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OVULE MORPHOGENESIS IN NORMAL AND MUTANT *ZEA MAYS*

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Comparison of maize mutants *pam1*, *mac1* and normal plants (variety Belaya noch') showed that the formation of female reproductive organs is very similar between them. One afunicular ovule develops in an ovary; it is ortho-campylotropous and bitegmic, with a hypostase. The postament, podium and nucellar cap are differentiated in the nucellus. The integuments, epidermal in origin, arise from a common initial zone, but their differentiation is separated in time. In the subepidermal layer of the apical part of the primordium, the complex of initial cells is distinguished. Some of them differentiate as archesporial cells. In normal plants and *pam1*, only one of them transforms into the megasporocyte, while up to 6–8 do in *mac1*. Polygonum-type embryo sacs are formed. In *pam1* the majority of megasporocytes do not undergo meiosis, while the surrounding ovular tissues continue their development. Analysis of the distribution of polysaccharides and proteins revealed similarities in their spatial and temporal coordination. The differences are quantitative, and probably conditioned by the number of megaspores and embryo sacs.

Key words: *Zea mays*, ovule morphogenesis, polysaccharides, proteins, *mac1*, *pam1*.

INTRODUCTION

Two mutations resulting in irregularities in the reproduction system of *Zea mays* – *mac1* (*multiple archesporial cells*) and *pam1* (*plural abnormalities of meiosis*) – were studied. *Pam1* is characterized by female sterility and the formation of multinuclear micro- and megasporocytes that show different anomalies of meiosis. This mutation blocks cytokinesis in sporogenous cells during cell cycles preceding meiosis; as a result, additional nuclei are formed in archesporial cells (Golubovskaya et al., 1994). While *mac1* mutants are male-sterile plants, in female organs a number of archesporial cells, megaspore tetrads and embryo sacs are formed (Sheridan et al., 1996).

MATERIALS AND METHODS

Maize ovules at different developmental stages were studied; *pam1* mutant seeds, *mac1* ears and Belaya noch' variety seeds were kindly provided by Professor I.N. Golubovskaya and Dr. G.V. Matveyeva. The ears were fixed in FAA, stored in 70% ethanol and embedded in paraffin. Material for light microscopy was sectioned 7–15 μm and stained with gentian violet and orange G, or Schiff's solution, or hematoxylin and alcian blue 8GS. Additionally, histochemical reactions for polysaccharides (Jensen, 1963) and procion blue RS and procion red 2BS (Ivanov and Lytinskaya, 1967) were applied.

RESULTS

The ear is initiated 40–45 days after the appearance of shoots. Two flower initials are produced in each spikelet, but only one flower is functional.

The ovary is tricarpellate, sessile and unilocular, with one ovule. The ovule develops on basal placenta. The style is very long and silk-like, with bifurcated stigma. At the base of the ovule a complex vascular system forms; two vascular bundles enter

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the pistil. The borders of the carpel do not fuse, and form a peculiar "canal stylaire" (stylar canal) (Guignard, 1901). After fertilization, as usual, it becomes discernible.

The ovule initiates at the base of the ovary about 50 days after the appearance of shoots. The ovular primordium is initiated by periclinal divisions of cells in the subepidermal placental layer. Later, anticlinal cell divisions start and ovular structures begin to differentiate. The funiculus is absent and the placento-chalaza is formed. The ovule curves during development (Fig. 1a,c,f,j,o,p). Following fertilization, one side of the ovule continues to grow, leading to asymmetrical positioning of the embryo in the seed. Owing to the curve of the nucellus, the micropyle comes closer to the placenta and is positioned almost level with the chalaza (Fig. 1p).

The hypostase is a boundary tissue between the nucellus, integuments and chalaza. It is represented by one layer of relatively large isodiametric cells arranged in the form of a disk (Fig. 1d). The hypostase develops a second layer as a result of periclinal divisions of cells. Its cells become flat, lignified and arranged in pairs. Later, mainly anticlinal divisions take place.

A row (or rows) of tabulate, flattened cells are formed under each archesporial cell, positioned in the center along the nucellus axis (Fig. 1g). During ovule development these rows elongate and curve, and the radial cell walls thicken (Fig. 1i,k). The majority of these cells transform into the postament. The postament is column-like tissue, located below the sporogenous or gametophytic structures. It curves during development (Fig. 1o,p).

The podium is disposed in the chalazal region of the nucellus above the hypostase and consists of small cells. Its lateral parts are formed by divisions of epidermal and subepidermal nucellus cells, whereas its base arises from the chalazal part of the column of tabulate flattened cells (Fig. 1o,p).

The nucellus is asymmetrical, more massive on the dorsal side. During development, its cells significantly enlarge. Their growth continues up to fertilization, and then the first signs of deterioration appear. Subsequently the destruction of nucellus tissue begins.

The nucellar cap is formed from epidermal cells of the apical parts of the nucellus. At megasporocyte prophase I, the cells divide periclinally in a centripetal sequence (Fig. 1h,i,k). When the mature embryo sac forms, the nucellar cap consists of 4–6 cell layers. The cells are almost flat, the nuclei are large and usually have nucleoli, and the radial cell walls are thickened. The nucellar cap persists after fertilization.

The maize has a bitegmic ovule. The inner integument develops before the outer one (Fig. 1d,e,g). They are of epidermal origin. The integuments are laid down as ring-shaped ridges surrounding the nucellus. The epidermal cells of the lateral part of ovule divide periclinally and form a hillock (Fig. 1d,e). It consists of 4 isodiametric square-shaped cells. Later these cells divide several times. The outer integument is formed in a similar manner (Fig. 1e,g). The initials of the outer integument are placed near the inner integument initial, but their development is slightly delayed. Thus the integuments have a shared base and arise from a common initial zone, but their differentiation is separated in time.

At the beginning of their development, the integuments consist of two layers of cells. The number of cell layers increases due to periclinal divisions of the outer epidermal cells (Fig. 1i,k). Thus the middle layer is derived from epidermal cells of the ovule. The biggest part of the integument lies close to the stylar canal, and forms an integumentary excrescence in it. The outer and/or inner integuments can form this structure.

The outer integument is always shorter than the inner one. The integuments grow unevenly, more intensively on the dorsal side, and they curve as the ovule curves. Up to the time the embryo sac is formed, the number of epidermal cells of the apical part of the inner integument increases, mainly radially, and the operculum is formed (Fig. 1o).

The formation of female generative organs is similar in the mutants and normal plants. In the subepidermal layer of the apical part of the primordium, a group of cells is distinguished: a complex of initial cells (4–5 in normal plants and *pam1*, and up 10–15 in *mac1*) (Fig. 1b). Some of them in the center of the complex begin to differentiate as archesporial cells (1–4 archesporial cells in Belaya noch' and *pam1*, and 2–15 in *mac1*) (Fig. 1d). In normal plants and *pam1*, some archesporial cells (in the central position of the group) transform into megasporocytes, up to 6–8 in *mac1*. In *pam1* the majority of megasporocytes do not undergo meiosis; they stop at prophase I, while the surrounding ovular tissues continue their development. In Belaya noch' and *mac1*, meiosis occurs without any irregularities (Fig. 1h,i,k,l). A Polygonum-type embryo sac is formed (Fig. 1l,m,n) (for more details see: Voronova and Batygina, 1997; Voronova et al., 2002).

Polysaccharides and proteins are observed in the ovary at the beginning of ovule formation

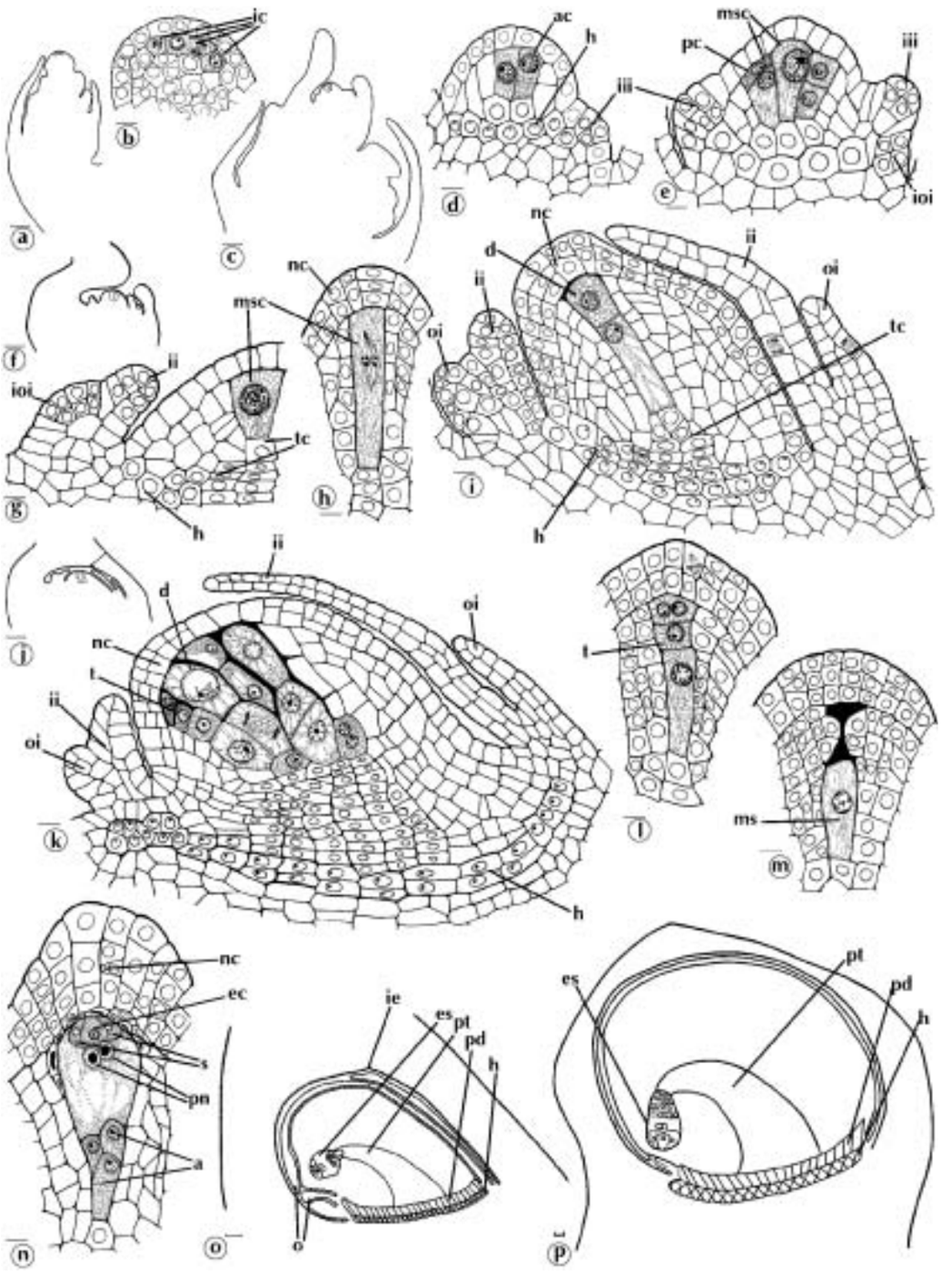

Fig. 1. Ovule development in *Zea mays*. (**a–e**) Differentiation of archesporium and initiation of integuments in normal (**a, b**) and mutant plants: *mac1* (**c,d**) and *pam1* (**e**), **(f–m)** Megasporogenesis, initiation of formation of nucellar cap, hypostase, postament, and podium in normal (**f–i**) and *mac1* mutant (**j,k**), **(n,p)** Ovule during embryo sac maturation. a – antipodal cell; ac – archesporial cell; d – dyad; ec – egg cell; es – embryo sac; h – hypostase; ic – initial cell; ie – integumentary excrescence; ii – inner integument; iii – initial of inner integument; ioi – initial of outer integument; ms – megaspore; msc – megasporocyte; nc – nucellar cap; oi – outer integument; pc – parietal cell; pd – podium; pn – polar nuclei; pt – postament; t – tetrad of megaspores; s – synergid; tc – tabulated cell. Bar = 0.05 mm in (a,c,f,j); 0.01 mm in (b,d,e,g–i,k–p).

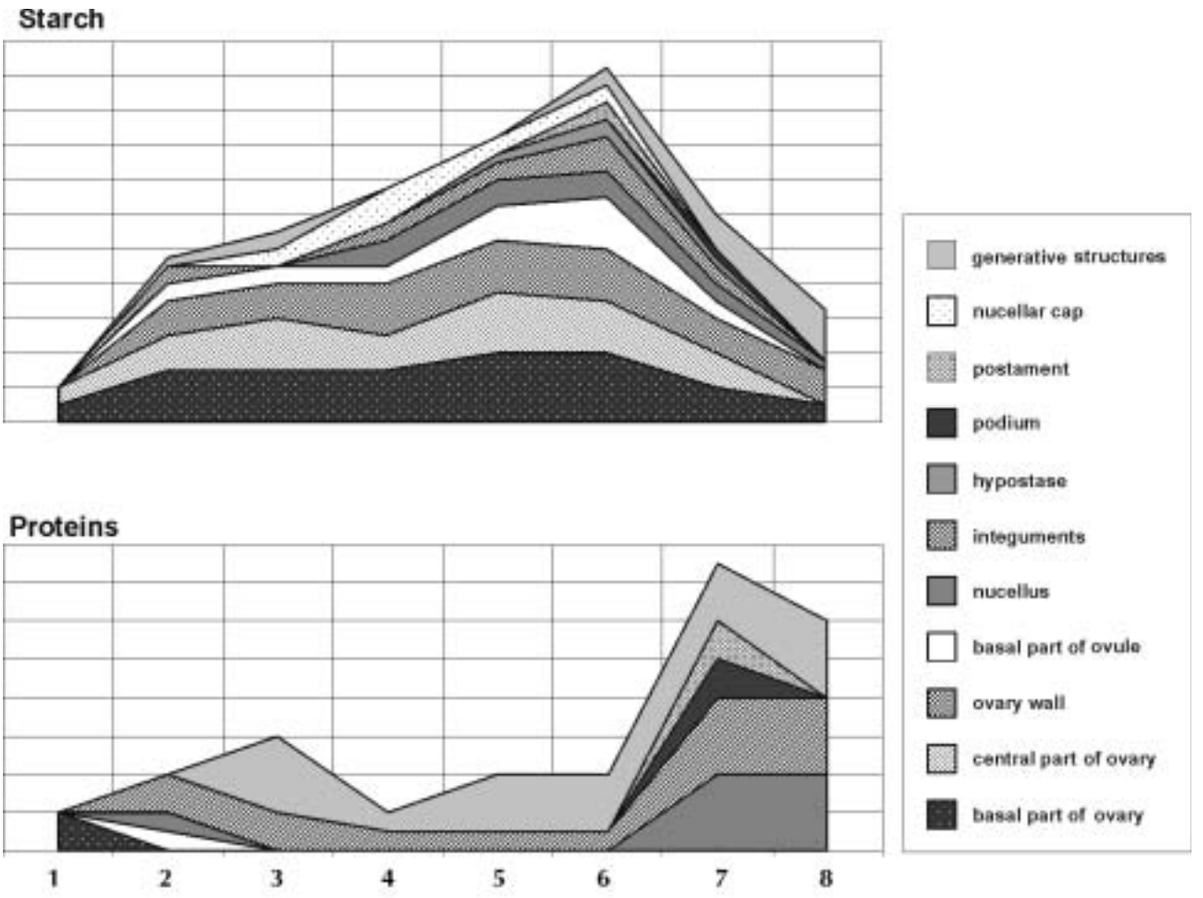

Fig. 2. Starch and protein distribution. 1 – ovular primordium; 2 – archesporium and inner integument differentation; 3 – prophase of meiosis I, outer integument and nucellar cap initiation; 4 – 2-4-nucleate embryo sac, ovule begins to curve; 5 – 8-nucleate embryo sac; 6 – mature embryo sac (before fertilization); 7 – after fertilization; 8 – a day after fertilization.

(Fig. 2). Starch accumulates at the base of the ovule, in the nucellus and the ovary wall, whereas proteins appear in integuments and in megaspores. Before fertilization, starch accumulates in ovular tissues, and in the embryo sac around the egg nucleus and polar nuclei; these findings are in line with those of other authors (Chebotaru, 1972; Korobova, 1982; Lammeren, 1986). The proteins are found in the cytoplasm of the central cell, in the nucleoli of polar nuclei and in antipodal cells. After fertilization, starch disappears from the ovular tissues and accumulates in the zygote, in the endosperm around the nuclei and in the pericarp; proteins are found in the zygote, in antipodal cells, in degraded integuments and in cells adjacent to the embryo sac.

DISCUSSION

Based on the development and the structure of the nucellus, it may be concluded that the maize ovule belongs to the pseudo-crassinucellate type (according to Davis, 1966), or medionucellate type (according to Shamrov, 1999).

Some authors have claimed that the maize ovule is semianatropous (Guignard, 1901; Lammeren, 1986), amphianatropous (Cooper, 1937), anatropous (Weatherwax, 1955; Korobova, 1982) or campylotropous (Palamarchuk, 1960). Our observations demonstrated that the maize ovule curves due to one-sided growth of the nucellus and integuments, and therefore the mature ovule becomes ortho-campylotropous (terminology according to Bocquet, 1959).

The nucellar cap develops very well in normal and mutant plants (Voronova and Batygina, 1997; Batygina and Voronova, 1999). We do not agree with the suggestion that the nucellar cap is absent in *mac1* mutants (Abramova et al., 2002).

The initiation of integuments in the apical part of the ovule primordium, and the formation of a large chalazal area at the base of the outer integument, indicates that the maize ovule is pachychalazal (Periasamy's 1962 classification) or exopachychalazal (Shamrov's 1999 classification).

The formation of the integumentary excrescence has been recorded, but the question of what forms this structure remains. Some authors (e.g., Palamarchuk, 1960) suggest that it is formed from the inner integument; Weatherwax (1955) asserts that it is formed only from the outer integument, which becomes shorter than the inner integument.

The formation of such structures as the hypostase, podium and postament (Batygina and Shamrov, 1994) has not been described earlier in maize. Their development is much the same as in *Trapa natans* (Titova et al., 1997), *Ceratophyllum demersum*, *Paeonia lactiflora*, *Nuphar lutea* and *Ribes aureum* (Shamrov, 1998). However, in maize and *Trapa natans*, periclinal divisions of the epidermal and subepidermal cells of the chalazal region of the nucellus form the lateral parts of the podium; in the other species mentioned, only subepidermal cells take part in the formation of the lateral regions of the podium.

Comparative cytoembryological studies of Belaya noch' and both mutants revealed similar ovule morphogenesis and differentiation of the complex of initial cells, from which one or more archesporial cells may be formed. In *pam1* the development of most megasporocytes is stopped, while the surrounding ovular structures continue their development. Meiosis in *mac1* and Belaya noch' occurs without anomalies; the embryo sac is formed in a manner typical of the Polygonum type.

Our research revealed similarities in the spatial and temporal coordination of the polysaccharide and protein distributions. The differences are quantitative, and most likely determined by the number of megaspores and embryo sacs, which use these substances. In *mac1* the formation of a large number of megaspores was recorded, and therefore more nutrition is needed for additional embryo sacs. Compared with normal plants, more polysaccharides are used and less are accumulated. In *pam1*, the embryo sac usually fails to form, so polysaccharides and proteins accumulate in the ovule, ovary base and wall.

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