Cell wall formation in multinucleate pollen grains of *Hordeum vulgare* anthers cultured *in vitro*

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Abstract

Cell wall formation in several-nucleate pollen grains of *Hordeum vulgare* anthers cultured *in vitro* was initiated at the intine. The walls grew centripetally and branched, dividing pollen protoplast into a several-celled embryoid.

INTRODUCTION

During androgenesis in cereals cell wall formation in anomalously developing, several-nucleate pollen grains was generally visible under the light microscope (Clapham, 1971; Wenzel and Thomas, 1974; Wang Ching-Chu et al., 1973; De Buyser and Picard, 1975; Zenkteler, 1976).

According to Willson et al. (1978), in anther culture of *Hordeum vulgare* the cell wall formed at the tri- to tetranucleate stage of microspores development, only occasionally bicellular microspores were also encountered. In our observations on the same material cell wall formation took also place in several- to multinucleate pollen grains. In this communication problem is studied at the subcellular level.

MATERIAL AND METHODS

Earley (*Hordeum vulgare* cv. *Alsa*) was grown in the greenhouse. The anthers with microspores either just before, or after the formation of

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vacuole (Młodzianowski and Idzikowska, 1978) were cultured *in vitro* in order to induce the process of androgenesis. After 3-24 days of culture fragments of anthers were fixed in 6% glutaraldehyde in 0.1 M cacodylate buffer at 6.8 pH for 18 hours, at 4°C, and postfixed with 2% OsO₄ in cacodylate buffer for 2 hours. After washing with the buffer, the preparations were stained with 2% uranyl acetate for 1 hour, dehydrated with ethanol, acetone and propylen oxide, and embedded in a mixture of Epon 812. Thin sections were cut on an LKB ultramicrotome and contrasted with uranyl acetate and lead citrate (Reynolds 1963, Venable et al., 1965). Electron micrographs were made on JEOLCO microscope, type 7A.

RESULTS AND DISCUSSION

In several-nucleate pollen grains of *Hordeum vulgare* cultured *in vitro* the cell wall did not form via the cytokinetic spindle. Cell wall formation was initiated at the intine of the pollen grains. The thickened areas of forming walls were rich in various kinds of membranous and amorphous inclusions (Fig. 2). Such inclusions were later supersede by fine fibrillar material (Fig. 3a). The fully formed cell wall looked normal, with middle lamella, primary wall and plasmodesmata running across it (Fig. 4). Only few osmiophilic globules (produced by endoplasmic

Fig. 1. Cell wall formation in multinucleate pollen grains of *Hordeum vulgare* cultured *in vitro*.

A. Fragment of multinucleate pollen grain, B. Cell wall formation, C. Fragment of multinucleate embryo

Fig. 2. Cell wall (CW) initial formed at the intine (I) contained many membranous and amorphous inclusions.
E — exine, ($\times 35\ 000$)

Fig. 3a. Cell wall inclusions superseded by fine fibrillar material (F). ($\times 35\,000$);
 b. Osmiophilic globules in endoplasmic reticulum (ER) and in cell wall (CW). GA —
 Golgi apparatus. ($\times 35\,000$).

Fig. 4. Well developed cell wall (CW) surrounding dense cytoplasm rich in ribosomes (R) of the multicelled embryo.

M — myelin-like structure, ER — endoplasmic reticulum, PL — plasmodesmata, S — starch. ($\times 22\,500$).

Fig. 5. Wall initial at the intine side (I) and margin of long wall (CW) joining each other. In both many different kinds of inclusions are present. Near the growing wall endoplasmic reticulum (ER). ($\times 21\,000$).

Fig. 6a. Branching cell wall (CW) inside the embryoid. N — nucleus. ($\times 18\,000$).
 b. Cellulose-containing wall formed on the outer surface of the cell.
 E — exine, ER — endoplasmic reticulum, GA — Golgi apparatus, Mi — mitochondrion. ($\times 38\,000$).

Fig. 7. Several-celled embryoid with visible cell wall pattern.
 CW — cell wall, PW — pollen wall, N — nucleus. ($\times 2700$).

Fig. 8. Nucleus with two nucleoli (N) near the second nucleus (N₂) in the same cytoplasmic area. ($\times 7000$).

Fig. 9. Cell wall (CW) section in different planes.
Mt — microtubules. ($\times 35\,000$).

reticulum?) could be observed in the cell wall area (Fig. 3b). Also myelin-like structures occurred near the wall (Fig. 4). The cell wall initials grew centripetally from several areas of the intine. On the cross sections they were sinuous and during their further growth they branched and joined to each other (Figs 5 and 6a).

What decides of the direction of wall growth is unknown, but it was ascertained, that the growing wall may separate cytoplasm fragments containing more than one nucleus (Fig. 7), which can fuse (Sunderland, 1974). Thus, it can be one of the reasons responsible for the development of diploid or poliploid cells from *Hordeum vulgare* pollen in anthers grown *in vitro*. The cellulose wall also formed on the outer surface of cells in the intine areas (Figs 3a and 6b). As soon as the exine wall bursts, the about 20-celled pollen grains of the embryonic cells all have their own wall (Fig. 1).

In cell wall formation such cell components participated as endoplasmic reticulum, plasmalemma and Golgi apparatus (Figs 5 and 6b). All these structures are known to be involved in cell wall formation and differentiation (Robards and Perveen, 1969). Near the cell wall microtubules were also observed (Fig. 9).

The centripetal development of the cell wall in *Hordeum* pollen is homologous with a primitive type of cell-cleavage known in lower plants (Picket-Heaps, 1969). A similar cell wall formation as in *Hordeum* pollen grains was observed during incomplete microspore division in anther cultures of *Datura* (Dunwell and Sunderland, 1976) and particularly in multinucleate cotton endosperm (Jensen et al., 1977). In the latter cell walls were initiated at the embryo-sac wall and grew towards the vacuole. Membraneous inclusions in the wall areas of this material were also observed. It may be that the pattern of wall growth in androgenic stimulated multinucleate embryoids of *Hordeum* and multinucleate endosperm of cotton is very similar.

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*Tworzenie się ściany komórkowej w wielojądrowych ziarnach pyłku
 pylników Hordeum vulgare hodowanych in vitro*

Streszczenie

W pracy opisano etapy tworzenia się ściany komórkowej w ziarnach pyłku *Hordeum vulgare* indukowanych warunkami hodowli *in vitro* w kierunku androgenyzy. Dopiero w kilkujądrowych ziarnach pyłku obserwowano zakładanie się ściany komórkowej. Tworzyła się ona nie w wyniku cytokinezy, lecz wrastała z intyny centrypetalnie w głąb wielojądrowego protoplastu. Dodatkowo mogła się „rozgałęziać” dzieląc protoplast na wielokomórkowy embrioid.