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Examination of Meiosis Anomalies in Anthers of *Vitis vinifera* L. cv. Çavuş

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Abstract: Meiotic anomalies in pollen mother cells in anthers of *Vitis vinifera* L. cv. Çavuş (2n = 38) were examined by light microscopy. In most microspore mother cells, the course of meiosis is regular. In some cells irregularities were observed, including chromosome bridges, univalents and fragments. Microspore tetrads are isobilateral and tetrahedral.

Key words: *Vitis vinifera* L. cv. Çavuş, microsporogenesis, meiotic division

INTRODUCTION

Due to the considerable economic importance of viticulture in the world and the variety and richness of grape co-products, the genus *Vitis* has been the subject of many diverse scientific studies.

Turkey, besides being the motherland of the species *V. vinifera* and viticulture, also has ecological conditions suitable for viticulture and possesses a rich source of grapevine genes.

In *V. vinifera* with functional female flowers, generally, microspore development occurs differently from that occurring in the types with hermaphrodite flowers. These types, forming a smaller group in terms of numbers, are generally characterized as possessing sterile pollen grains[1,2]. However, due to the limited number of studies on this topic[3-5], the determinants of sterility and their causes could not have been completely clarified. Hilpert[6,7] indicated that defective developments occurred in early meitoic stages in *V. vinifera*. Analysis of meiotic chromosomes carried out by Jelenkovic and Olmo[8], in F1 hybrids of *V. vinifera* (2n = 28) and *V. rotundifolia* Michx (2n = 38) revealed the presence of univalents, bivalents, trivalents, tetravalents and pentavalents.

Staudt and Kassrawi[9] made a study on the spontaneous tetraploid mutation in Riesling grapes and meiotic division in diploid types. Me *et al.*[10] mentioned that bivalent and trivalent, tetravalent chromosomes in diakinesis and Metaphase I, respectively, indicating also Metaphase II with lagging chromosomes. Viljoen and Spies[11] who explored the genetic relation among the species *V. vinifera, V. rotundifolia* and *V. rupestris*, studied the meiotic chromosomes of F1 hybrids that are formed by the crossbreeding of those aforementioned three species. Zhang *et al.*[12] carried out cytogenetic analyses in the diploid and tetraploid pollens of the hybrid *V. labrusca* X *V. vinifera.* Cresti and Ciampolini[13] studied the ultrastructural characteristics of pollen development in *V. vinifera* (cv. Sangiovese). Silva *et al.*[14] on the other hand, examined the meiotic division, pollen fertility and pollen germination in Brazilian Rubi type of *V. vinifera. V. vinifera* types with functional female flowers whose gene centers are Mediterranean and East European countries, are of importance in the breeding of new types due to their fruit characteristics. One of those types is the high quality table grape cultivar, Çavus, with its large grains, thin hull, its peculiar aroma, being relatively seedless. While the effects of ionized radiation applications on sterile pollen grains in Çavus types of grapes have been explored[15], there have not been any studies on the defects of meiotic division. Although it is possible to create seedless or partly seedless grapevines through artificial methods, genotype based sterilities occur due to flower morphology, structures and developments of pollen and seed drafts and further research on these structures have important effects particularly on the success of breeding initiatives. This study aims at putting forth the defects in the meiotic division of pollen mother cells in anther for providing more efficient usage of Çavus type of grape which has considerable importance among Turkey's grapevine gene sources in breeding programs.

MATERIALS AND METHODS

In this study, which explores the abnormalities in the meiotic division of pollen mother cells in various anthers of the type *V. vinifera* cv. Çavus (2n = 38), the anthers were obtained from the experimental fields of the Faculty of Agriculture, Ankara University in Ankara, Turkey. Between the dates April 9 and May 4 samples were taken

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everyday in the morning between 9. 00 and 10.30 from various flower stages, including the bud. Approximately, 1150 anthers were examined and also, 9200 numbers of the cells were analysed. Samples were firstly fixed in Carnoy (ethanol/acetic acid, 3:1) fixing solution for 2 h. Following that process, having been passed through alcohol and xylol series, materials were then embedded in paraffin after the process of paraffin saturation. Sections in length and breadth of 8-12 µm in thickness were cut from those materials and after getting stained with hematoxilen with Heidenhain Fe[16], they were covered with Canadian balsam.

RESULTS AND DISCUSSION

In each loculus of anther, eight pollen mother cells in general were observed in the transverse sections. The cells in question are the ones with dense cytoplasm and big nuclei. While some of those were to experience a normal meiotic division, some recorded an abnormal formation due to the thickening of the nucleus membrane (Fig. 1a) and the increase in the number of nucleolus (Fig. 1 b).

In those kinds of cells, nucleus membrane was stained to a considerably dark extent and 3-8 nucleolus were counted inside. This sort of increase in nucleolus number points out a noteworthy level of RNA synthesis in the nucleus instead of DNA. At this point, a hypothesis comes into mind that the great amount of the synthesized RNA might have congested nucleus pores and probably, necessary enzymes could not have entered in the nucleus.

In the prophase stages of meiosis, while some mother cells experienced a normal division, some recorded various abnormalities. In some nuclei, it was observed that chromatin filament was stacked on the nucleolus and could not resolve immediately (Fig. 2a). It was seen that in some nuclei, despite the disappearance of nucleolus, chromosomes could not still become organized (Fig. 2b) and they even could not separate from one another for a long time in some other of the observed nuclei.

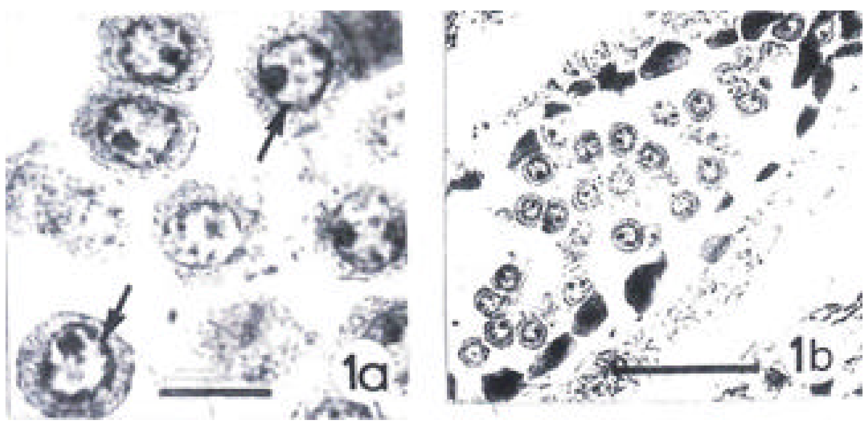

Fig. 1a: Thickening of nucleus membrane taken place in pollen mother cells (arrows). (Bar=0 µm) b: Increasing number of nucleolus in the nucleus. (Bar = 50 µm)

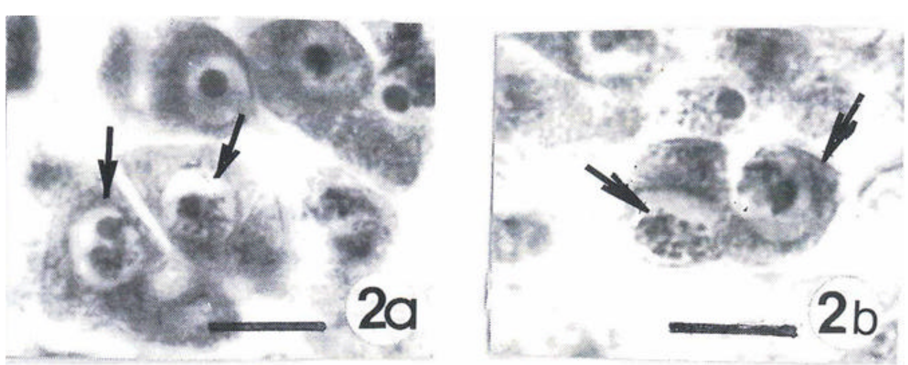

Fig. 2a: Folded chromatins in the nucleus of pollen mother cell which could not get organized in the prophase stage. b: Chromosomes not completely organized despite the disappearance of nucleus. (Bars = 10 µm)

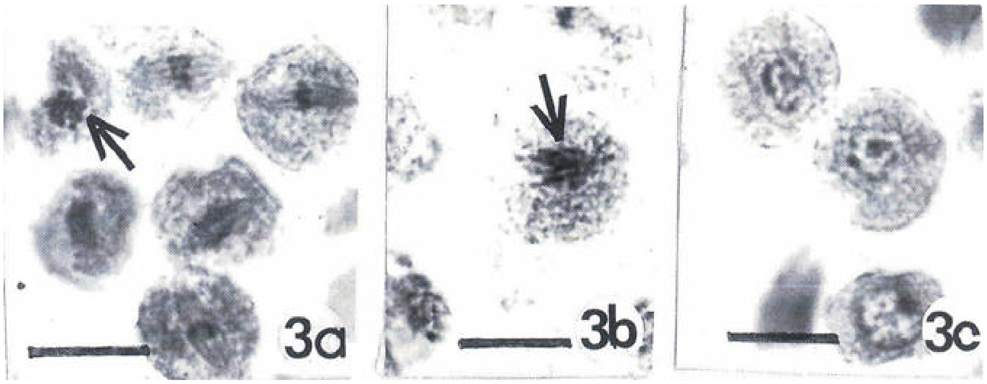

Fig. 3a: Chromosomes standing in groups in Metaphase I. b: Chromosomes standing dispersed. c) Univalents in Metaphase I. (Bars = 10 μm)

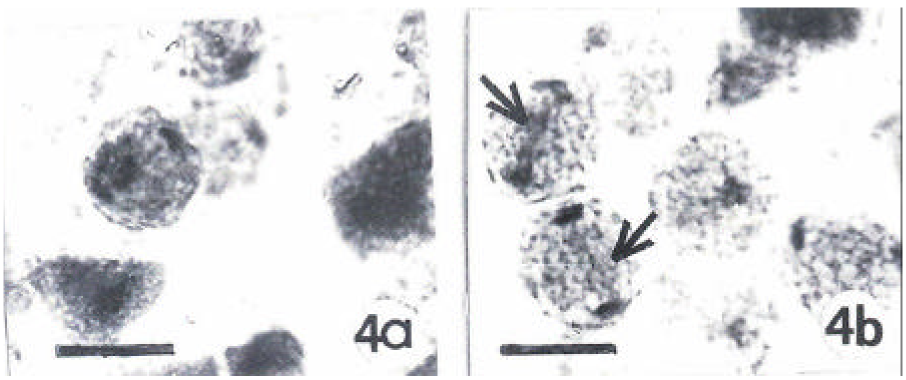

Fig. 4a and b: Chromosomes that could not migrate to the poles at the same time in Anaphase I. (arrows). (Bars = 10 μm)

In the metaphase stage, some of the chromosomes were observed to look like groups (Fig. 3a), some of them were found to be standing dispersed (Fig. 3b). In some cells, chromosome groups were observed both at the center and in the poles. Also, in some others, it was seen that chromosomes were stacked on a certain side of the cell. This kind of collapse of the chromosomes in one pole of the cell makes one think about the assumption of non-formation of poles.

Hilpert[6] and Me *et al.*[10] mentioned that chromosomes, being trivalent and tetravalent as well, were observed to form usually bivalents in Metaphase I. It was determined that the aforementioned case also applies to *V. vinifera* X *V. rotundifolia* hybrid[11]. Staudt and Kassrawi[9] reported that, similarly, in Riesling type of grape, they observed circular bivalents in diakinesis.

Having found out in Anaphase I that chromosomes did not synchronize in their attachments to spindle strings and their withdrawals to the poles, we also observed the existence of chromosomes forming bridges and those that could not reach the poles. Chromosome bridges probably might have occurred due to unequal crossing-over.

Hilpert[7] also mentioned that chromosomes could not reach to the poles due to the defects occurring in Anaphase I. Zhang *et al.*[12] expressed that from Prophase I to Prometaphase I, the synapsis was not complete in univalent and multivalents and because of that, homolog chromosomes did not split in Telophase I.

Nevertheless, also combined chromosomes univalent and bivalent shapes were found in this phase (Fig. 3c). It was observed that in the anaphase I stage, clinging on to the spindle filaments and being drawn to the poles did not occur at the same time in many of the chromosomes. In the samples observed, chromosomes at different distances from the equator to the poles were seen as well as inseparable ones (Fig. 4a and b).

In Telophase I stage, after the chromosomes reached to the poles, nucleus membranes appeared. However, spindle preserved their appearance for a long time without any spoiling (Fig. 5a). Meanwhile, spoiling to a certain extent was recorded in some of the nuclei. The nucleus membrane transformed ingrowths. The nuclei lost their normal shapes, some of them appeared to have many nucleolus after the transformation (Fig. 5 b and c). In the

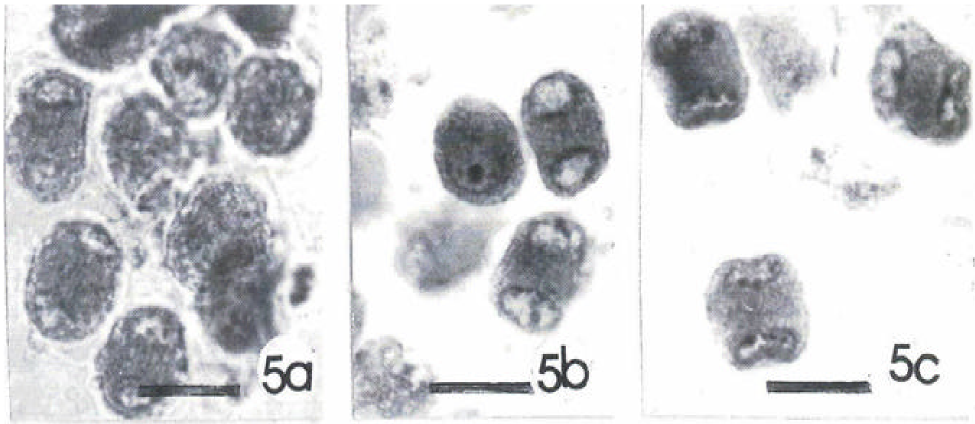

Fig. 5a: As seen, spindle filaments are still unspoiled in Telophase I despite the formation of the nucleus membrane. b and c: Abnormal appearances of the formed nuclei . (Bars = 10 μm)

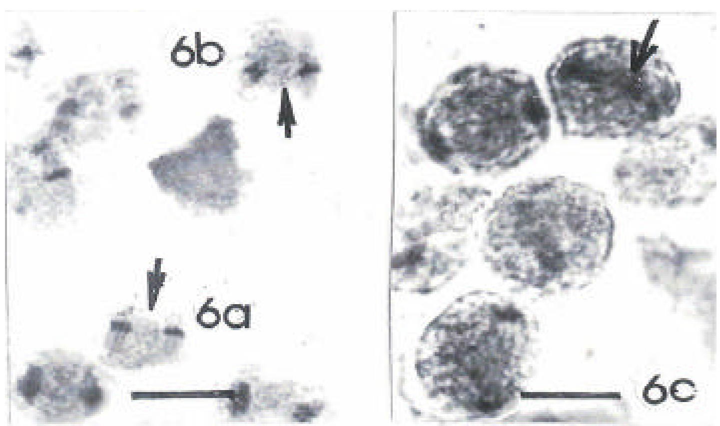

Fig. 6a: The position of four-poled parallel (arrow). b: and non-parallel (arrow) spindle filaments in post meiotic division. c: Anaphase II with chromosome bridges (arrow). (Bars = 10 μm)

formation of 4-folded poles, both parallel and perpendicular positions of the spindle filaments were observed (Fig. 6 a and b).

The irregularities seen in the first meiotic division were seen in the second as well. In anaphase II bridges were observed (Fig. 6c). Chromosomes and small chromosome groups scattered in the cytoplasm were also seen.

It was observed that at the end of meiotic division, usually 4 microspores were formed isobilateral and tetrahedral in shape. In some of the tetrads, many micronuclei were determined. The high numbers of micronuclei in the tetrads indicate a lack of regular meiotic division.

Probably, the origin of the micronuclei in the tetrads are the lagging chromosomes in Anaphase I and the origin of the lagging chromosomes in Anaphase I are the univalants in Metaphase I. As a result of all those observations and research, it is concluded that the meiotic division is regular in some of the pollen mother cells in various anthers of the type *V. vinifera* cv. Çavus *Vinifera* type of grape. However, irregularities were found out in many of the samples examined. It was concluded that those irregularities would imply possibly low pollen productivity and the plant might affect fruitseet formation through seed formation.

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