The influence of centrifugation on the course of microsporogenesis in *Tradescantia bracteata* (L.)

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Abstract

Centrifugation is one of the laboratory techniques used to separate the mixture into fractions. Microsporogenesis is a process leading to development of microspores in pollen grains. The process consists in meiotic division of pollen mother cells. The aim of the study was to assess the impact of centrifugation on the course of the microsporogenesis process and subsequent development of the pollen grain in Tradescantia bracteata and to check whether centrifuged pollen was able to germinate. The material used in the experiment included stamens of the spiderwort (*Tradescantia bracteata*) from the family Commelinaceae. The investigations showed a correlation between centrifugation and the arrangement of the anther tissue and cellular structures in pollen grains.

Key words:

microsporogenesis, centrifugation, *Tradescantia bracteata*

Introduction

Centrifugation is one of the laboratory techniques used to separate the mixture into fractions, which is used in various fields of medicine. The rotor of the centrifuge force is generated several hundred thousand times superior to the Earth's gravity. Shall fall particles and positioning them in balance by a concentration gradient. In the process of spinning followed by the migration of particles in the gradient, when their molecular density is balanced density separation solution a group of molecules is shorted specific place. After completion of the centrifugation may be desired fractions were collected and used for further experiments. In molecular biology centrifugation is used to separate the various cell organelles, DNA, cell membranes, depending on the formulation and the purpose of separation. This process has an impact on the functionality of individual organelles.

Microsporogenesis is a process leading to formation of haploid cells, i.e. microspores that later transform into pollen grains, which are male gametophytes. This process takes place in pollen sacs and involves meiotic division of pollen mother cells.

Meiosis in stamens takes approximately 36 hours, and prophase I is its longest phase. After two meiotic divisions, a microspore tetrad with a reduced number of chromosomes in the cell nucleus is formed. Next, pollen grains develop from these cells. The onset of meiosis and its subsequent phases leading to tetrad formation take place almost synchronously in the entire anther and in all anthers of a stamen whorl.

The sporogenous tissue initially has a structure of an ordinary meristematic tissue. The cells of the tissue are characterised by a large nucleus, dense cytoplasm, and a thin cellulose-pectin cell wall with numerous connections with tapetum cells. Through these connections, nutrients and stimulatory substances reach the cells. The transformation begins at the onset of the meiotic prophase. Prior to morphological chromatin transformations, the amount of DNA and associated proteins is doubled in the pre-meiotic nucleus [1]. In the meiotic prophase, the sporogenous tissue begins to separate from the surrounding tapetum. The plasmodesmatal connections are ruptured due to the thickening of the inner and radial meiotic cell walls. In sporogenous cells, i.e. microsporocytes, pinocytosis takes place and droplets of tapetal material are engulfed by a protruding microsporocyte plasmalemma. The vesicle moves inside the cytoplasm towards the perinuclear zone. The pinocytic activity is therefore associated with a rapid increase in the cytoplasm volume in microsporocytes [2,3].

Protoplasts of all adjacent microsporocytes are interconnected with broad cytoplasm bands via newly formed channels in the cell walls. As a result of the formation of the channels, microsporocyte protoplasts constitute a closely linked unity. Macromolecular compounds and cellular organoids, e.g. mitochondria, can be easily transferred along the cytoplasm bands. Cytoplasmic channels facilitate transfer of nutrients and stimulatory compounds into all sporogenous cells, both those adjacent to the tapetum and those located more deeply. The tissue resembles a polyenergid cell with all of its nuclei at a similar development stage and starting meiosis at the same time. Further chemical changes occur in the walls of prophase I microsporocytes. During prophase I, a polysaccharide, i.e. callose, is deposited on the cellulose-pectin microsporocyte wall. The amount of callose increases rapidly, thereby forming a continuous layer, which tightly envelops the protoplast by the end of the meiotic prophase. At that time, all cytoplasmic connections and plasmodesmata disappear. The cells assume a spherical shape and partly detach from each other. Each microsporocyte becomes a separate unit. Callose walls are also formed during meiotic divisions; hence, each cell in the tetrad is separated from the other cells with a thick callose wall. The tetrad has a common callose wall formed during the prophase and new internal walls [4,5]. The callose walls present in the microsporogenesis process were referred to as special walls by Beer in 1911 [6,7,8].

Tetrads disintegrate into individual cells, i.e. microspores. The tetrad callose walls are rapidly hydrolysed, and released microspores form new walls. The callose wall persists for several tens of hours on the meiocytes, which are then subjected to substantial transformations. The callose wall-building material is poorly permeable. Consequently, only fine particles of basic nutrients can penetrate the meiocyte. Due

to their partial isolation, sporogenous cells can begin differentiation in a completely different direction than the other anther cells [9,10].

The thick and abundant meiocyte cytoplasm reduces its metabolic activity between mid-prophase I and the end of meiosis. During this time, the ability of binding RNA precursors and meiocyte cytoplasm proteins declines [11,12,13,14].

Meiotic division of the cytoplasm can proceed via two mechanisms and is dependent on two types of cytokinesis: successive (subsequent) cytokinesis and simultaneous cytokinesis (evolutionarily older). There are also a number of variations and intermediate cytokinesis forms [15,16].

Microspores constituting the tetrad can typically exhibit tetrahedral, isobilateral, T-shape, and, sporadically, linear arrangement. In monocotyledonous plants, the hydrolysis of tetrad callose walls and disintegration into single microspores takes from a few to several dozen hours. After formation of microspores, the microsporogenesis process is terminated and microspores further develop into pollen grains.

Tredescantia bracteata is a herbaceous plant from tropical and subtropical climate. In temperate climate, it is grown as a potted plant. The length of its bud is strongly correlated with the developmental stages of sporogenous tissue in stamens.

3-mm long buds contain pre-meiotic microsporocytes in the stamens, 3-4,5 mm exhibit young microspores, and 4,5-5,5-mm buds have vacuolated microspores with lateral location of the cell nucleus. Larger buds contain bicellular pollen grains. The 3-4,5 mm buds were centrifuged, surface-sterilised, and on kept a medium for 3 and 6 days for observation of possible developmental changes (Tabl. I, phot. 1).

Tradescantia brecteata has a typical monocot flower with three sepals, three corolla petals, and a pistil composed of three carpels. There are six stamens arranged in two whorls. Each stamen is composed of a filament and a head. The head is divided into two anthers joined by the connective comprising vascular bundles. The anther, in turn, has two pollen sacs (microsporangia). It is surrounded by epidermis and an underlying *endothecium with properties characteristic of mechanical tissue. Below the endothecium, there are transitional layers. They have a parenchymatous character and can usually be crushed.* The next layer is the tapetum (tapetal tissue). The interior of each microsporangium is filled with sporogenous tissue (archespore), whose cells are transformed into microspores during the meiotic division and next into pollen grains. Mature *Tradescantia* pollen has an ellipsoid shape with two axes of symmetry.

In very young 3-mm long *Tradescantia* buds, the central part of pollen sacs is occupied by archesporial cells. These cells closely adhere to each other and to the other surrounding layers of the anther wall. The changes in the sporogenous tissue are accompanied by simultaneous changes in the tapetum. The tapetum in a young anther is a layer separating archesporial cells from the transitional zone of the anther wall. It is built of isodiametric cells in cross section with a single spherical, centrally located nucleus and dense cytoplasm. The tapetum stains intensely with safranin and light green, unlike the other layers of the anther walls.

When five layers have been formed in the anther wall, archesporial cells cease to divide and increase in size. Their centrally located nuclei enlarge as well. The archesporial cells transform into meiocytes (microsporocytes).

Meiotic division in sporogenous tissue starts simultaneously in all stamen cells and proceeds synchronously. At the onset of prophase I, meiocytes are filled with dense cytoplasm and have a large centrally located nucleus (Fig. 1, phot. 2). Cell nuclei contain a nucleolus with signs of chromosome spiralisation. Furthermore, meiotic cells exhibit a thick callose layer. During metaphase I, a karyokinetic spindle forms in dividing meiocytes and the bivalents are located in the equatorial plane. Next, during anaphase I, the chromosomes move towards the poles. At the end of telophase I, two nuclei are formed and the spindle disappears. The meiocyte is divided by the centrifugal cell wall and a dyad is formed.

After the second meiotic division, each dyad cell is divided following centrifugal cytokinesis and a microspore tetrad is formed (Fig. 1, phot. 3). Therefore, meiosis with successive cytokinesis takes place in *Tradescantia bracteata*.

The microspores in the tetrad are separated from each other, but they are surrounded by a common

callose wall. They have a large nucleus containing a distinct nucleolus. In the tetrad stage, tapetal cells undergo polyploidisation. Their cytoplasm does not divide, and the nuclei divide mitotically. The tapetal cells become multinucleated, e.g. in *Tradescantia bracteata,* they are bi- or trinucleated. The *endothecium* increases as well and its transverse walls exhibit bands of thickenings stained red in PAS reaction.

In the further development stages, tetrads disintegrate into single cells (Fig. 1, phot. 4). This process, likewise the further development of microspores, does not proceed synchronously within one pollen sac.

During their development, the microspores are enlarged, become rounded, and are surrounded by a new wall, i.e. the sporoderm. Moreover, a vacuole appears initially inside the microspore and later expands, pushing the nucleus towards the wall (Fig. 1, phot. 5).

During the early stage of microspore vacuolisation, the tapetal cell walls disappear. The tapetal cytoplasm forms a periplasmodium, which infiltrates between the microspores.

During the mitotic division of the microspore, the vacuole shrinks and finally disappears. Two daughter nuclei are formed; one of them is smaller and is referred to as a generative nucleus. Initially, it is located near the sporoderm; next, it moves into the interior of the vegetative cell cytoplasm. The other one, i.e. a vegetative nucleus, accumulates storage substances, grows, and occupies a greater part of the pollen grain (Fig. 1, phot. 6).

The tapetum is further destroyed and completely degraded at the time of pollen grain maturation in the pollen sac (Fig. 2, phot. 1).

The generative cell in a mature *Tradescantia bracteata* pollen grain is completely suspended in the cytoplasm of the vegetative cell surrounded by a thick sporoderm and containing typical cytoplasmic components (Fig. 2, phot. 2).

The aim of the study is to analyse the impact of centrifugation on the course of microsporogenesis and development of pollen grains in *Tradescantia bracteata* and to check whether pollen subjected to centrifugation can germinate and produce the pollen tube.

Material and methods

The investigations were carried out on stamens of the spiderwort (*Tradescantia bracteata*) representing the family *Commelinaceae*. Buds were collected in the greenhouse of the Department of Plant Anatomy and Cytology, Maria Curie-Skłodowska University. The developmental stage of sporogenous tissue was assessed in crushed preparations in acetocarmine stain. The spiderwort buds were measured, their anthers were isolated, and crushed preparations were made. Thus, preparations with visible microsporocytes and different pollen development stages were obtained. The stages were assigned to the respective length of the flower buds. The spiderwort buds were divided into groups. Permanent preparations were made from control buds kept in water for 3, 5, 8, and 10 days. Some preparations were made from material fixed immediately after centrifugation. The research material was centrifuged for 20 minutes at 4000 rpm in a laboratory centrifuge (type WE-2) with a rotor radius of 120 mm.

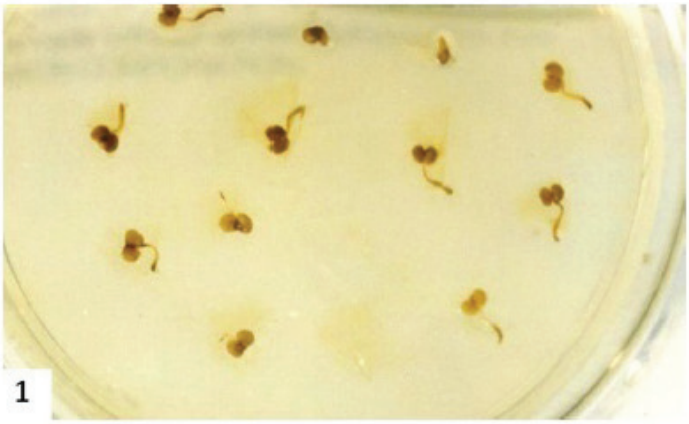
Observations

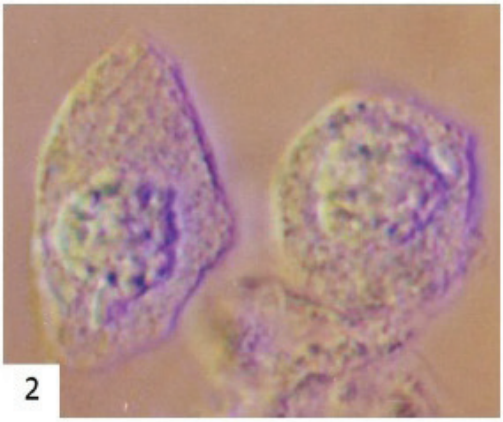
Development of stamens subjected to centrifugation.

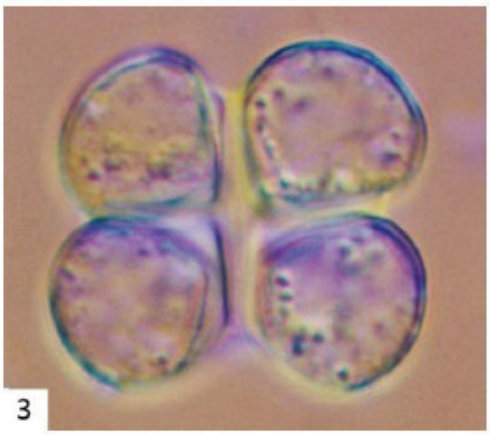
A part of the *Tradescantia* material was subjected to centrifugation, which caused dislocation of microspores relative to the tapetal cytoplasm, organelles in the microspores, pollen grains in the anther loculus, and protoplast elements in these cells. The susceptibility of microspores and pollen to the centrifugal force varies depending on their weight and the developmental stage of sporogenous tissue.

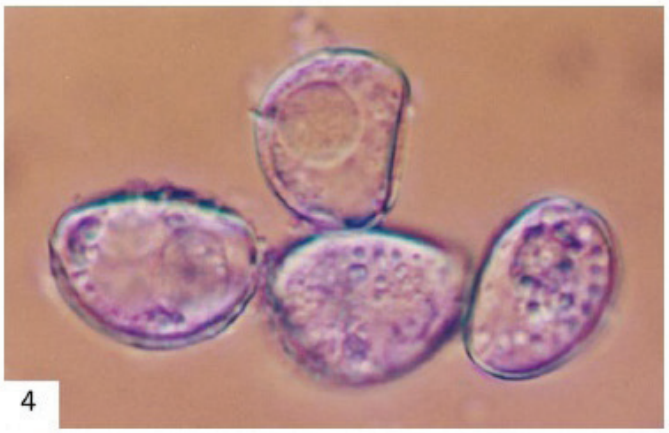
In the early meiotic prophase, tapetal cells have no walls, i.e. they are in a stage preceding formation of the periplasmodium, which infiltrates between meiocytes.

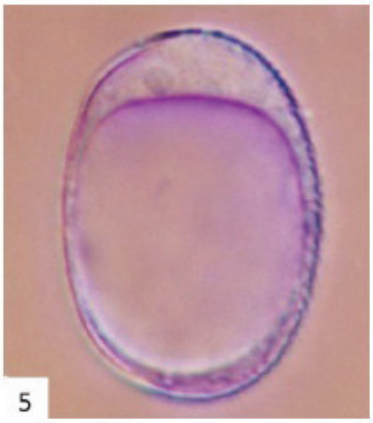
DNA replication proceeds in cell nuclei and is followed by mitosis; however, cytokinesis does not take place and the cells remain multinucleated. During microsporocyte meiosis, tapetal cells in some anthers are already dead. The *endothecium* cannot be distinguished from the other intermediate layers. The cells are flat and have no cellulose reinforcements.











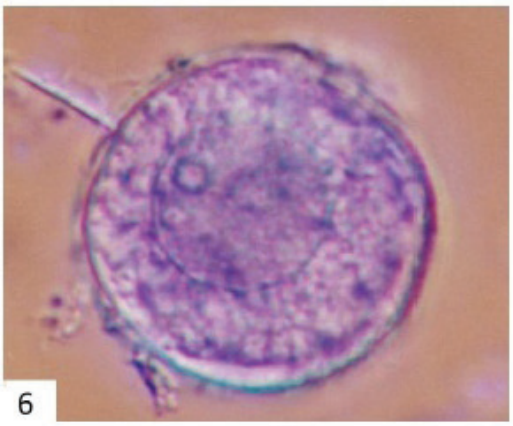


Fig. 1.
Phot. 1. Plate with *Tradescantia* buds inoculated on the medium. Magn. 1.7x
Phot. 2. Meiocyte in the w prophase I stage with central location of the nucleus. Magn. 800x
Phot. 3. *Tradescantia bracteata* microspore tetrad. Magn. 800x
Phot. 4. Four young microspores after tetrad disintegration. Magn. 800x
Phot. 5. Microspore filled by an enlarging vacuole. Magn. 800x
Phot. 6. Young pollen grain with a visible vegetative nucleus. Magn. 800x

In anthers containing microspore tetrads and young microspores, the tapetal cells are sufficiently loose to be dislocated during centrifugation. The direction of the centrifugal force is indicated by the displacement of cell protoplasts as well as the arrangement of tetrads and young microspores within the tapetal cytoplasm. Due to the centrifugation, post-meiotic cells moved along the direction of the centrifugal force, whereas the amorphous tapetal cytoplasm was located above them (Fig. 2, phot. 3).

After the dislocation, the microspores form a dense mass with evenly distributed tapetal nuclei. The next layer is formed of the tapetal cytoplasm with bodies and granules as well as amorphous cytoplasm without tapetal cell nuclei (Fig. 2, phot. 4).

The centrifugation applied in the stage of young vacuolated microspores caused displacement of the nuclei in one direction (Fig. 2, phot. 5). Round tapetal cells with a large nucleus and a nucleolus as well as single nuclei are visible between the microspores (Fig. 2, phot. 6). Epidermis with elongated nuclei distributed centrally or near the outer wall is clearly visible in the anther wall. At this stage, the centrifugation did not cause separation of the tapetum from the microspores. At a later stage of microspore development, centrifugation is not able to displace the microspore relative to the tapetal cytoplasm.

In the stage of binucleate pollen immediately after the mitotic division into generative and vegetative cells, there are only visible traces of displacement of the nuclei in the anther wall cells. In contrast, there are no such traces in the pollen grain cytoplasm. The tapetum in some anthers is already degenerated. Only generative pollen nuclei are displaced in the cytoplasm of vegetative cells.

In later stages of pollen development, the vegetative cell nuclei were displaced, whereas the generative nuclei were not detached from the sporoderm (Fig. 3, phot. 1). The nuclei of the anther wall cells were also displaced (Fig. 3, phot. 2).

Under the impact of the centrifugal force, plastids in the mature bicellular pollen grain are concentrated at one of the cell poles, whereas the tiny vacuoles are accumulated on the opposite side. There are also well visible residues of starch-containing tapetal cytoplasm (Fig. 3, phot. 3).

In later pollen development stages characterised by slight vacuolation, the centrifugation caused displacement of entire generative cells within the vegetative cell cytoplasm and resulted in similar aggregation of plastids at one of the cell poles and small vacuoles at the opposite pole (Fig. 3, phot. 4).

Description of material centrifuged after 3 – and 6-day cultivation.

After three days of culture, the distribution of the microspores in the tapetal cytoplasm of some stamens was identical to that observed immediately after centrifugation. The microspores moved along the direction of the centrifugal force and formed a thick layer. The layer was covered by the tapetal cytoplasm containing orbicles and vesicular spaces resembling vacuoles. This was the lightest fraction. The strongest displacement along the direction of the centrifugal force was observed in the case of starch grains from tapetal amyloplasts and crystalline forms.

After six days of cultivation, the content of the anthers degenerated. Due to necrotic changes, separation of pollen grains from the tapetal cytoplasm was not discernible. No displacement of generative cells in the vegetative cell cytoplasm was visible either.

Discussion and conclusions

Centrifugation of young buds of stamens of *Tradescantia bracteata* causes marked dislocations in the mutual arrangement of the tapetal cytoplasm and sporogenous tissue, which results in changes in the traits of pollen. At a constant centrifugal force and a temperature of 21°C, the range of the displacement depends on the developmental stage of the sporogenous tissue and tapetum. The dislocations are inconsiderable at the stage of meiosis but substantial in the stage of microspores, when a layered system resulting from the fractionated centrifugation procedure is formed. Microspores and cell nuclei of the amoeboid tapetum form the lowermost layer, as these structures are the heaviest. Above, there are three layers of the tapetal cytoplasm with distinctly different characteristics. The lowest layer in contact with microspores consists of amorphous content and suspended pollen grains. The middle layer contains many fibrous

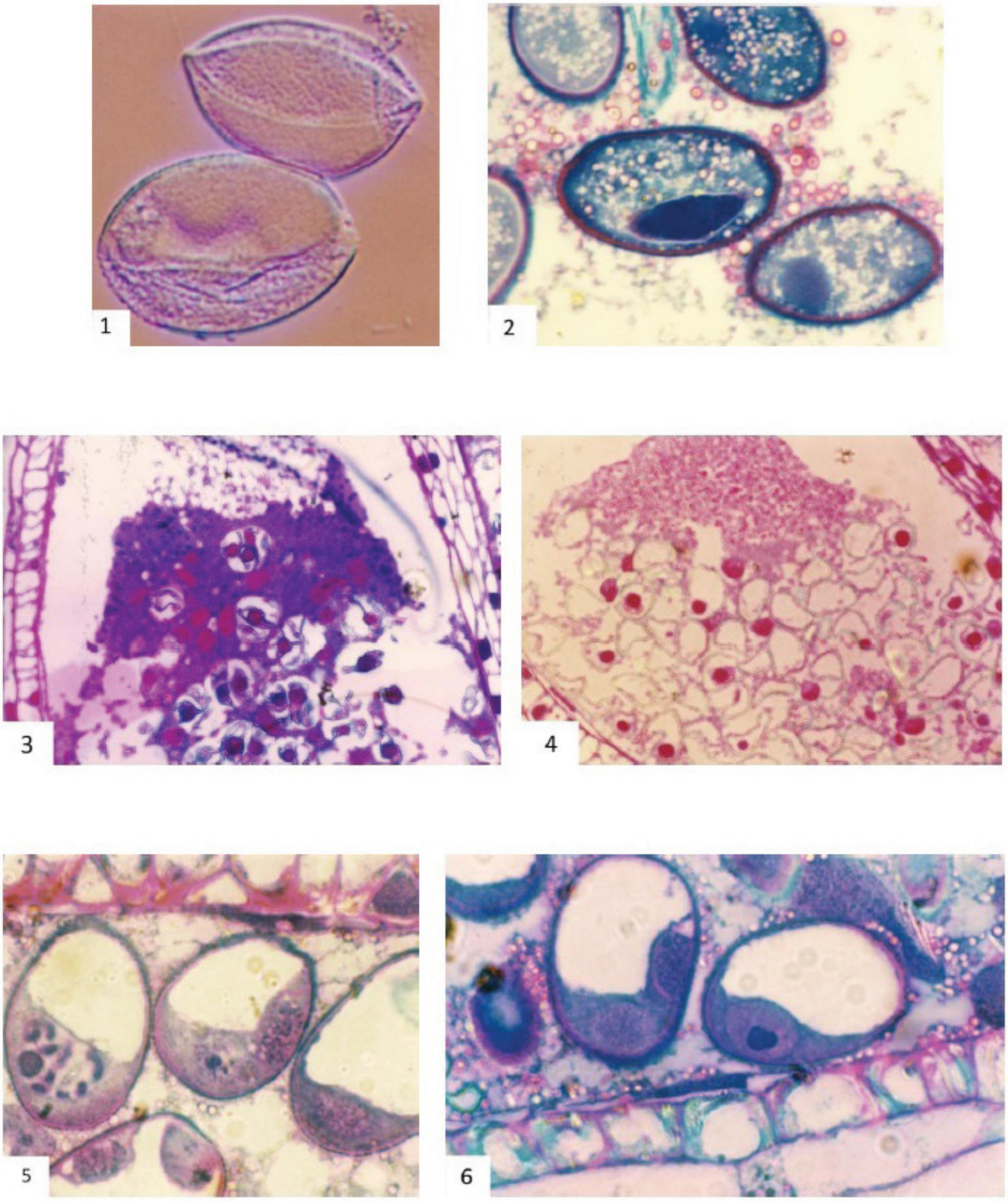


Fig. 2.
Phot. 1. Maturing *Tradescantia bracteata* pollen grains. Magn. 800x
Phot. 2. Bicellular pollen grain with a visible generative cell. Magn. 1200x
Phot. 3. Longitudinal section of the *Tradescantia* anther. Visible layered arrangement of the content. Magn. 1200x
Phot. 4. Longitudinal section of the *Tradescantia* anther. Layered arrangement of the cytoplasm, tapetal cell nuclei, and microspores. Magn. 1200x
Phot. 5. Young microspores with dislocated vegetative nuclei. Magn. 1200x
Phot. 6. Tradescantia microspores with dislocated vegetative nuclei. Magn. 1200x

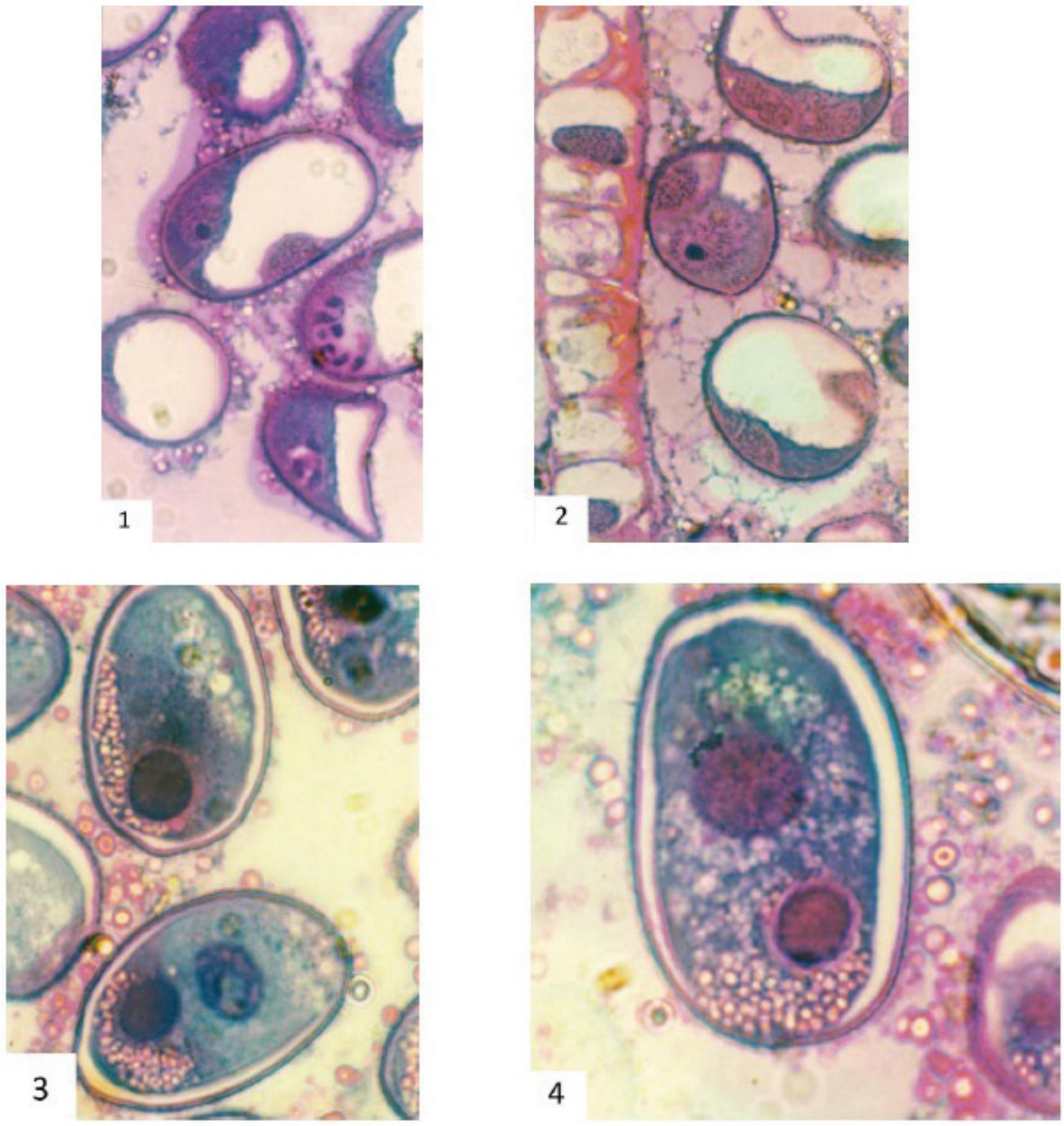


Fig. 3.
Phot. 1. Pollen grains with generative cells at the sporoderm and dislocated generative nuclei. Magn. 1200x
Phot. 2. Binucleate pollen grains with visible dislocation of vegetative nuclei. Magn. 1200x
Phot. 3. Mature *Tradescantia* pollen grains containing plastid clusters dislocated along the centrifugal force. Magn. 1200x
Phot. 4. Partly vacuolated pollen grain with dislocated generative cells within the vegetative cell cytoplasm. Magn. 1200x

elements, and the top layer has only an amorphous form without clear morphotic elements.

In older stamens, the density of the tapetal cytoplasm increases and the same centrifugation conditions cause only dislocations of cell nuclei in the microspore cytoplasm along the direction of the centrifugal force, while the bicellular pollen grains exhibit dislocation of the relatively heavier generative cells. The displacement of the nuclei is supported by data on the meiocyte cytoskeleton. In the late tetrad stage, there was no extensive skeleton of microtubules stabilising the cell cytoplasm in the other stages. The centrifugal force operating during centrifugation can more easily displace cell organelles that are heavier than the cytoplasm.

Microspores and pollen grains developed despite the altered conditions in the pollen sac. However, we observed changes in the appearance of the tapetal cytoplasm and developmental disturbances in some microspores and pollen grains involving symmetrical mitotic divisions and impairment of sporoderm formation. In some pollen grains, an incomplete cell wall was formed and sporopollenin was deposited only along the cell periphery. The centrifugal force also induced disappearance of the tonoplast in the microspores. Changes in centrifugation conditions (temperature rise, higher acceleration, prolonged centrifugation time) led to apoptosis of meiocytes and microspores.

In the experiment, the *Tradescantia bracteata* pollen formed in stamens subjected to centrifugation was not capable of germination and formation of the pollen tube.

Based on our experiments there is no reference in the literature. Our results raise the need for further verification tests whether the use of the method of spin living tissues, does not impair their vital functions in the event of any subsequent use of the biological material to procedures such as reproduction. In future experiments, we plan to see how the change of power, time and temperature spin will affect the functionality of the same organelles.

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