

## Embryo sac development in *Stellaria media*

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### Abstract

Osmiophilic granules were observed during certain stages of intermitotic periods in one, two and four nucleate embryo sacs in *Stellaria media*. The granules, some inside the short ER cisternae, were mainly distributed along the walls of nucleate embryo sacs. It is assumed that the osmiophilic granules may be involved in the deposition of substances within the wall of the megagametophyte.

### INTRODUCTION

The ultrastructure of megasporogenesis and embryo sac development has been described in several plants (Ponzi, Pizzolongo, 1978; Boer-de-Jeu, 1978; Bednara, 1977; Russell, 1979; ref. Jensen, 1972).

### MATERIAL AND METHODS

Ovules of *Stellaria media* (Caryophyllaceae) were fixed in 3% glutaraldehyde at pH 6.8, 0.05 M cacodylate buffer, postfixed in 2% OsO<sub>4</sub>, embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate and examined in Tesla B616 electron microscope. Squash preparations of ovules fixed in ethyl alcohol and acetic acid (3:1) were fluorochromed with aniline blue (R o d k i e w i c z, 1970).

### RESULTS

The meiocyte in *Stellaria* ovule develops under the subepidermal layer (Fig. 1). After meiosis the chalazal megaspore gives rise to the embryo sac of monosporic, *Polygonum* type of development. The cytoplasm of

the meiocyte is quite dense, containing plastids and mitochondria but very few dictyosomes and ER cisternae during the examined stage of early prophase (Fig. 1).

A layer of material showing fluorescence following aniline blue treatment is deposited within the meiocyte wall during the meiotic prophase I. The fluorescing material (callose) is unequally distributed forming a thick submedian belt (Fig. 4) which persists throughout meiosis. The belt, together with a fluorescing cross wall which was formed after the second meiotic division, eventually constitute the upper portion of the functional megaspore wall (Fig. 5). The chalazal wall of this megaspore is partially devoid of fluorescing material and transversed by plasmodesmata which seem to be lacking in the other walls of the megaspore (Fig. 7).

Following meiosis, the chalazal megaspore grows and gives rise to a mononucleate embryo sac within which large portions of cytoplasm are gradually digested within enlarging vacuoles (Fig. 2, 3).

The development of the eight nucleate embryo sac consists of three mitotic cycles. A similar sequence of morphological events occurs in each of the intermitotic phases. During the early intermitotic phase, elongated cisternae of rough ER develop in the cytoplasm of the nucleate embryo sac. Later, these cisternae fragment into shorter pieces which can be found adjacent to the embryo sac wall. Small, electron opaque osmiophilic granules, presumably lipoidal in nature, are apparent within certain cisternae of embryo sac and some of the nucellar cells (Fig. 8). However, the granules may be associated with organelles other than ER. At this stage a number of larger osmiophilic granules are appressed to both the inside and outside of the embryo sac plasmalemma (Fig. 6). Those granules which occur external to the plasmalemma occupy an electron-lucent area along the cell wall. At the conclusion of the intermitotic phase the nucleate embryo sac is almost devoid of ER cisternae.

After the third mitotic cycle cell walls are formed, resulting in the initiation of the cellular embryo sac development. At the early stage this embryo sac is filled with dense cytoplasm. The entire surface of the central cell wall which borders the nucellus is covered by small ingrowths (Fig. 9). When the embryo sac grows and differentiates, cell wall ingrowths are retained only within the upper part of the side wall at the level of the egg apparatus (Fig. 10).

#### DISCUSSION

Osmiophilic granules were observed during certain stages of intermitotic periods in one, two and four nucleate embryo sacs in *Stellaria*. Similar osmiophilic structures have been reported in both the synergids and central cell of *Aquilegia* embryo sac. Fougère-Rifot (1978)

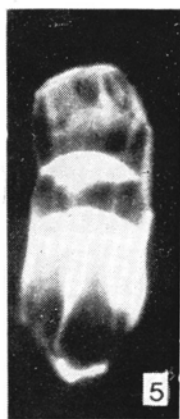
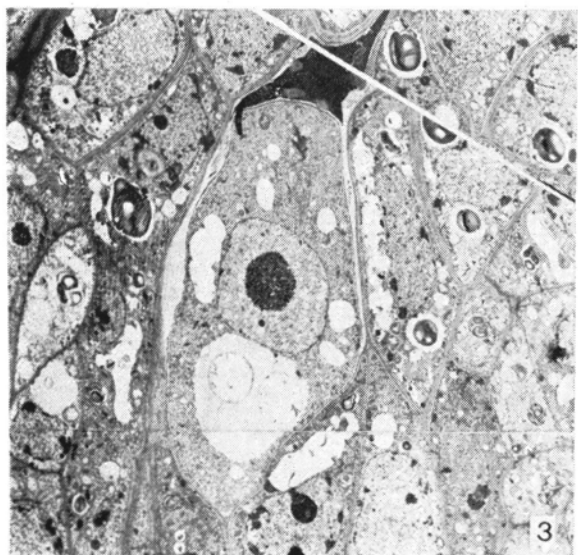
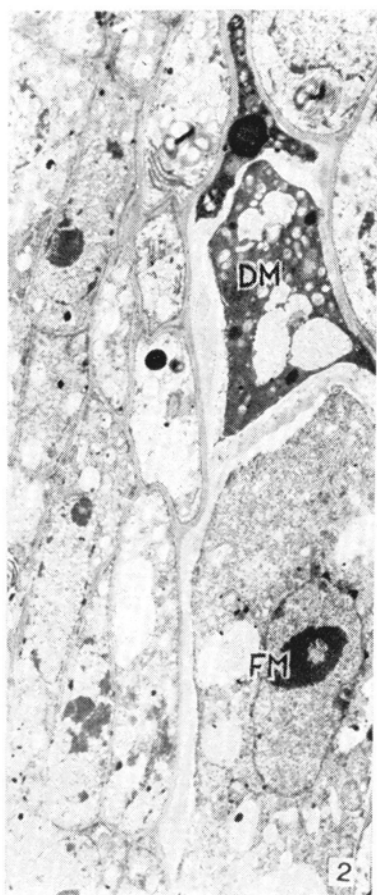
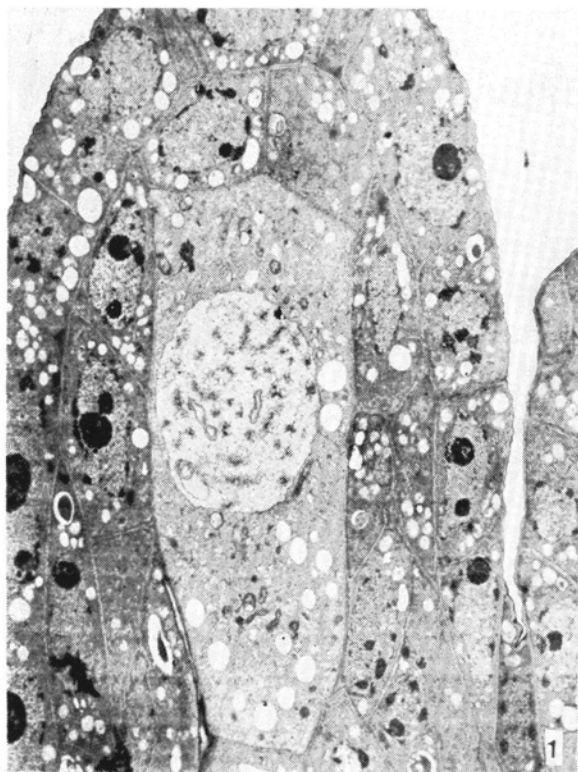


Fig. 1. Nucellus in *Stellaria media* ovule showing meiocyte in meiotic prophase I. ( $\times 800$ )

Fig. 2. Triad with functional megaspore (FM) and degenerating megaspores (DM). ( $\times 1000$ )

Fig. 3. Embryo sac mother cell with large vacuole in chalazal part. ( $\times 800$ )

Figs. 4 and 5. Aniline blue fluorescence of late meiotic prophase I meiocyte (4) and triad (5) with strongly fluorescent wall in functional megaspore. ( $\times 300$ )

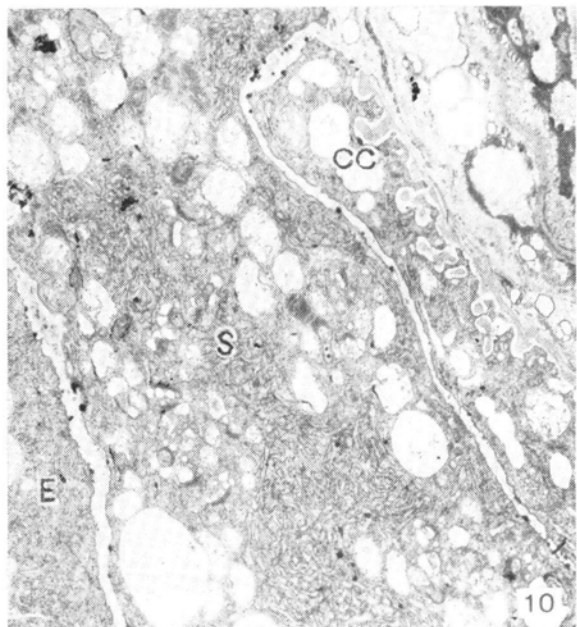
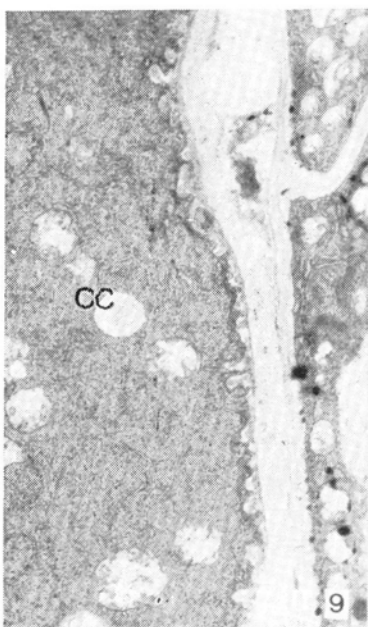
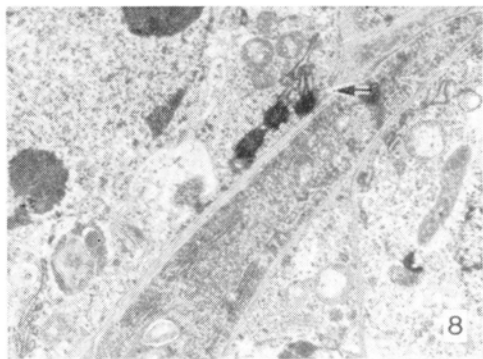
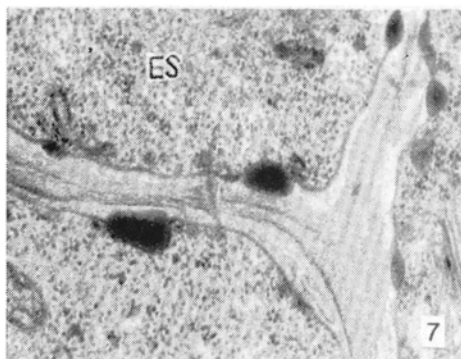
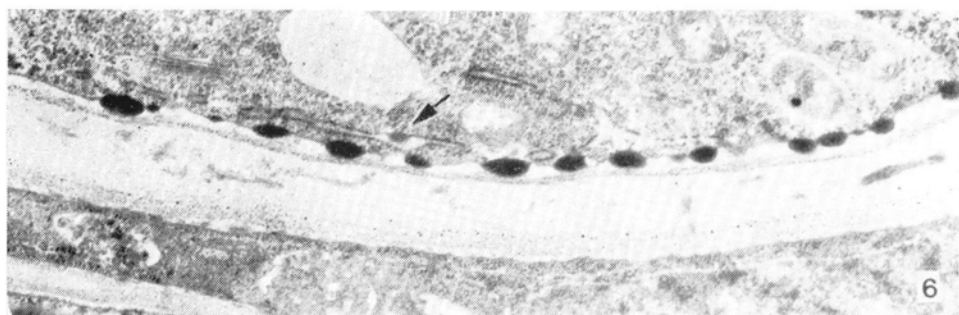


Fig. 6. Two nucleate embryo sac of *Stellaria media*; electron lucent area next to cell wall contains osmiophilic granules; a granule (arrow) present within an ER cisterna. ( $\times 10000$ )

Fig. 7. Embryo sac mother cell (ES); osmiophilic droplets projecting into cell wall. ( $25000 \times$ )

Fig. 8. Nucellar cells; osmiophilic droplets (arrow) within RE cisternae. ( $10000 \times$ )

Fig. 9. Early stage of cellular embryo sac; wall ingrowths in central cell (CC) wall. ( $8000 \times$ )

Fig. 10. Micropylar part of maturing embryo sac; synergid (S) with irregularly shaped ER cisternae; central cell (CC), ingrowths in CC wall; small part of egg cell (E). ( $8000 \times$ )

suggests that these granules are transported from cell to cell by plasmodesmata. We think that the distribution of granules along the walls of nucleate embryo sac suggests that they may be incorporated into the wall. In this connection, an association of osmiophilic structures with the wall of a variety of pollen has been reported (Vasil, Aldrich, 1970; Dickinson, 1976; Rodriguez, 1978). Thus, it seems possible to assume that the osmiophilic granules may be involved in the deposition of substances within the wall of the megagametophyte.

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