

The cell wall of the pollen mother cell at the tetrad stage in *Convallaria majalis* L.

MARIA CHARZYŃSKA*, IWONA PANNENKO

Department of Plant Physiology II, Biology Faculty, Warsaw University,
Krakowskie Przedmieście 26/28, 00-927 Warsaw, Poland

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Abstract

The cell wall at the tetrad stage in *Convallaria majalis* L. has been studied by light microscope histochemical techniques. The standard PAS reaction has shown the persistence of the primary pecto-cellulosic pollen mother cell wall localized around the callosic special wall (determined both by Bauer's reaction and the fluorescence technique with aniline blue) of individual tetrads. A PAS-positive spore precursor wall (primexime) is formed while the tetrad of microspores is still enclosed by intact callose and pollen mother cell walls. The pecto-cellulosic wall and callose layer dissolve simultaneously to release the microspores into another loculi.

Key words: pollen grain, pollen mother cell wall, tetrad stage, *Convallaria*

INTRODUCTION

The pollen mother cells in angiosperm anthers have primary pecto-cellulosic cell walls. During the first prophase of the meiotic division, a distinct "special callose wall" composed of β -1,3-glucans is secreted between the plasma membrane of each meiocyte and its original wall and, finally, a continuous layer of callose is formed around the microspore mother cell protoplast.

At the termination of meiosis the four resulting microspores become separated from each other by a callosic wall which is formed in both successive and simultaneous types of cytokinesis. Soon after the tetrad formation, the callose wall is rapidly dissolved and the microspores are released from the tetrad into anther loculi. The callose structure, deposition

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and dissolution during microsporogenesis have been extensively examined with the use of a callose-specific fluorescence technique with aniline blue and callose functions in pollen ontogeny have been widely discussed (see reviews of Heslop-Harrison 1972 and Bhandari 1984).

The fate of the primary cell wall of the pollen mother cells is less known. The behavioral patterns of this primary meiocyte wall seem to differ among angiosperm species. Some light and electron microscopic studies indicate that the pecto-cellulosic wall of the pollen mother cells disintegrates before the end of meiotic prophase during deposition of the callose wall (Heslop-Harrison 1972). This wall has been described, in a reduced form, at the tetrad stage by Echlin and Godwin (1968) in *Helleborus foetidus* and by Pacini and Juniper (1979) in *Olea europaea*. Recently Bhandari et al. (1981) demonstrated histochemically the persistence of the primary pollen mother cell wall at the tetrad stage in *Allium tuberosum* and *Cyclamen persicum*.

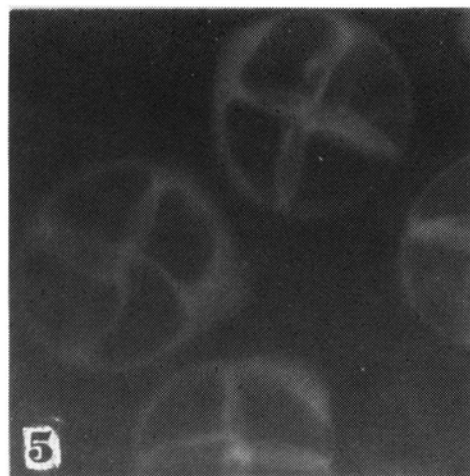
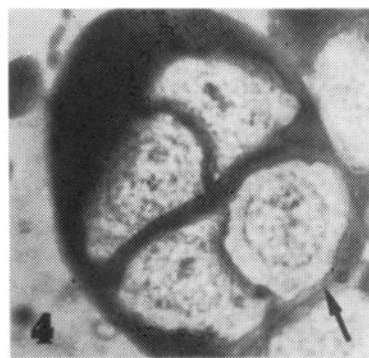
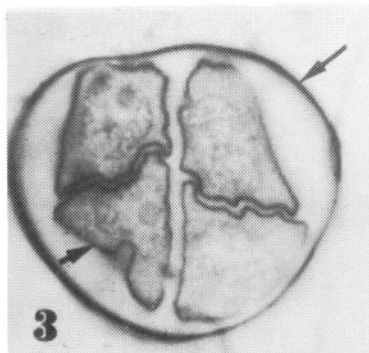
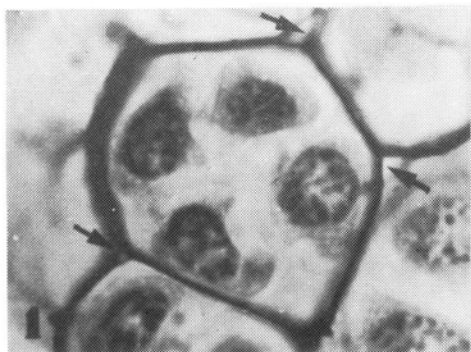
In the present work some light microscope histochemical techniques have been used to examine the nature of cell walls at the tetrad stage in *Convallaria majalis* L.

MATERIAL AND METHODS

Convallaria majalis L. plants grown at the Warsaw University Botanical Garden were used in this study. Anthers were fixed in CrAF 0.5-1-20 (chromic acid, acetic acid and formalin, 0.5%, 1.0% and 20% w/v, respectively) for 12 h. According to the standard procedure, the material was

PLATE I

Fig. 1. Tetrads of microspores of *Convallaria majalis* after completion of meiotic division. PAS-positive primary pollen mother cell walls are visible. Between the walls of contiguous tetrads small intercellular spaces (arrows) are formed. Fig. 2. Tetrad stage, later than in Fig. 1. The section contains only two microspores: primary pollen mother cell walls as well as each microspore's own wall (primexine) are PAS-positive. The intercellular spaces (arrow) are larger than previously and the tetrads tend to pull slightly apart. Fig. 3. Release tetrad of microspores surrounded by preexisting pollen mother cell wall (large arrow). Cell wall of each microspore is also visible (small arrow). PAS reaction. Fig. 4. The special callose wall of tetrad stained by Bauer's reaction. Cellulosic wall of pollen mother cell is also Bauer-positive (arrow). Fig. 5. Smear of tetrads. Only special callose wall shows aniline-blue induced fluorescence. The stage of tetrads is the same as in Fig. 3. Fig. 6. Relaxation of microspores from a tetrad after dissolution of pollen mother cell pecto-cellulosic and callosic walls. PAS reaction



dehydrated through the ethanol-xylene series and embedded in paraffin. Transverse anther sections, 6 μm thick, were cut with the use of a Reichert microtome and mounted on glass slides.

The sections were stained by the periodic acid/Schiff (PAS) reaction and by the Bauer-Feulgen method (Pearse 1968) originally described by Bauer for glycogen, this was followed by hydrolysis and oxidation of the polysaccharide with 4 per cent chromic acid for a short period and demonstration of the resulting polyaldehydes with Schiff's solution. Callose walls, like cellulosic walls, have been shown to react strongly during this procedure.

Unfixed pollen tetrads were stained with 0.01% water-soluble aniline blue (Eschrich and Currier 1964) and examined for white-yellow fluorescence (characteristic of callose) by UV fluorescence microscopy.

Photomicrographs were taken using 15 NPORWO film in a Zeiss Nf pK microscope.

RESULTS AND DISCUSSION

After termination of meiosis in *Convallaria majalis*, at the early tetrad stage, both the primary pecto-cellulosic pollen mother cell wall and callose wall were present. The pollen mother cell walls surrounding contiguous tetrads were held together and only small intercellular spaces between them were visible (Fig. 1). During microspore maturation, while the tetrads were still connected with one another, microspores enclosed within the callose wall developed their own wall (exine) (Fig. 2). The tetrad were gradually separated from each other and eventually the individual tetrads were released into anther loculi. At this time, the pollen mother cellulosic cell wall occurred as an external coat of each tetrad (Fig. 3). Dissolution of this wall took place simultaneously with the dissolution of the callose layer to release the microspores (Fig. 6).

The recognition of the dual nature of the tetrad wall, as well as the indication of the microspores' own wall were possible because of a positive PAS reaction (red colour) with the primary cellulosic pollen mother cell wall and exine, and the negative or weak response to this staining in case of the callose walls (Figs. 1, 2, 3 and 6). The positive PAS reaction of microspore walls is connected with the cellulosic composition of primexine (Knox 1984a) and may also be detected in the early sexine of microspores after their release from the tetrad (Knox 1984b). The callose wall in the tetrads of *Convallaria majalis* was determined by aniline blue fluorescence (Fig. 5) as well as by Bauer's reaction (Fig. 4).

The presence of the pecto-cellulosic pollen mother cell wall during microsporogenesis until the late tetrad stage, in addition to the callose

special wall, suggests its function in pollen ontogeny at least in some angiosperm species. It is interesting, that in all of the species in which the persistence of this wall has been described to date, it is correlated with the secretory type of tapetum, but it is not clear whether this interdependence can be generalized in angiosperms.

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Występowanie ściany komórki macierzystej ziaren pyłku w stadium tetrad u Convallaria majalis L.

Streszczenie

Stosując barwienia cytochemiczne w mikroskopie świetlnym wykazano, że u *Convallaria majalis* L. PAS-dodatnie pierwotne ściany celulozowo-pektynowe komórek macierzystych ziaren pyłku nie ulegają degradacji podczas mejozy ale zostają zachowane do późnego stadium tetrad, otaczając ścianę kalozową pojedynczych tetrad, której obecność wykazano w reakcji Bauera i techniką fluorescencji z zastosowaniem błękitu anilinowego. Ściany własne mikrospor (przegazyna) barwiące się w reakcji PAS, wytwarzane są w obrębie tetrad. Ściana celulozowo-pektynowa komórek macierzystych ziaren pyłku zostaje rozpuszczona równocześnie ze ścianą kalozową tetrad, uwalniając mikrospory do komory pyłkowej.