: Tapetum cell with two nuclei. Bars = 10 µm.

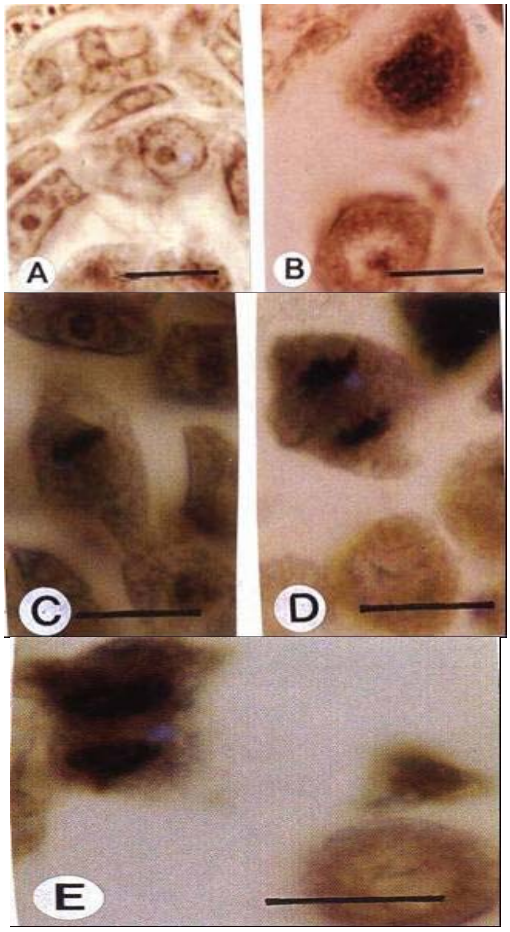
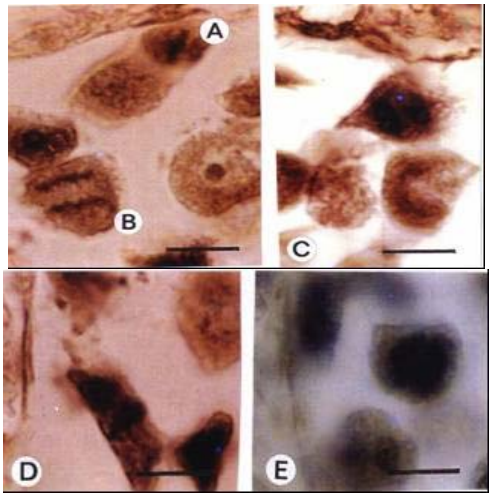


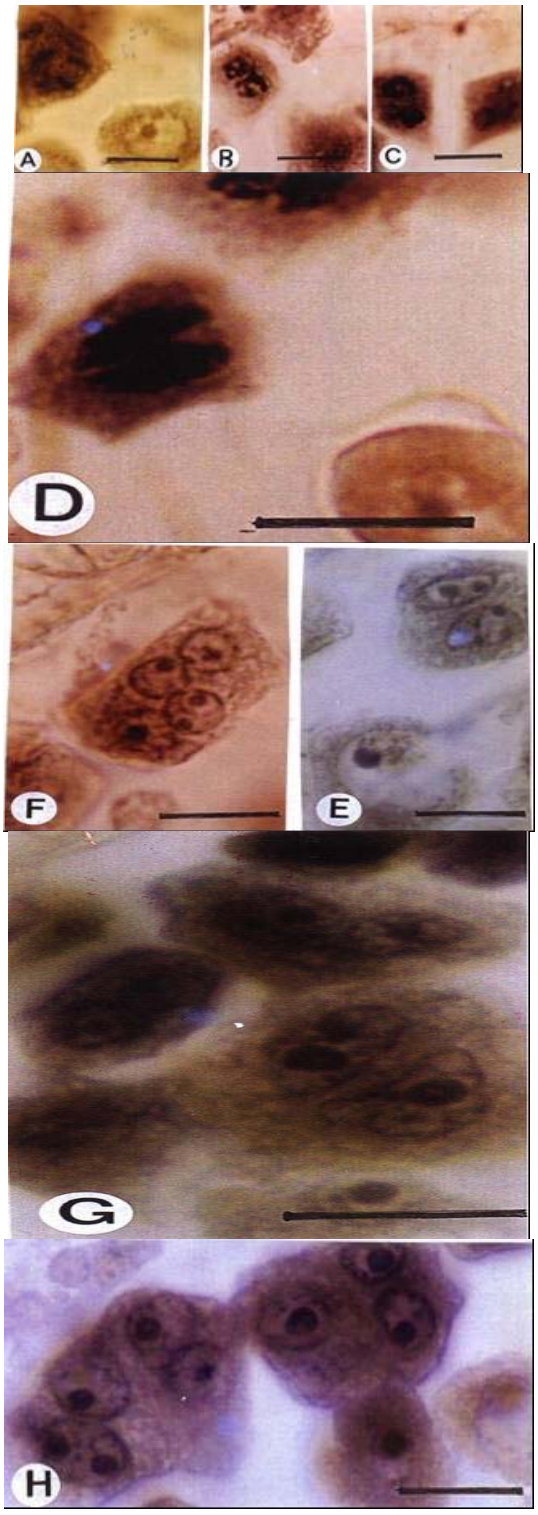
Fig. 5. Defective mitosis division (sticky division) in tapetum cells. A: Metaphase, B: Anaphase, C: Chromosom bridges at the end of anaphase, D and E: Two nuclei unifying due to the bridges. Bar = 10µm.



RESULTS

Anthers were identified in tetrasporangiat structure. Microspore mother cells at the early prophase stage of meiosis were surrounded by a single layer of epidermis in the anther wall surrounding the sporagenous tissue, one row of endothecium, one to two rows of middle layer and one row of tapetum layer (Fig. 2).

Fig. 6. Secondary nucleus splits (A,B,C,D) and unifications (E,F,G,H) in tapetum cells (E). Bars = 10µm.



Tapetum was in the form of secretory tapetum, comprising cells with large nucleus and dense cytoplasm. It was observed that, during further development, epidermis was broken into pieces**,** tapetum cells developed abnormally and pollen mother cells grew up to larger sizes in some samples. According to average values, tapetum cells and pollen mother cells were calculated as 11.75 µm and 11.25 µm, respectively. These cells were found to consist of often two, occasionally one and rarely three-six nuclei (Fig. 3) and differences were observed in nucleus size as well. Eight pollen mother cells (PMCs) in general were identified in loculus (pollen bags) in anther cross sections. An increase in volume was observed in the nuclei of tapetum cells when PMCs were going through the prophase stage of meiosis, and chromosomes were aligned in an equatorial plane at metaphase stage.

While chromatids were normally being pulled to poles at anaphase stage, their spirals were loosened and nucleus was formed at telophase. As nuclear division was not followed by cytoplasmic, two cells were noted to exist in the same cell (Fig. 4). At this period, when PMCs were going through the prophase stage of meiosis, the content of starch increased in epidermis, endothecium and middle layer cells. It was noted that normal mitosis division and defective mitosis division (sticky division) in tapetum cells occurred at the same time (Fig. 5).

At the beginning of anaphase, as homolog chromatids was not separated completely, they looked unified. As these sister chromosomes were not pulled to the poles regularly, bridges were formed in between and due to the high number of those bridges, a tetraploid nucleus was formed at the end of telophase with the combination of two diploid cells. Nuclei of tetraploid cells formed after normal and defective mitosis division, again experienced secondary normal and defective divisions of nuclei, unification and formed poliploid tapetum cells with many nuclei (Fig. 6). It was observed that most of the tapetum cells were not yet spoiled once meiosis was completed and tetrads were formed (Fig. 7).

Epidermis and single row endothecium were identified in mature anther wall and pollen grains were identified in loculus (Fig. 8). Whereas some of endothecium cells observed fibrous thickness, this was not the case in some other endothecium cells. Also during the development of anther, middle layer cells were crushed and grown in width.

DISCUSSION

Tapetum cells, responsible for microspore nutrition, have also been implicated in abortion. These cells show certain anomalies at some stage of their development cycle. The early disintegration of these cells is characterized by an

Fig. 7. Tetrads formed at the end of meiosis division and tapetum cells that have not spoiled. Bar = 20µm

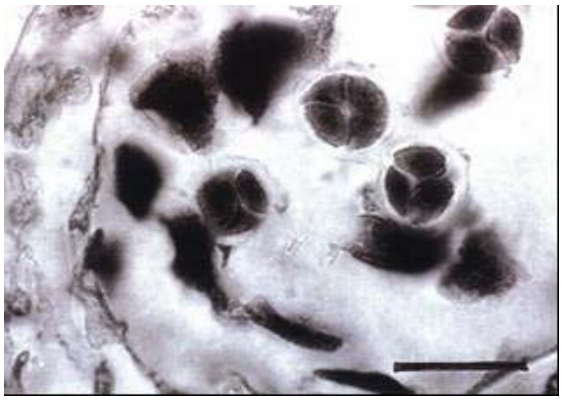
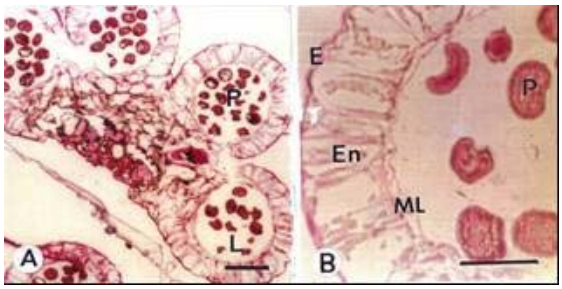


Fig. 8. A: Section in breadth from a mature anther. Bar = 50µm. B: Wall layers and pollen tetrads in a mature anther (E: Epiderma, Ml: Middle layer, P: Pollens, En: Endothecium, L: Loculus) Bar = 10µm



increase in vacuolation and the occurence of lipid bodies.

In our study, endomitosis was not identified in the tapetum cells of *V. vinifera* cv. Çavuş despite the occurence of normal and defective mitotic divisions, nucleus splits and unificated. The number of nuclei in tapetum cells changed generally between two to six, sizes of nuclei were different and cells were stained very dark. Another finding is that in most of the samples, tapetum cells were dispersed far from each other.

Numerous investigations indicated that tapetum through modified physical and physiological factors initiates the process of polen abortion acting at the meiotic stage (Bhandari, 1984). In many plants, tapetum is often degenerated before pollens leave the flower. However, in Çavuş type of grape, it was identified that tapetum cells have not spoiled till the dispersal of pollens. Many researchers have stated that in some other male sterile plants as well, a permanent tapetum with many nuclei exists (Graybosch & Palmer, 1985, 1988; Gorman & McCormick, 1997; Chaubal *et al*., 2000). Furthermore, persistent tapetum blocks nutrient transportation, which is necessary for the development of microspores into viable polen grains.

In conclusion, some defects during the division of tapetum cells in Çavuş type might affect pollen development. It is considered that these defects may lead to low pollen fertility and affect the proportion of seed setting and grain formation. Our studies to find the reasons of sterile pollen development are continued with comparing the thin structure of tapetum cells, the differentiation of organelles in the cells and the anther walls of the samples that have gone through normal and defective meiotic divisions, respectively.

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