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A COMPARATIVE ULTRASTRUCTURAL STUDY OF THE MEGAGAMETOPHYTES IN TWO STRAINS OF *ZEA MAYS* L. BEFORE AND AFTER FERTILIZATION

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CONTENTS

SUMMARY

Two inbred lines of *Zea mays* L. are compared with respect to general morphology and fine structure of the cells of the megagametophyte before and after fertilization. In both inbred lines the semi–anatropous sessile ovules contain one multicellular megagametophyte in a tenuinucellate nucellus. In contrast to the inbred line Black Mexican Sweet corn (BMS) the nucellus cells near the micropyle of strain A188 are not arranged in regular rows. Here the micropyle is formed by a lobed inner integument and hence pollen tube penetration is more cumbrous in strain A188. At the moment of fertilization both synergids may still be intact but the one which will be penetrated by a pollen tube has often partly degenerated. There is a comparable and distinct distribution of organelles in the synergids of the two inbred lines. The overall structure of the egg cell cytoplasm of strain BMS and A188 show many similarities such as the location of the cytoplasm, the distribution, frequencies of occurrence and occupation rates of most organelles and the structural changes initiated by fertilization. Plastids in BMS, however, are smaller and occur in significantly higher frequencies and occupation rates in BMS. In the zygotes of BMS and A188 a polarity is established by a shift of the nucleus and the cytoplasm towards the antipodal cell side. The metabolic activity increases after fertilization as can be deduced from increasing amounts of RER and higher densities of polysomes and dictyosomes. The composition of the cytoplasm of the central cell of BMS is comparable to that in A188. Plastids, however, differ in having more and smaller starch grains in BMS. Before fertilization the polar nuclei and the major part of the central cell cytoplasm are always found at the antipodal side of the egg apparatus. The abundance of well developed organelles points to a high metabolic capacity. Fertilization evokes an increase of cellular activity and a shift of the endosperm nucleus and cytoplasm towards the lateral cell side. Antipodals contain much cytoplasm and numerous well differentiated organelles. The ultra structure of the cytoplasm points to a high synthesis and secretion of organic compounds.

INTRODUCTION

Growth, differentiation and senescence are the characteristics of the complex morphogenesis in the life cycle of plants. When haploid and diploid generations alternate, cell differentiation leads to divergent cell functions in the gametophyte and sporophyte. In Spermatophytae, the cells of the mature micro– and megagametophytes represent an end phase of cell differentiation whereas the zygote is the onset of a new sporophytic generation. The development of the zygote to a heterotrophic young sporophyte offers the opportunity to study the principles of morphogenesis; in a short period of time the initiation of polarity, mer-

istem formation and organogenesis gives rise to a relatively small organism. The onset of embryogenesis has been studied in detail in plants of various families of both the Dicotyledonae and the Monocotyledonae (MAHESHWARI, 1950, 1963; JOHRI, 1984). The details of fertilization, the developmental pathways and the final organization of the embryos vary widely among families of the Monocotyledonae and even within the family of the Poaceae such as barley (CASS and JENSEN, 1970;NORSTOG, 1972, 1974;CASS, 1981), wheat (CHANDRA and BHATNAGAR, 1974; HU, 1964; SMART and O'BRIEN, 1983) and Texas wildrice (EMERY and GUY, 1979).

In maize the shape of the embryo sac, the organelle distribution within the embryo sac and the shape of the developing embryos appear to vary between different strains as has been observed by means of light microscopy (MILLER, 1919; AVERY, 1930; RANDOLPH, 1936; KIESSELBACH, 1949; COOPER, 1951; SASS, 1955) and electron microscopy (DIBOLL, 1964, 1968a, b; CHEBOTARU, 1970; RUSSELL, 1979; VAN LAMMEREN, 1981; VAN LAMMEREN and KIEFT, 1983; VAN LAMMEREN and SCHEL, 1983). Unlike in many other plants (MAHESHWARI, 1950), the first cell divisions of the zygote and the young embryo seem to lack a clearly defined sequence and orientation although, within a strain, the eventual shapes of the embryos are quite similar (RANDOLPH, 1936).

To study the initial phase of maize embryogenesis an inventory was made of the cell shapes and organelle distributions within the embryo sac. Two inbred lines were chosen for the present study which is introductory for the experimental in vitro studies on callus formation and somatic embryogenesis. One inbred line is the sweet corn Black Mexican Sweet (BMS). The second is the starchy corn A188. These strains have been selected because of their favorable properties for experimental manipulation in vitro (SHERIDAN, 1977; GREEN and PHILLIPS, 1975).

The present paper describes the changes in the fine structure of the embryo sac just before fertilization. Then, the interactions of the pollen tubes and the tissues of the pistil including the ovule will be presented. Thirdly the post–fertilization events which occur in the embryo sac will be regarded from a structural and functional point of view. The two inbred lines are compared to detect intraspecific variations in cytology.

MATERIALS AND METHODS

The plant material used in this study was obtained from the *Zea mays* L. inbred lines Black Mexican Sweet (BMS) and A188 which were kindly provided by R.J. Lambert, University of Illinois, Illinois, USA and by C.E. Green, University of Minnesota, St. Paul, USA respectively. Plants were grown under greenhouse conditions; before emergence of the silks, cobs were masked with small bags to prevent uncontrolled pollination. Ovaries were dissected either from unpollinated plants or at defined intervals after hand–pollination. Sagittal

sections of the ovaries containing the whole embryo sac were fixed with 2,5–6% glutaraldehyde in 0,1 M Na-cacodylate buffer, pH 7.0, for 2 hours at room temperature. Sections were rinsed in the buffer and postfixed in a saturated aqueous $KMnO_4$– solution for 5–15 minutes or in a 1% OsO_4– solution in cacodylate buffer, pH 7.2, for 2–4 hours at room temperature. After rinsing, sections were dehydrated in a series of ethanol, ranging from 30% to 100%, followed by a graded series of propylene oxide. The material was then transferred to a propylene oxide – Epon 812 (40:1) mixture and kept overnight at a relative humidity of 30% to permit the propylene oxide to evaporate slowly. Finally, sections were transferred to fresh resin in gelatin capsules. Polymerization occurred for 16 hours at 35 °C, for 8 hours at 45 °C and for 24 hours at 60 °C. Ultrathin sections were cut on an LKB Ultrotome III and, in the case of OsO_4 fixation, poststained in lead citrate for 2–5 minutes and in uranyl acetate for 5–25 minutes. Sections were observed using a Philips EM 301 transmission electron microscope at 60 kV. For the detection of pollen tubes in silks and ovary cavities, sagittal sections (8–50 μm) of freshly frozen pistils were cut with a microtome-cryostat (Daman/IEC division, Mass., USA) at minus 18 °C. Two percent 'Wasserblau' (Merck, Darmstadt, FRG) in 20% aqueous solution of K_3PO_4 was added to the thawed sections to obtain fluorescence of callosic substances. The observations were recorded on Agfachrom 50L.

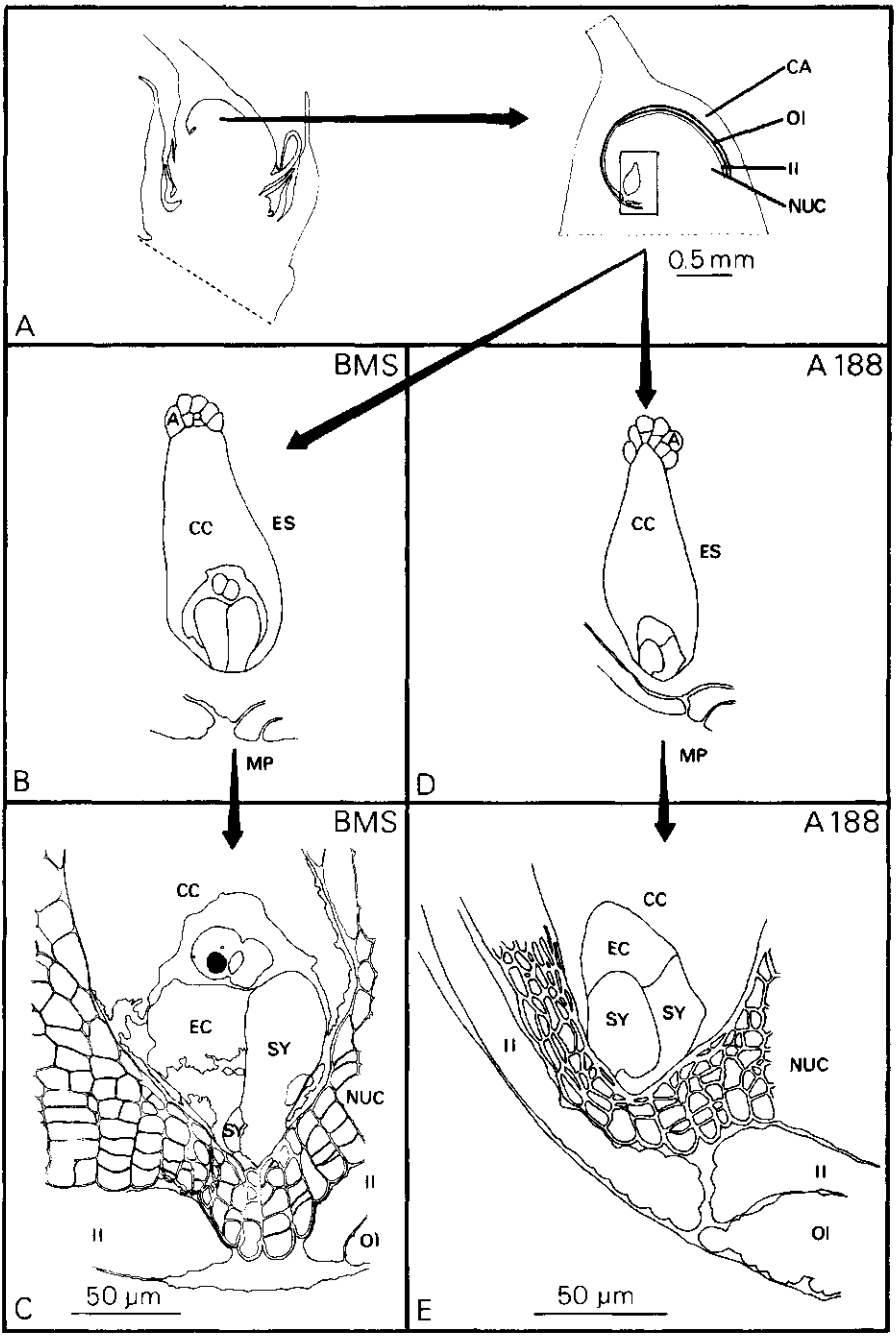
Results

The position of the embryo sac in the ovule

The position of the embryo sac in the nucellus with respect to the micropylar entrance is not similar for the two strains (Fig. 1). In comparison with BMS the embryo sac of strain A188 has a more oblique position towards the micropyle and fewer nucellus cells are in between the embryo sac and the inner integument.

In both strains the nucellus cells of the micropylar region divide several times but more regular rows are formed in BMS (c.f. Figs. 2a and b). In BMS, division and enlargement of nucellus cells also occur in a more symmetrical fashion in the micropylar region. In strain A188, cell division and enlargement are less intensive both near the base of the embryo sac and at the side directed towards the single integument. Therefore, the thickness of the nucellus covering the embryo sac is unequal at the two sides of the egg apparatus. Several nucellus cells which border on the mature embryo sac are flattened because of the enlargement of the embryo sac. Here a total collapse of the cells is preceded by a process of cytoplasmic desintegration (Fig. 1c).

When a pollen tube penetrates the ovule of A188 there appears not to be one straight way towards the embryo sac like in BMS (Figs. 9a and c). Serial sections of the micropylar region of A188 demonstrate the existence of a lobed micropyle (Fig. 10a). One of the folds in the inner integument lies opposite the egg apparatus forming the passage for the pollen tube (Fig. 10a 5, arrow).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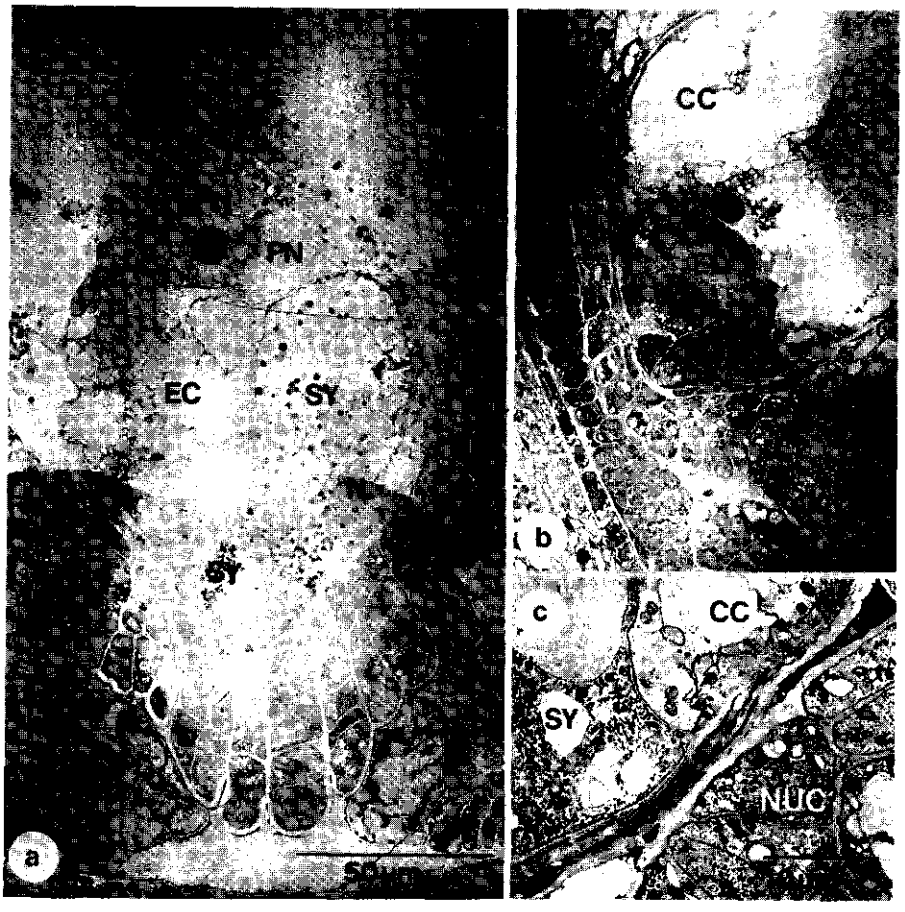
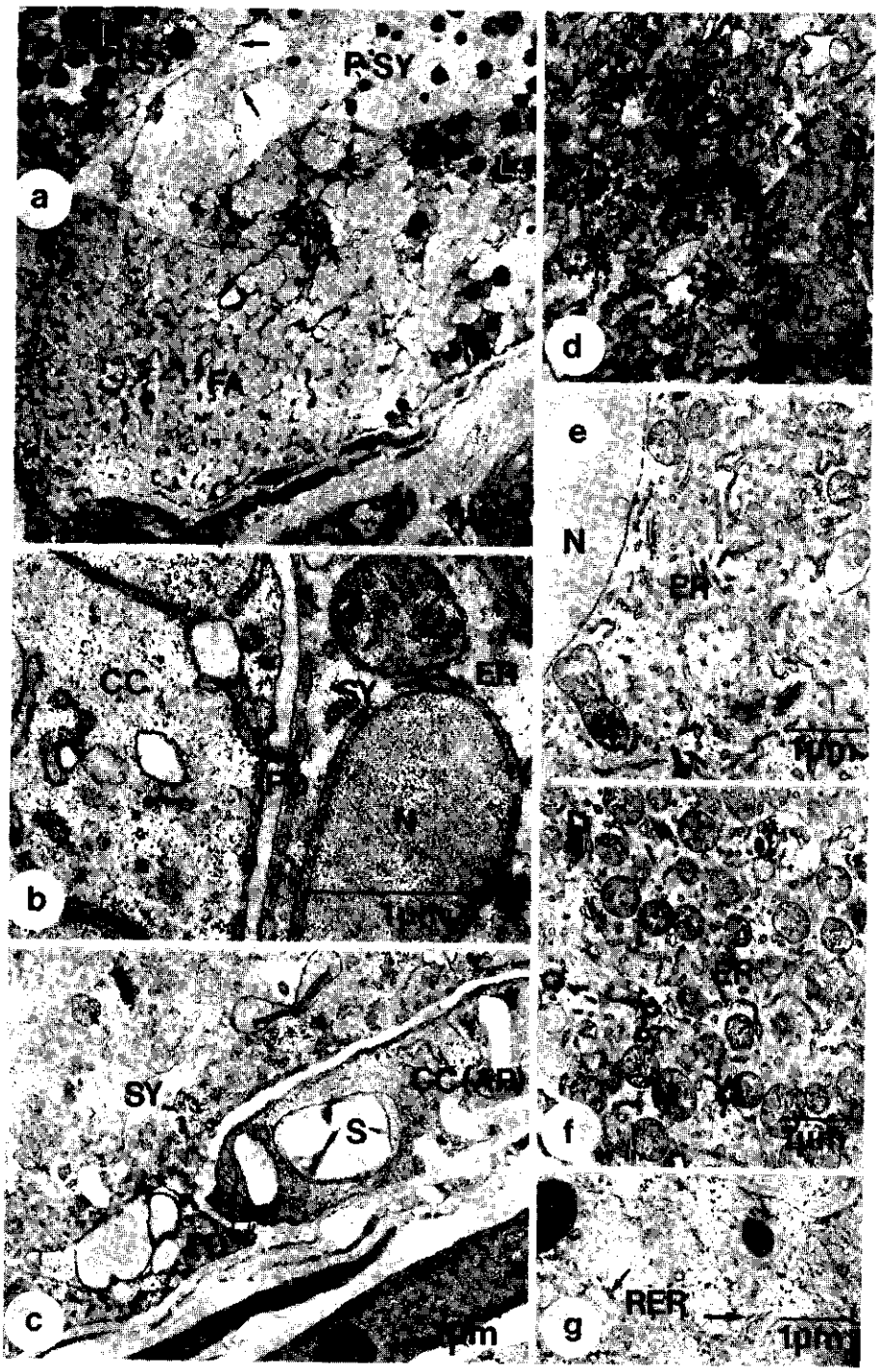


FIG. 2. Median sections through the micropylar parts of the megagametophytes of *Zea mays*, strain BMS (**a**) and A188 (**b,c**). The polar nuclei of the central cell are surrounded by cytoplasm and lie on top of the egg apparatus. In contrast to the synergids the egg cells are highly vacuolated. Their cytoplasm is found in the micropylar half. Note the regular arrangement of the nucellus cells near the micropyle of BMS (**a**). Several nucellus cells collapse because of the enlargement of the embryo sac.

The cells of the embryo sac before fertilization

Intact synergids At the time of fertilization both synergids may still be intact. Sometimes, however, one synergid degenerates before fertilization. This synergid will receive the pollen tube. Synergids are about as tall as the egg cell.

FIG. 1. Schematic representation of the megagametophyte positions in the ovaries of *Zea mays*, strain BMS and A188. **A** The pistilate spikelet and the ovary with its modified campylotropous ovule. The outer integument does not completely surround the ovule. **B, D** The embryo sac consists of approximately 20 antipodals, a large central cell and the egg apparatus. **C, E** With regard to the micropyle there is a difference in position of the egg apparatus in BMS and A188.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At their micropylar side, where they are attached to the wall of the embryo sac, wall protrusions invade the cytoplasm forming the filiform apparatus (Fig. 3a). Some wall protrusions are also formed at the micropylar part of their joint cell wall. Here, the scarcely spread plasmodesmata give cytoplasmic contact. Plasmodesmata leading to the central cell occur frequently in the apical pockets as indicated in Fig. 3c. In other regions there is less cytoplasmic contact with the central cell and plasmodesmata have only rarely been observed leading to the egg cell. Cell walls are clearly formed at the micropylar half of the synergids but at the upper half, cell walls are rudimentary or absent. The distribution of the organelles is not random in the intact synergids and for most organelles the distribution appears similar in the two strains studied. Vacuoles, however, occur more frequent in strain BMS where they occupy the major part of the cell volume (Fig. 2a). In both strains, the nuclei of the synergids always appear to be adjacent to the cell wall in the mid–region or the micropylar half of the cell (Figs. 2a, 4a). They can be surrounded by strains of ER (Fig. 3b). The organelle distribution within one synergid of strain A188 is shown in Figs. 3d,e,f. The majority of the spherical mitochondria accumulates near the filiform apparatus (Fig. 3f), whilst in the middle and upper parts of the cell the tubular endoplasmic reticulum (TER) is more extensive. Proper fixation reveals the existence of rough ER (Fig. 3g). Polysomes are spread over the cytoplasm. Large plastids are dominantly located in the upper half of the cell but small ones are also found near the filiform apparatus. The accumulation of starch has not been detected. Dictyosomes are spread over the whole cytoplasm although more are found at the base of the cell. Lots of granules bud off at the periphery of the cisternae. Osmiophilic droplets occur in all cells of the megagametophyte but they are most numerous in the synergids. Here they are a concomitant feature of cytoplasmic desintegration and they can be found as large spherical droplets in the cytoplasm and as small ones in the cell membrane (Fig. 3a–arrows) and in the outer membranes of the mitochondria. The quantification of the sizes of nuclei, mitochondria and plastids is summarized in Table I.

Egg cell At maturity the megagamete is a highly vacuolized cell surrounded by the two synergids and the central cell (Figs. 2 and 4a). At its micropylar side it is attached to the wall of the embryo sac next to the filiform apparatus. Here, plasmodesmata give cytoplasmic contact with the central cell as illustrated for strain A188 in Fig. 4b. Only few plasmodesmata lead to the synergids. At the antipodal side a clear cell wall is absent. The position of the cytoplasm and the organelles within the egg cell is very characteristic at the unfertilized stage.

FIG. 3. Longitudinal sections through various parts of the pre– fertilization synergids of *Zea mays*, strain BMS (**a**,**g**) and A188 (**b**–**f**). Wall protrusions forming the filiform apparatus invade the cytoplasm (**a**), which shows free (L) and membrane bound (arrows) osmiophilic droplets as a sign of an initiated degeneration. Plasmodesmata lead towards the central cell (**b**) especially in the apical pockets (**c**). Note the distribution of plastids, ER and mitochondria in the antipodal (**d**), mid (**e**) and micropylar (**f**) region of one synergid. Profiles of RER are detected frequently (**g**, arrows).

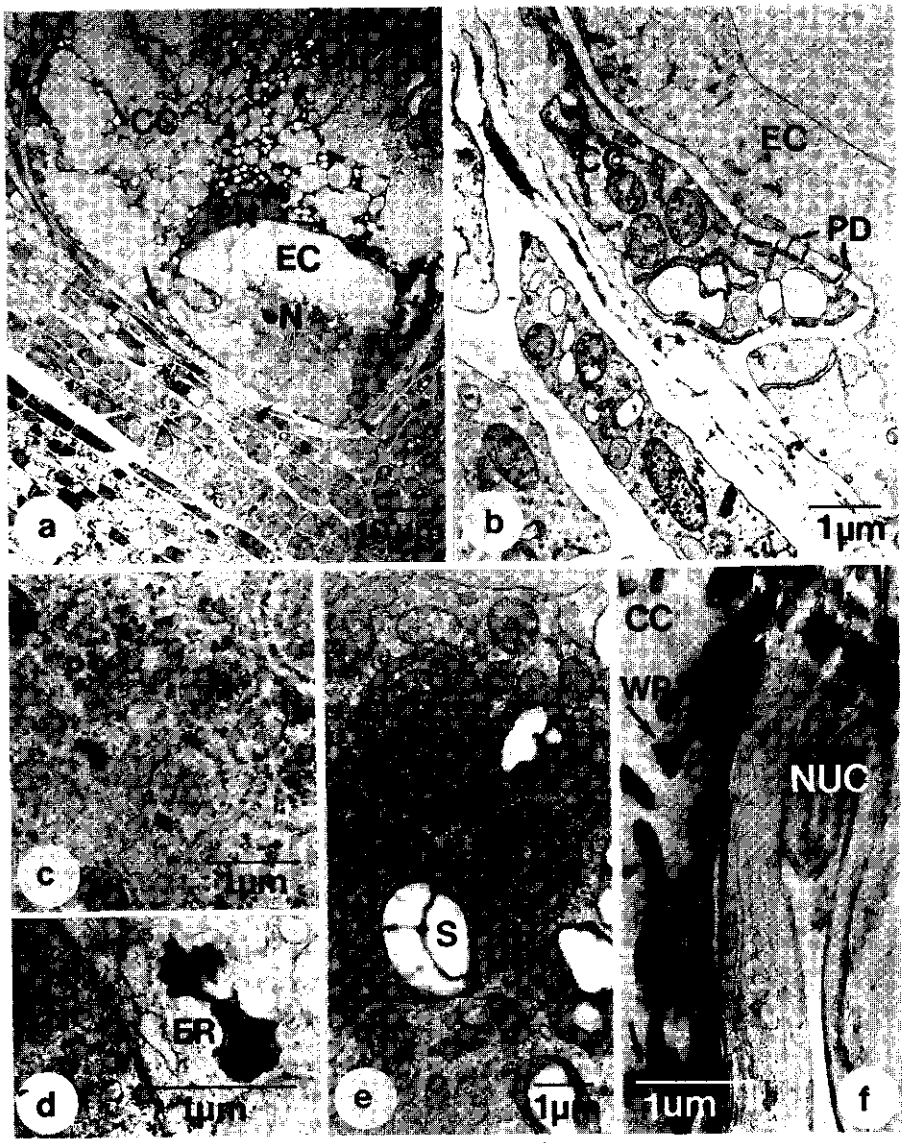


FIG. 4. Longitudinal sections through the micropylar halfs of the embryo sac of *Zea mays*, strain BMS (**c,d,f**) and A188 (**a,b,e**). At its micropylar side the egg cell is connected with the wall of the embryo sac (**a**, arrow–heads). Plasmodesmata from the egg cell towards the central cell are restricted to the apical pockets (**b**). Polymorphic mitochondria, polysomes and starch containing plastids dominate in the egg cell cytoplasm (**c,e**); the ER is in contact with the nuclear membrane (**d**). Numerous wall protrusions (arrows) enlarge the surface of the cell membrane of the central cell. The electron–dense staining results from a selective periodic acid oxidation and the thiocarbohydrazide–silver proteinate reaction after Thiéry (1967) to detect polysaccharides.

In both strains the larger part of the cytoplasm and organelles surrounds the spherical nucleus and can often be found near the wall of a synergid in the basal, micropylar half of the cell. Vacuoles of various sizes occupy the upper, antipodal half. A shift of the cytoplasm and the nucleus towards the apex of the cell results in a basal vacuolation and is an early consequence of fertilization as will be shown later on.

Among the organelles mitochondria are most striking in the egg cell cytoplasm. They are often found solitary but data obtained from serial sections show that they can also be arranged around each other, like shells forming a globular structure with a spherical mitochondrion in its centre (Figs. 4c and e). The latter phenomenon is only observed in this cell of the embryo sac and it occurs most frequent in strain A188. Many ribosomes and polysomes lie between those mitochondria. Smooth ER and RER are scattered throughout the cytoplasm and the ER makes contact with the outer nuclear membrane (Fig. 4d). The ER may have swollen cisternae in both strains. Polysomes and monosomes occur regularly but dictyosomes contribute for a minor part in the composition of the cytoplasm. There are also few plastids. They are large, contain several starch grains and sometimes they tend to be clustered.

Diagram 1 gives a schematic representation of the organelle shapes in both the egg cells and the zygotes of strain BMS and A188. Diagram 2 and Table II summarize the quantification of sizes, frequences and occupation rates of plastids and mitochondria in the egg cell. It appears that mitochondria and plastids do not differ in average sizes significantly although the graphic presentation of sizes demonstrates the existence of larger mitochondria and plastids in strain A188 (c.f. Diagram 2, BMS **1,5**; A188 **1,5**). The occupation rates of the mitochondria and the plastids, as presented in Table II were calculated from organelle surface measurements within a defined area of cytoplasm in which the surfaces of the nucleus and vacuoles were not included. In both strains, about 35% of the cytoplasm is occupied by mitochondria. The rates measured for plastids were significantly lower: for BMS 20 $\pm$ 3%, for A188 12 $\pm$ 4%.

Central cell The cytology of BMS and that of A188 central cells is similar in many aspects. The central cell encompasses the major part of the egg apparatus. The cell wall which borders on the nucellus is well developed, especially in the micropylar region (Fig. 4a, arrows) where it bears small polysaccharide containing protrusions (Fig. 4f, arrows). At the antipodal side of the egg apparatus, however, the cell walls between synergids, egg cell and central cell are often incomplete or undetectable. Here the male gamete is supposed to leave the synergid to fuse with the central cell. In the pre-fertilization phase of the mature megagametophyte the two polar nuclei and the major part of the cytoplasm are always found at the antipodal side of the egg apparatus (Fig. 2a,b; 5a). The nuclei are always close to each other and might already be partly fused before fertilization (Figs. 5b,c). There is continuity between the nuclear envelope and the ER (Fig. 5d). The major part of the central cell is occupied by vacuoles of various sizes. A thin layer of cytoplasm covers the cell membrane adjacent

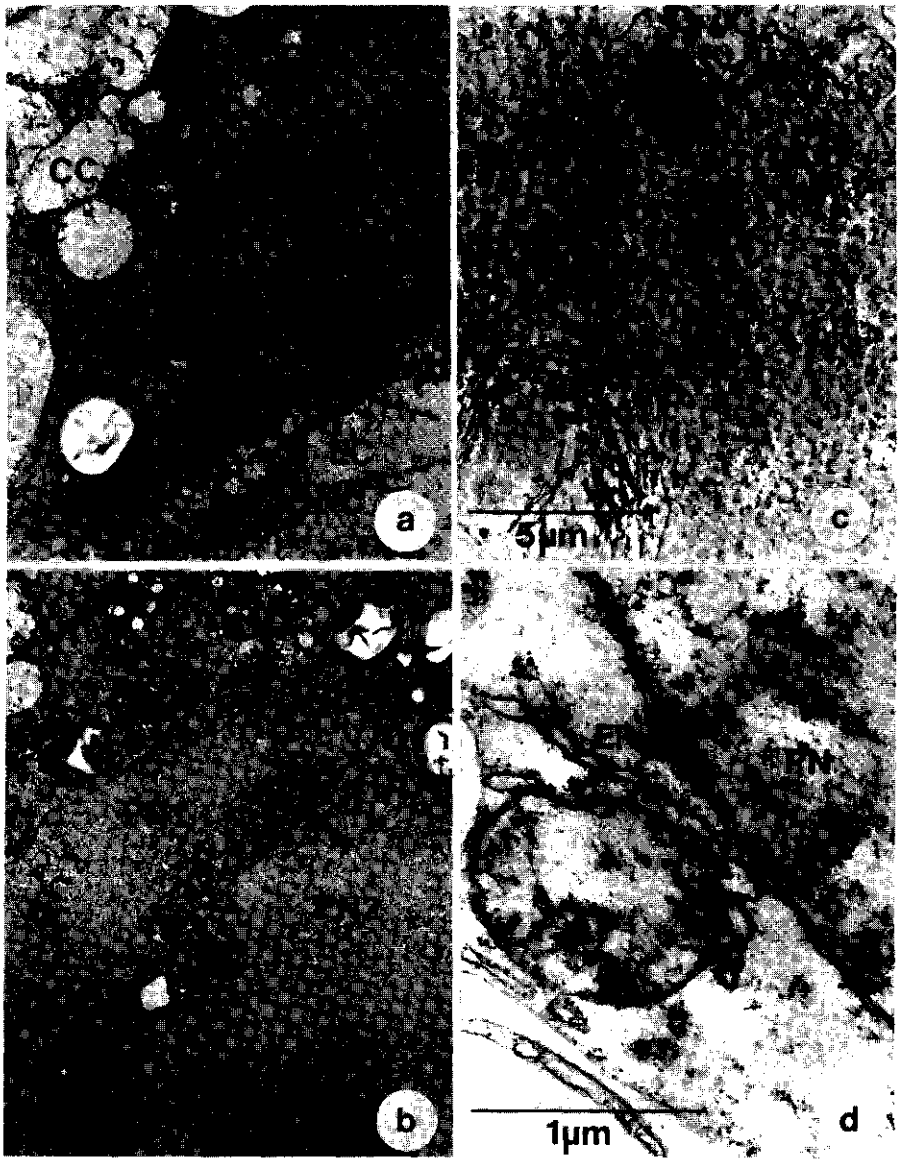


FIG. 5. Polar nuclei in the central cells of *Zea mays*, strain BMS (**c,d**) and A188 (**a,b**) are always at the antipodal side of the egg cell (**a**). The nuclei are close to each other (**b**) and can be partly fused before fertilization (**c**). There is a continuity between the nuclear envelope and the ER (**d**).

to the cell wall of the embryo sac that borders on the antipodal and nucellus cells. Plasmodesmata occur regularly between the central cell and the other cells of the megagametophyte but not between the central cell and the surrounding nucellar tissue.

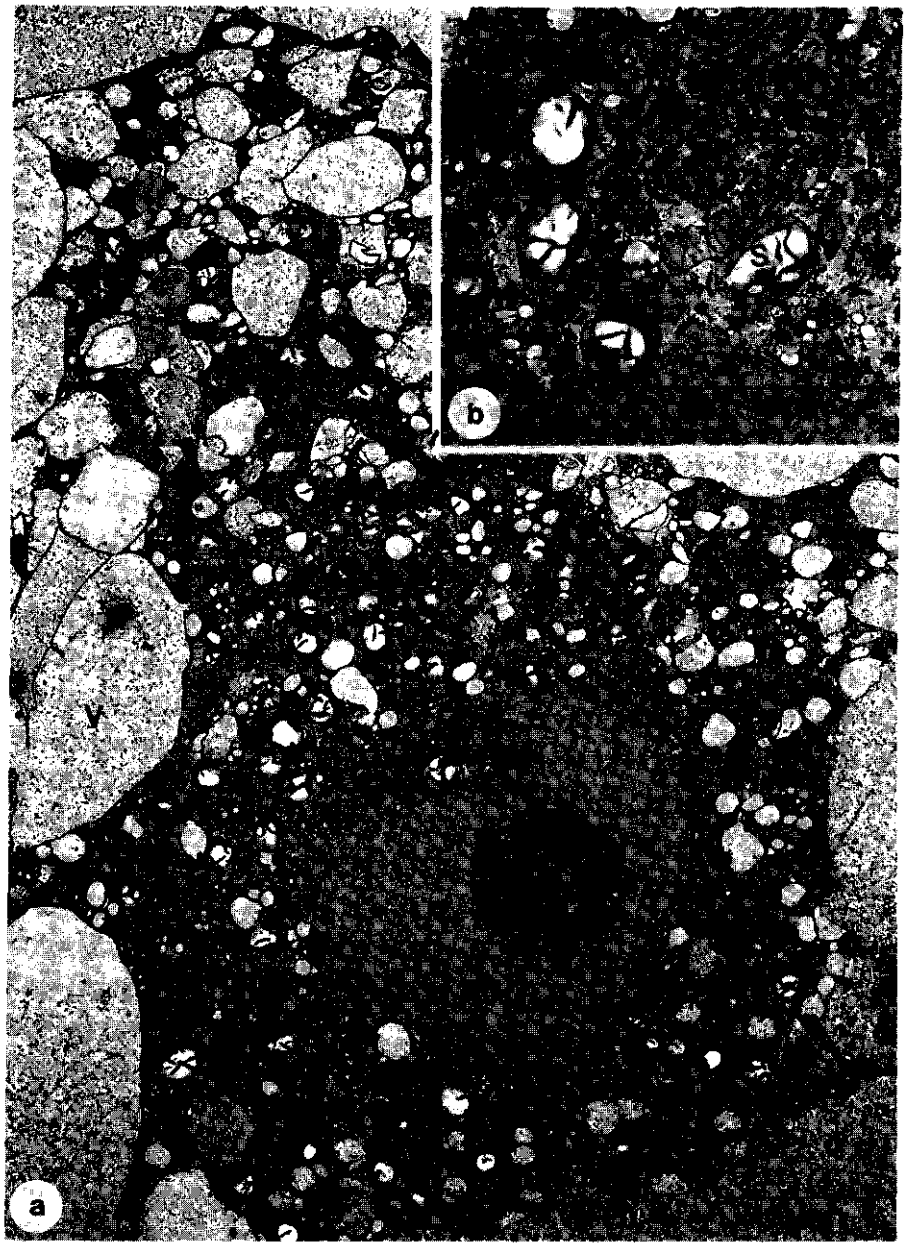


FIG. 6. An overall picture and a detail of the majority of the central cell cytoplasm of *Zea mays*, strain A188 (**a,b**). Mitochondria have well developed cristae; the ER is often tubular; dictyosomes have small vesicles and the polymorphic plastids often contain starch.

For both strains there is a high density of organelles in the cytoplasm around the nuclei as is shown in Fig. 5 and 6. Mitochondria are sometimes polymorphic but mostly ellipsoid and have well developed cristae. The endoplasmic reticulum appears to be mainly tubular in strain A188 whereas in strain BMS long profiles of ER have often been seen in sections. Dictyosomes have small vesicles and are never numerous at this stage. Plastids vary considerably in size and shape and often contain starch grains in strain A188. The organelles of the central cell are schematically represented in Diagram 3. Diagram 2 and Table II summarize the quantification of sizes, frequences and of the occupation rates of mitochondria and plastids in a part of the cytoplasm near the egg apparatus. The mitochondria of the BMS and A188 central cells have about the same size. There are no differences in cytoplasmic occupation rates. For plastids significant differences can neither be detected in mean organelle sizes nor in cytoplasmic occupation rates. When, however, central cells are compared with the egg cells it appears that the occupation rate of mitochondria is significantly higher in the egg cell of strain A188 than in the central cell of that inbred line.

Antipodals Multiplication of the three antipodal cells results in approximately 20 antipodals which form part of the full-grown embryo sac (Figs. 1b, 1d). Several cells contain more than one nucleus because complete cell separation was not realized after mitosis. Cell walls, adjacent to the nucellar tissue, may

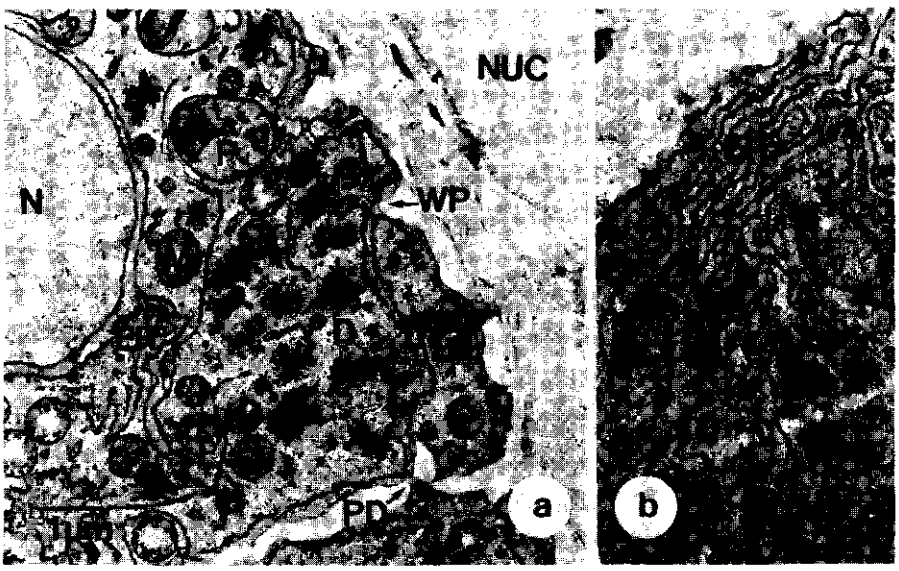


FIG. 7. Antipodals of *Zea mays*, strain BMS (**a,b**) contain numerous organelles among which an abundance of ER, dictyosomes and mitochondria. The ER is frequently arranged in long, interconnected sheets parallel to each other (**b**). Dictyosomes can be in close association with the ER. They have many cristae but secretory granules do not bud off frequently.

have small protrusions (Fig. 7a) as observed in the central cell, too. Plasmodesmata allow contact with other antipodals and with the central cell but there is no cytoplasmic contact with the nucellus cells. Some antipodals have large vacuoles, others have only small ones. The cytoplasm contains numerous organelles among which an abundance of ER, dictyosomes and mitochondria (Fig. 7a). In both strains the ER is frequently arranged in long interconnected sheets parallel to each other (Fig. 7b). Intra–cisternal continuity throughout the cytoplasm from the viscinity of the outer nuclear membrane up to the periphery of the cell is obvious. Nuclei may be spherical or invaginated. They are partly enclosed by sheets of ER. Plastids have small thylakoid membranes and may contain starch grains as detected in BMS. In both strains, BMS and A188, a high number of polysomes, many large, well developed dictyosomes, and mitochondria with well developed cristae were observed.

The quantification of the sizes of some organelles is summarized in Table I. The antipodal nuclei are the smallest nuclei in the embryo sac. Antipodal mitochondria and plastids are small, too, like in the synergids. Due to the quantification procedure high standard deviations occur (see legend Diagram 2) and therefore, despite different average sizes, significant distinctions could only be demonstrated incidentally. Only the plastids of BMS antipodals are significantly smaller than the plastids in the egg cell and central cell.

Penetration of the pollen tube into the pistil and the embryo sac

The pollen tube growth is initiated after the contact of the pollen grain with the multicellular, receptive hairs located on two opposite sides of the silk. The pollen tube may penetrate the body of the style directly but it usually enters by way of one of these stigmatic hairs (Fig. 8a).

Once inside the tissue of the style, pollen tubes grow intercellularly towards a transmitting tissue which is associated with the strands of vascular tissue and then between the cells of that tissue down the silk towards the ovary (KROH et al. 1979; HESLOP–HARRISON et al. 1985). Pollen tubes are traced by the detection of fluorescent callose deposits in the pollen tube (Fig. 8b,c,d). In these studies about 7 pollen tubes were detected in a cryosection (25 μm) of an ovary. The pollen tubes follow the inner surface of the ovary wall until the micropyle is reached (Fig. 8b,c). Not all the pollen tubes grow straight towards the micropyle. Some were observed between the integuments and others at the opposite side of the ovule but eventually all come to the micropyle (Fig. 8d). The pollen tubes are detected by the staining of callose deposits, with 'Wasserblau' but when a pollen tube penetrates the nucellus, callose can no longer be detected.

In the ovules of strain BMS the micropyle consists of only one opening in the inner integument through which one or more pollen tubes have to reach the nucellus. Only one pollen tube was observed to pass the cuticle of the nucellus. It penetrates the nucellar tissue and grows directly towards the egg apparatus. No cells of the nucellus are damaged when the pollen tube grows through the cell walls and the intercellular spaces (Figs. 9a,c). The pollen tube penetrates the embryo sac by growing into the filiform apparatus through the joint cell

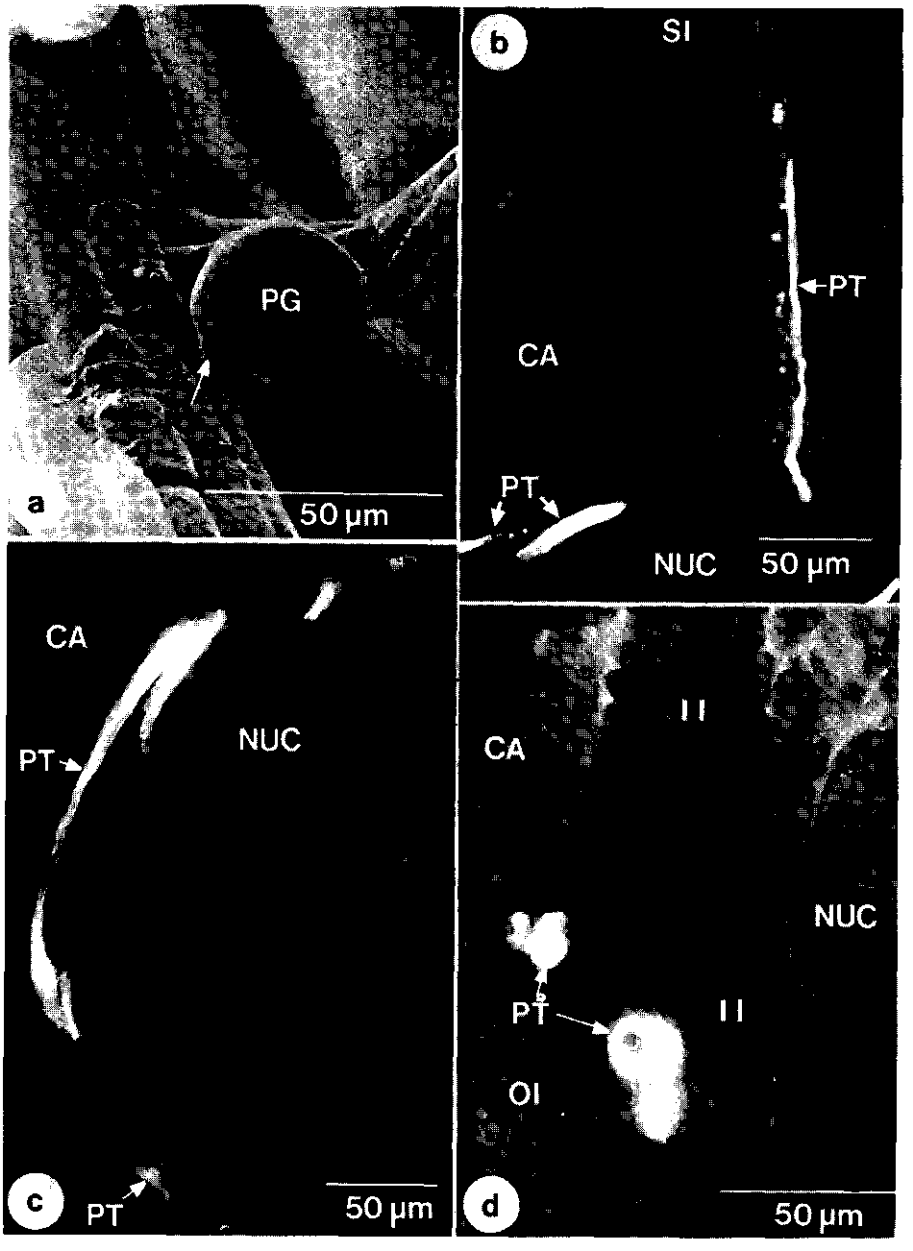


FIG. 8. A pollen grain (PG) of *Zea mays*, strain BMS, adheres to a stigmatic hair. It has germinated and the pollen tube penetrated the body of the silk directly (**a**, photograph courtesy Dr.Ir. H.J. Wilms). Then pollen tubes grow through the transmitting tissue of the silk into the ovular locule (**b,c**) towards and between the integuments at the micropyle (**c,d**).

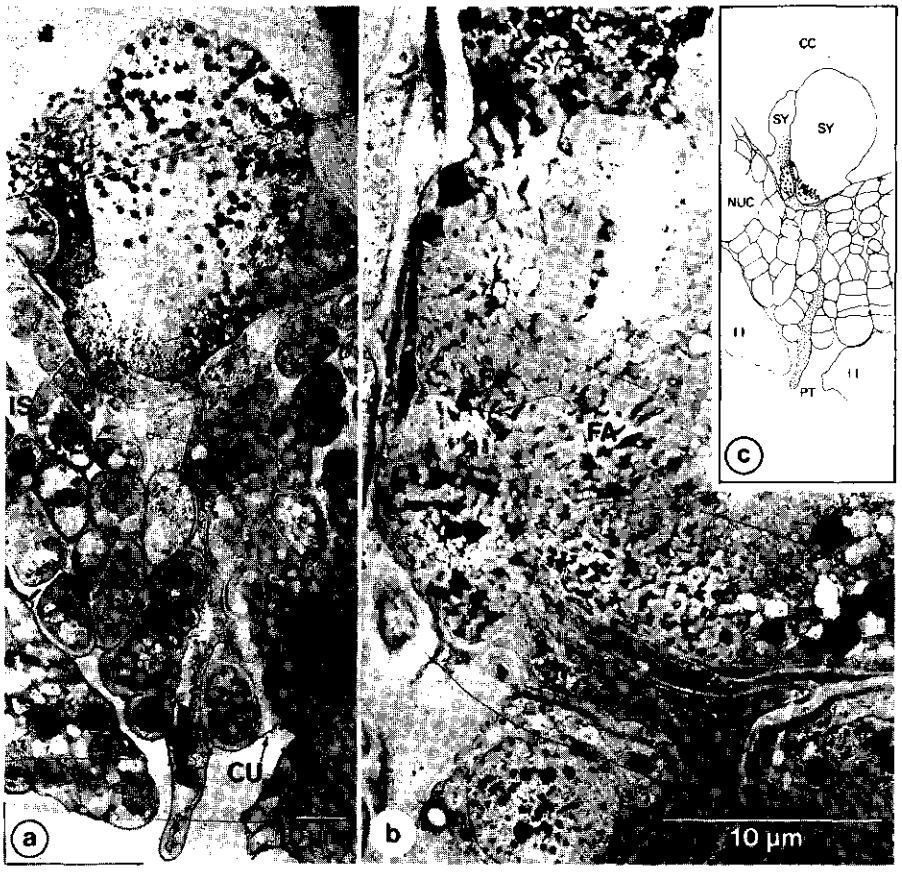
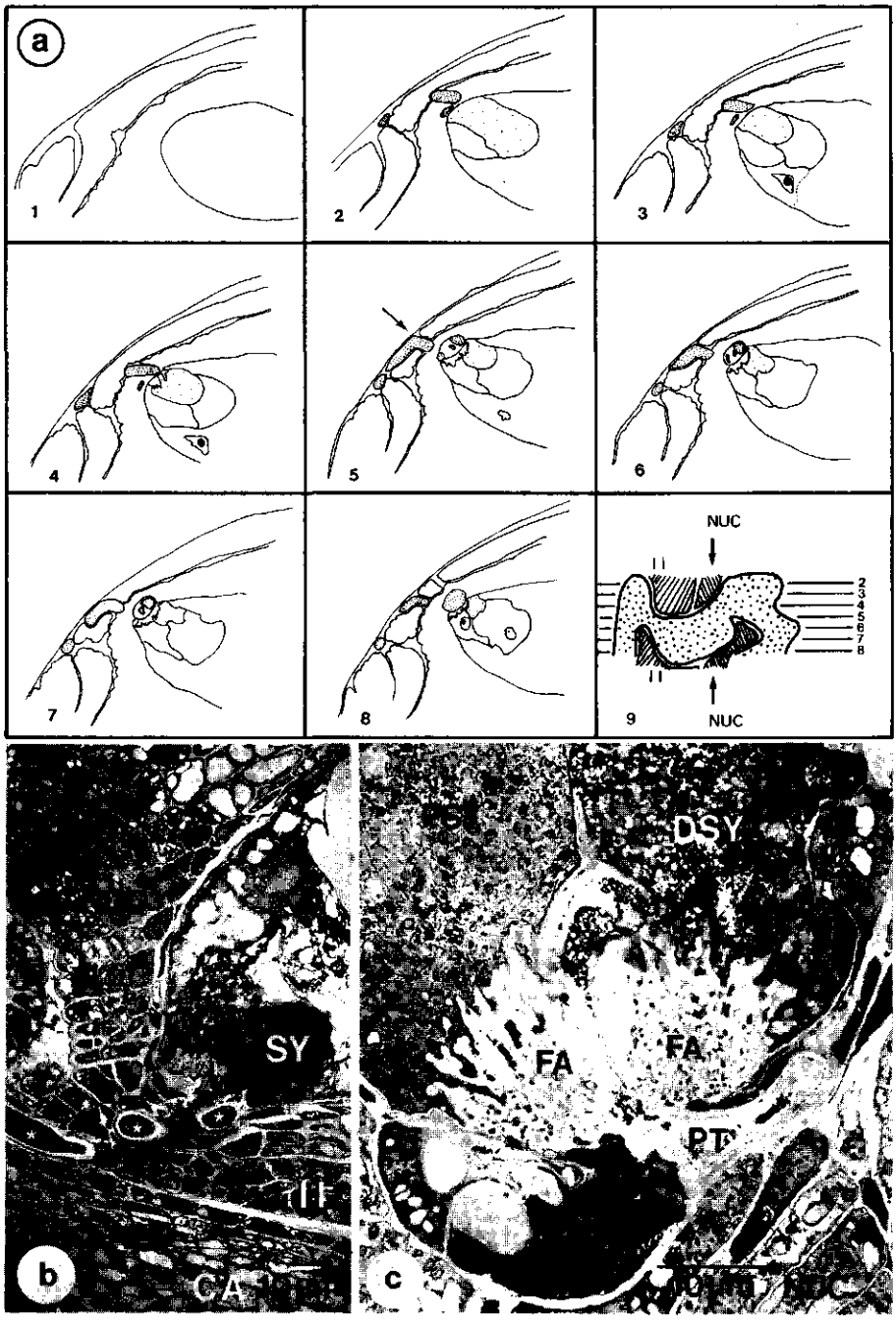


FIG. 9. Penetration of the nucellus and embryo sac by the pollen tube in *Zea mays*, strain BMS; overall view (**a**), detail (**b**) and drawing (c). The pollen tube penetrates the cuticle of the nucellus, then it grows through intercellular spaces into the nucellus and eventually it enters the filiform apparatus through the joint cell wall of the two synergids. Pollen tube cytoplasm has been discharged into one synergid.

wall of the two synergids. Then the pollen tube enters a synergid along side the joint cell wall (Fig. 9b). It stops growing and discharges a part of its contents into the synergid which gets filled up completely. When the synergid is penetrated it may have been degenerated already.

When a pollen tube penetrates the ovule of A188 there isn't one straight way towards the embryo sac like in BMS. Serial sections of the micropylar region of A188 demonstrate the existence of a lobed micropyle which has several openings (Fig. 10a,b). The pollen tube winds its way and cannot be observed in one plane of sectioning. One of the folds in the inner integument lies near to the egg apparatus and forms the eventual passage for the pollen tube (Fig.10a,5, arrow). Once in the nucellus, the pollen tube appears to bend again searching its way to the embryo sac. Reaching the base of the filiform apparatus the pollen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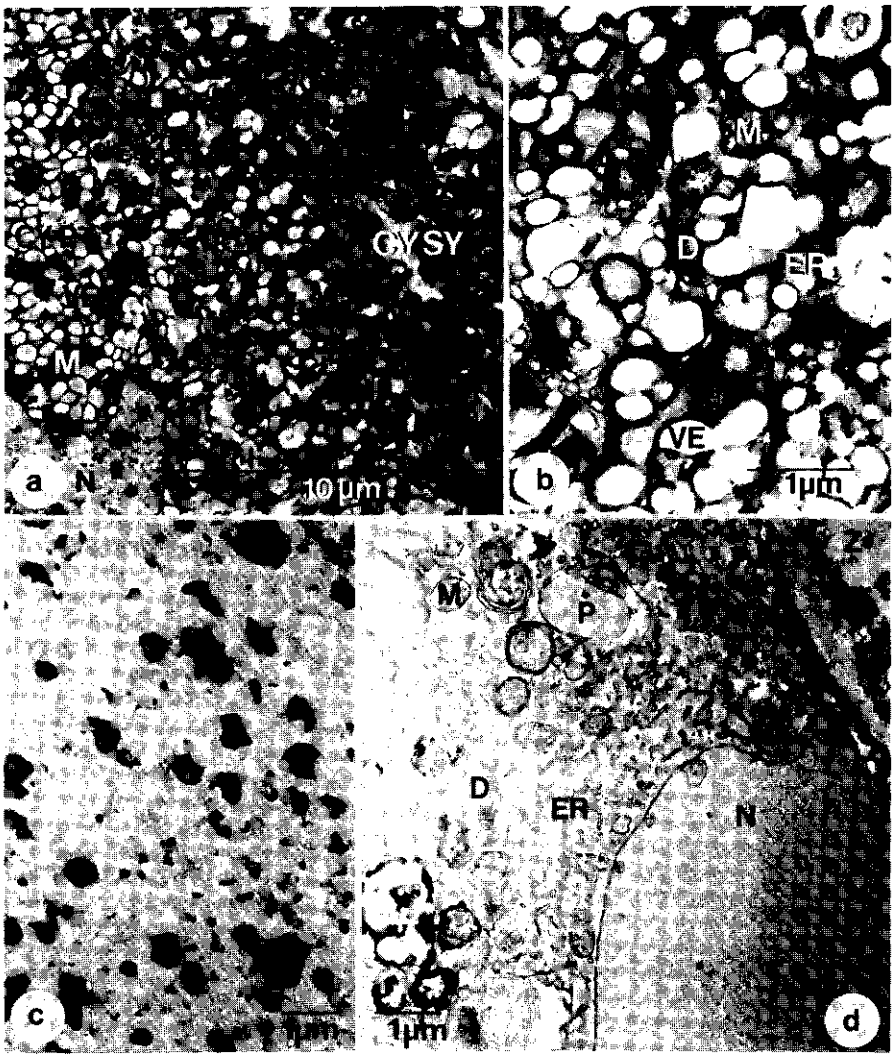
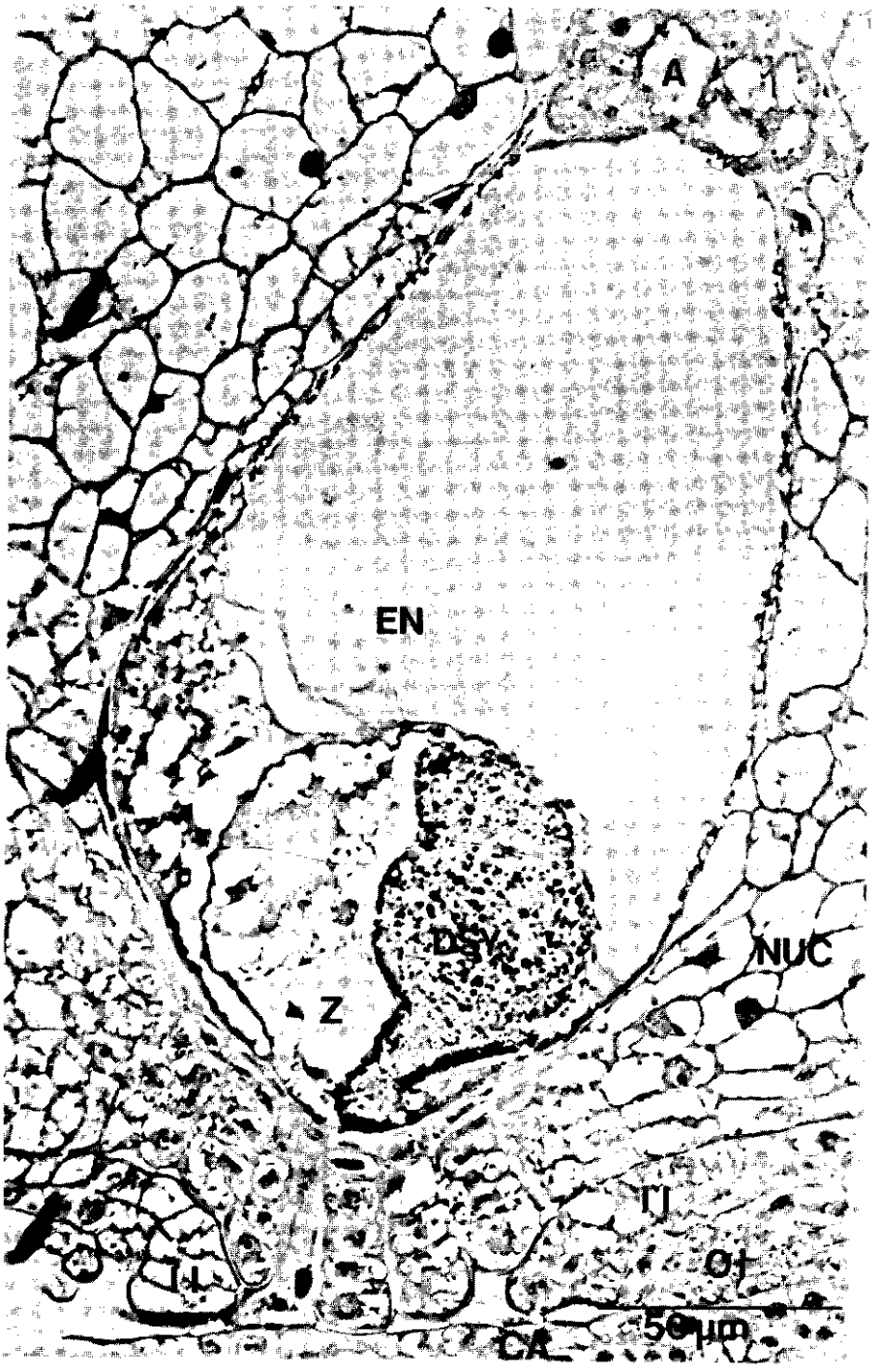


FIG. 11. Post–fertilization structure of the synergids of *Zea mays*, strain A188. Except for a small region, the pollen tube cytoplasm and the vegetative nucleus do not mix with the cytoplasm of the degenerated synergid (**a**). The cytoplasm mainly consists of dictyosomes, vesicles and mitochondria (**b**). Within a few hours, that cytoplasm degenerates and osmiophilic droplets and vesicles remain (**c**) whereas the persistent synergid is still intact (**d**).

FIG. 10. Penetration of the pollen tube into the ovule of *Zea mays*, strain A188.**a**) Serial, longitudinal sections through the micropylar region visualize the course of the pollen tube (**a 1–8**, pollen tube dotted) which runs up and down through the inner integument and nucellus. The arrow indicates the site of pollen tube entry; **a–9** gives the two–dimensional compilation of the drawings. **b–c**) Electron micrographs of micropylar region with pollen tube. Note the bending of the pollen tube (**b** asterisks), the penetration of the filiform apparatus (**c**) and the sub–terminal opening of the pollen tube (**c**-arrow).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50 µm

tube sometimes enlarges and eventually penetrates a synergid like in strain BMS (Fig. 10b,c)

For strain A188 it takes about 21 hours from pollen tube germination up to penetration of the embryo sac and about 26 hours for strain BMS. However, the periods mentioned are largely dependent on the greenhouse conditions.

The cells of the embryo sac after fertilization

Synergids When the pollen tube has penetrated a synergid and discharged its contents into that synergid, the cytoplasm of both cells is not mixed (Fig. 11a). In some parts of the cell the synergid cytoplasm can still be detected. The cytoplasm coming out of the pollen tube reaches up to the tip of the synergid and can be discerned by its characteristic appearance (Fig. 11b). Translucent vesicles, originating from the dictyosomes, predominate in the cytoplasm. Mitochondria have long cristae but the accumulation of small osmiophilic droplets which are often attached to the cristae and inner membranes of the envelope indicates the onset of degeneration. Plastids, however, are still intact and some strands of ER are scattered throughout the cytoplasm.

Soon after fertilization the discharged cytoplasm degenerates to such an extent that organelles can hardly be detected (Fig. 11c). Large, osmiophilic droplets and numerous small vesicles are the main constituents of the cytoplasm. The persistent synergid may be structurally intact until the division of the zygote (Fig. 11d) whereas even before fertilization signs of degeneration were observed (Fig. 3a).

Zygote Just after fertilization the egg cell and zygote are not remarkably different in cell size or in shape and yet they are easily distinguished by observing the location of the cytoplasm. As shown previously for the egg cell, the majority of the cytoplasm surrounds the nucleus in the micropylar half of that cell (Fig. 2). As a striking result of fertilization the cytoplasm and the nuclei of the zygotes of both strains BMS and A188 are translocated towards the antipodal side of the cell (Fig. 12,13,17a). The syngamy proper and the karyogamy, however, were not observed and organelles of the male gamete were not distinguished. Fertilization causes no significant change in size of the zygote but in the cytoplasm an increasing complexity is observed (Diagram 1). This holds for the ER, the dictyosomes and the polysomes rather than for the plastids and mitochondria of which the sizes (Diagram 2), occupation rates and frequences (Table II) do not change significantly. As was observed in the egg cells of BMS and A188, the mitochondria are often stacked, forming globular structures. In the zygotes they have large cristae (Figs. 14a,b). The ER tends to concentrate in some areas (Fig. 14c).

FIG. 12. Phase contrast light micrograph of a longitudinal section through the embryo sac of *Zea mays*, strain BMS. After fertilization the prophase nucleus and the cytoplasm are located in the antipodal half of the zygote whereas the endosperm cytoplasm shifted lateral.

FIG. 13. Longitudinal section of the zygote of *Zea mays*, strain A188 with the nucleus and the surrounding cytoplasm at the antipodal side of the cell. Note the abundance of well differentiated mitochondria. The pollen tube cytoplasm in the synergid has already degenerated.

By the time of mitosis, i.e. about 8–10 hours after fertilization, the distribution of organelles changes in the zygotes of both strains. During early prophase ribosomes occur frequently as monosomes, polysomes or they are attached to the ER. At prophase the larger part of the cytoplasm surrounds the nucleus (Fig.

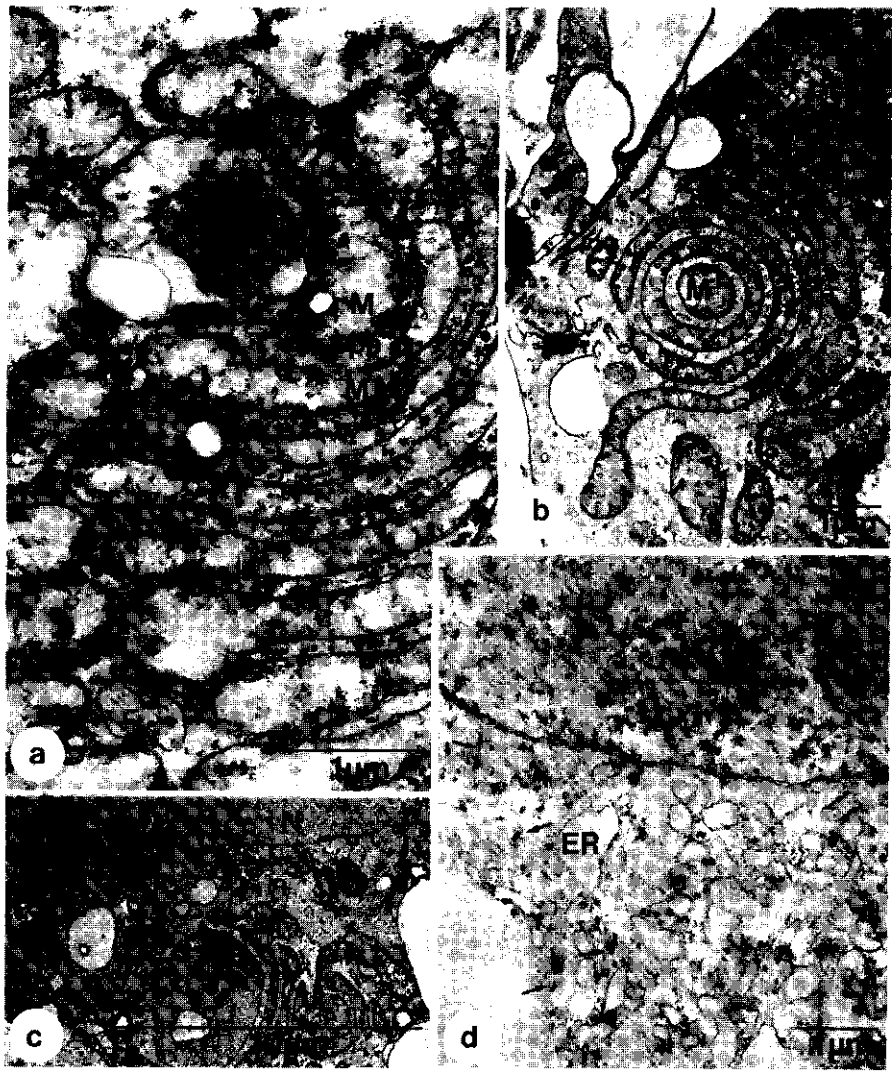

FIG. 14. Electron micrographs of some parts of the cytoplasms of the zygotes of *Zea mays*, strain BMS (**a,d**) and A188 (**b,c**). In both strains the mitochondria can stack, forming globular structures (**a,b**). Proper fixation (glutaraldehyde–OsO_4) reveals the existence of numerous polysomes between the mitochondria (**a**); The ER concentrates in some areas and has a tubular appearance (**c**); it dilates at prophase (**d**). Than, microtubules occur regularly (**d**, arrows; ultra–thin section made after reimbedding of the semi–thin section of fig. 12).

12). Most types of organelles except MTs, ER and ribosomes recede from the vicinity of the nuclear membrane (Fig. 14d). Serial sections reveal the existence of dilated sheets of ER forming a network around the nucleus. Both plastids and mitochondria decrease in size and increase in number then. During pro–

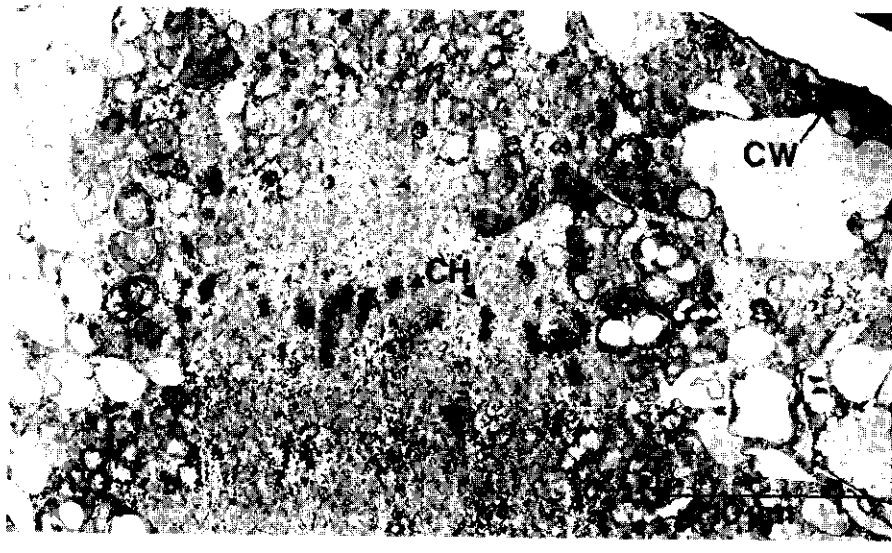

FIG. 15. The zygote of *Zea mays*, strain BMS at mitosis i.e. approximately 8 hrs after fertilization. The metaphase plate is located at the site of the nucleus near the antipodal side of the cell.

metaphase the phragmoplast is formed perpendicular to the long axis of the zygote and at or near the former site of the nucleus (Fig. 15). Just after telophase, the cell segregation is initiated from the cell plate and it proceeds towards the cell membrane. So division results in two unequal cells: an apical cell which is small and has only few vacuoles and a large basal cell in which vacuoles occupy the major part of the cell volume. Figure 16 summarizes some of the early events of fertilization and embryogenesis in the embryo sac of strain BMS up to the two-cellular stage of the embryo.

Endosperm The fusion of the male gamete with the central cell is the impulse for a series of events through which the endosperm will develop. As was observed in both strains fertilization soon evokes a shift of the first endosperm nucleus surrounded by the major part of the cytoplasm towards the lateral and micropylar part of the cell (Fig. 17a). Nuclear divisions occur fast and frequently in the young endosperm of both strains resulting in a coenocyt with eight nuclei at the moment of zygote division, i.e. about 10 hours after fertilization. In the early stage of endosperm formation the nuclei divide synchronously. They lie predominantly near the outer cell wall in the micropylar half of the former central cell. The cytoplasm is concentrated around the nuclei as is drawn in Figs. 16b and c. The position of the organelles near such a nucleus is shown in Figs. 17b and c.

It appears that the plastids and the mitochondria of BMS and A188 do not change in size significantly after fertilization (see Diagram 2: c.f. 3,4 and 7,8). The large plastids which are found in the cytoplasm of the central cell, however,

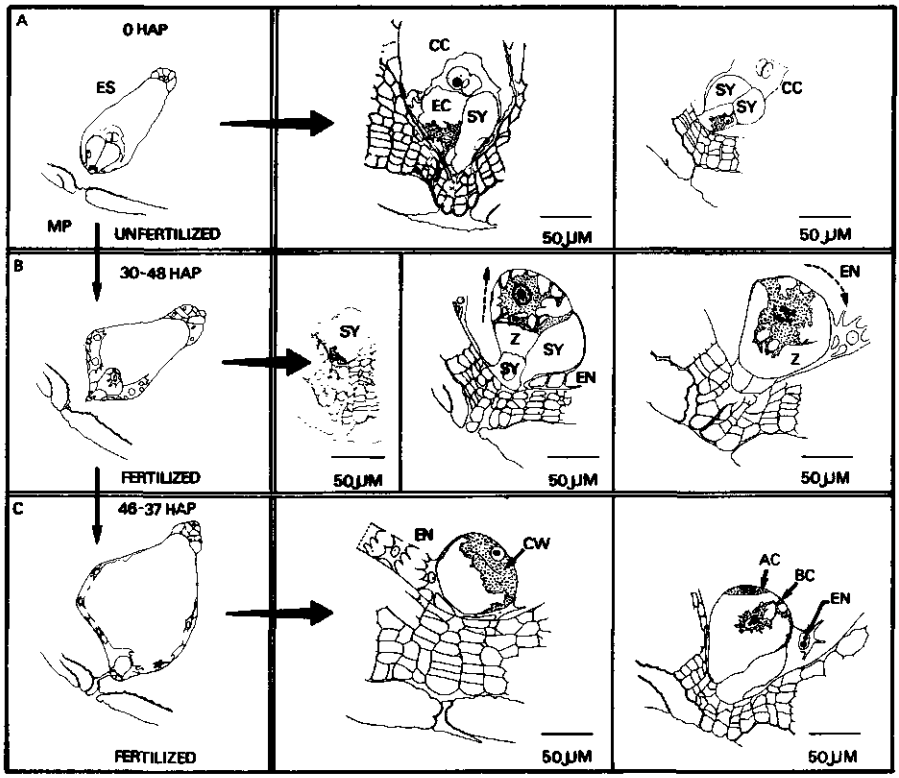

FIG. 16. Summary of the early events of fertilization and early embryogenesis in *Zea mays*, strain BMS. Note the characteristic positions of the cytoplasms of the egg cell and central cell at 0 hours after pollination (**A**) and the shift of the cytoplasms after fertilization (**B**, striated arrows). Mitosis results in a small apical cell and a large vacuolated basal cell (**C**). Than the endosperm is multinucleate.

are not found in the endosperm. The occupation rates of mitochondria and plastids of BMS and A188 do not differ significantly in the central cell and the endosperm (Table II). When the mitochondria of the central cell and the endosperm of A188 are compared with the mitochondria of the egg and the zygote respectively, there is no significant difference in size (Diagram 2: c.f. 3,4 and 1,2), but there is a significant difference in occupation rates being twice as high in the egg and zygote (Table II). As a result of fertilization, dictyosomes increase their complexity and a higher concentration of polysomes was detected in the young endosperm of BMS and A188 (Diagram 3). Long profiles of ER were found running parallel to the cell membrane and near the nuclei.

Antipodals Fertilization has no notable influence on the size and shape of the antipodals of BMS and A188 (Fig. 18a). Plasmodesmata between antipodals

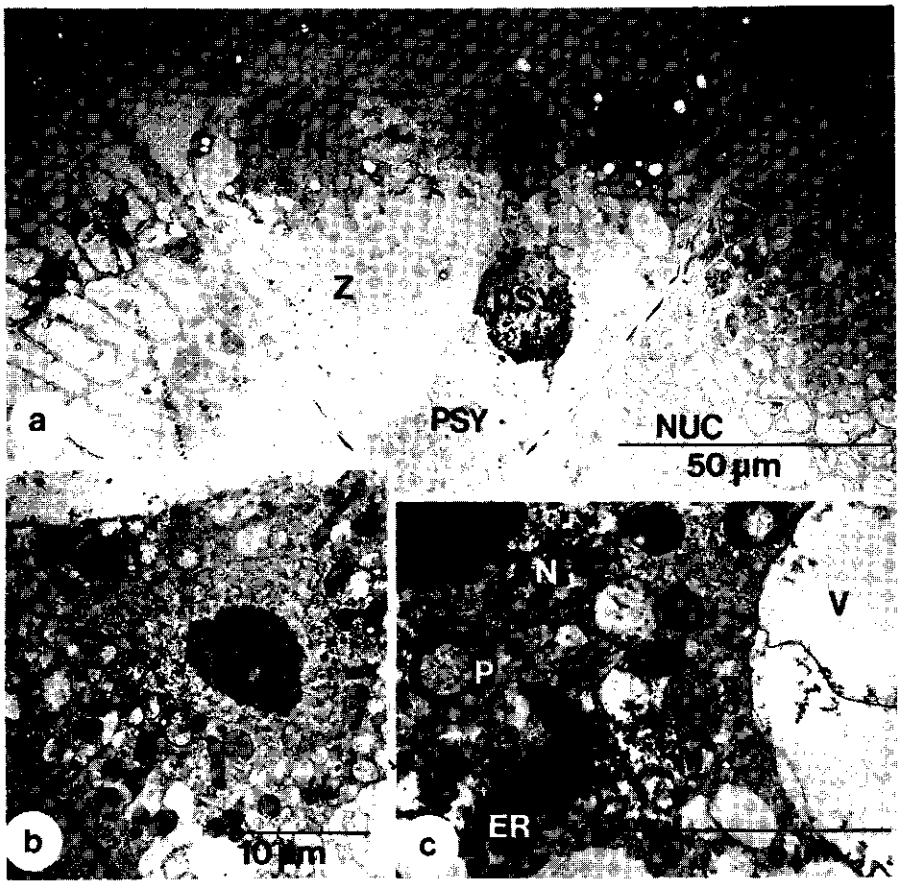

FIG. 17. Post-fertilization cytology of the embryo sac in *Zea mays*, strain A188 (**a**) and BMS (**b,c**). The nucleus of the zygote is located at the antipodal side of the highly vacuolated cell. The persistent synergid is still intact but shows increasing amounts of lipid droplets indicating the onset of degeneration. The endosperm is nucleate at this stage. The majority of the cytoplasm which contains well differentiated organelles surrounds the nuclei (**b,c**).

and leading towards the central cell still exist. Vacuoles can be prominent or nearly absent. The nuclei of the cells are sometimes lobed, even after fertilization. The texture of the chromatin in areas with euchromatin and heterochromatin is most clear in these cells of the megagametophyte. There are no signs of degeneration in the antipodals during the first period of endosperm development. Proper fixation reveals high amounts of ribosomes either bound to proliferating ER of free in the cytoplasm (Fig. 18b). Dictyosomes appear to bud off amounts of vesicles (Fig. 18c). The overall structure of the cytoplasm points at a high activity in the tissue.

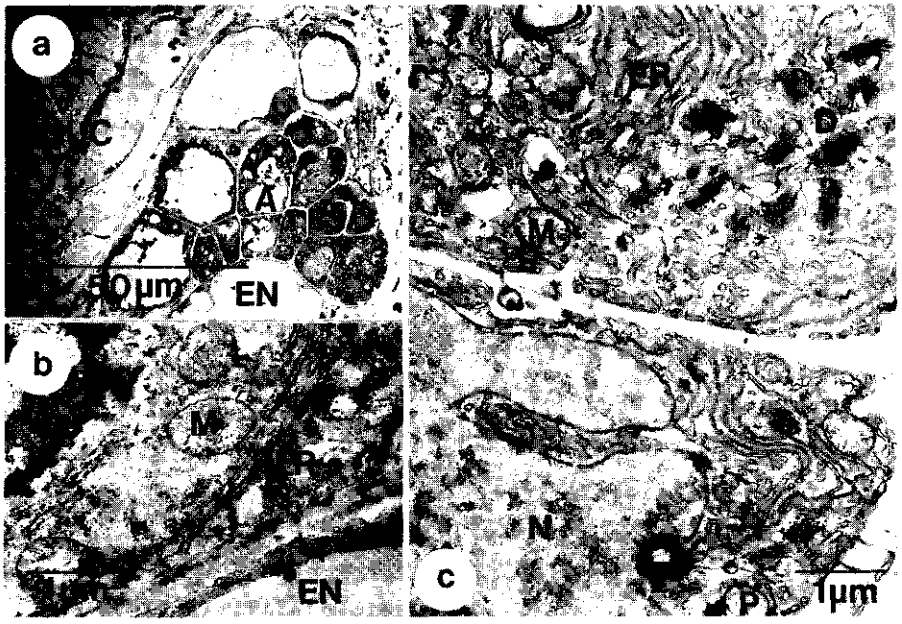

FIG. 18. Antipodals of *Zea mays*, strain A188 (**a,c**) and BMS (**b**) after fertilization. Well developed dictyosomes (**c**) and high amounts of ribosomes either bound to intensively proliferating ER (**b,c**) or free in the cytoplasm point to a high activity in the tissue.

	cells / organelles	Synergids	Egg cell	Central cell	Antipodals
BMS	Nuclei	70	185	310	30
	Mitochondria	0.36 ± 0.07	0.78 ± 0.66	0.62 ± 0.34	0.42 ± 0.07
	Plastids	1.23 ± 0.6	2.79 ± 1.64	4.75 ± 4.17	1.24 ± 0.39
A188	Nuclei	141	200	680	23
	Mitochondria	0.22 ± 0.07	1.60 ± 1.15	0.84 ± 0.65	0.24 ± 0.07
	Plastids	1.57 ± 1.06	5.38 ± 2.93	5.00 ± 3.15	0.68 ± 0.35

TABLE I. Average sizes* of nuclei, mitochondria and plastids in the cells of the unfertilized megagametophyte of *Zea mays*, strain BMS and A188. Measurements are performed with an image analyser (Kontron, MOP–30) and expressed in μm^2. *See note in legend of diagram 2.

Organelles \ cell types		Egg cell	Zygote	Central cell	Endosperm
Mitochondria	BMS	*36 ± 12%* 56 ± 21	*25 ± 7%* 32 ± 25	*27 ± 13%* 40 ± 20	*24 ± 3%* 39 ± 24
	A188	*33 ± 14%* 19 ± 11	*24 ± 5%* 15 ± 11	*16 ± 1%* 17 ± 6	*14 ± 4%* 17 ± 4
Plastids	BMS	*20 ± 3%* 5.5 ± 2.1	*12 ± 5%* 2.8 ± 0.8	*16 ± 8%* 7.3 ± 5.0	*10 ± 3%* 6.4 ± 3.5
	A188	*12 ± 4%* 1.7 ± 0.5	*22 ± 7%* 3.1 ± 1.8	*23 ± 7%* 4.6 ± 1.2	*23 ± 11%* 7.8 ± 4.6

TABLE II. Occupation rates (italics) and frequencies of occurrence (capitals) of mitochondria and plastids in the egg cells, zygotes, central cells and endosperm of *Zea mays*, strain BMS and A188. Each value and its standard deviation are based on five independent measurements. Frequencies of occurrence are expressed by the 100 μm^2.

DISCUSSION

Prefertilization and fertilization phase

After pollination and the germination of the attached pollen, the pollen tubes grow through the cortex of the silk towards one of the two parenchyma layers which are associated with the two vascular bundles of the silk (KROH et al., 1979; HESLOP–HARRISON et al., 1985). The parenchyma layers are interpreted as being a transmitting tissue because the pollen tubes grow further down the silk through the intercellular spaces of the fusiform cells of that tissue. Intercellular growth of pollen tubes has also been reported for other members of the Poaceae (see RANDOLPH, 1936; CHO, 1956: BONNET, 1961; BATYGINA, 1966; CHANDRA and BHATNAGAR, 1974). In maize, not all the pollen tubes which grow down in the transmitting tissue reach the ovule. The late–entering tubes are eliminated either at a stigma abscission zone on the base of the silk or at a constricted zone of the transmitting tracts in the upper ovary wall (HESLOP–HARRISON et al., 1985). Once inside the ovary the pollen tubes penetrate the inner epidermis and the cuticle of the ovary wall and they grow in between an integument and the ovary wall towards the micropyle as has also been observed with *Hordeum* (CASS and JENSEN, 1970). During the penetration of cell walls and cuticles the pollen tube probably excretes enzymes to digest cellulose, hemicellulose, pectine and cutin. At the same time it is dependent on metabolites provided by the sporophyte. Because the pollen tube growth, which will result in porogamy, is clearly directed towards the micropyle, a chemoattraction has often been suggested (see VAN WENT and WILLEMSE, 1984) The starch which was observed to accumulate in the micropylar region of the integuments and in the micropylar region of the nucellus might function in that pollen tube attraction. The carbohydrates are excreted into the intercellular spaces to provide the pollen tube with energy.

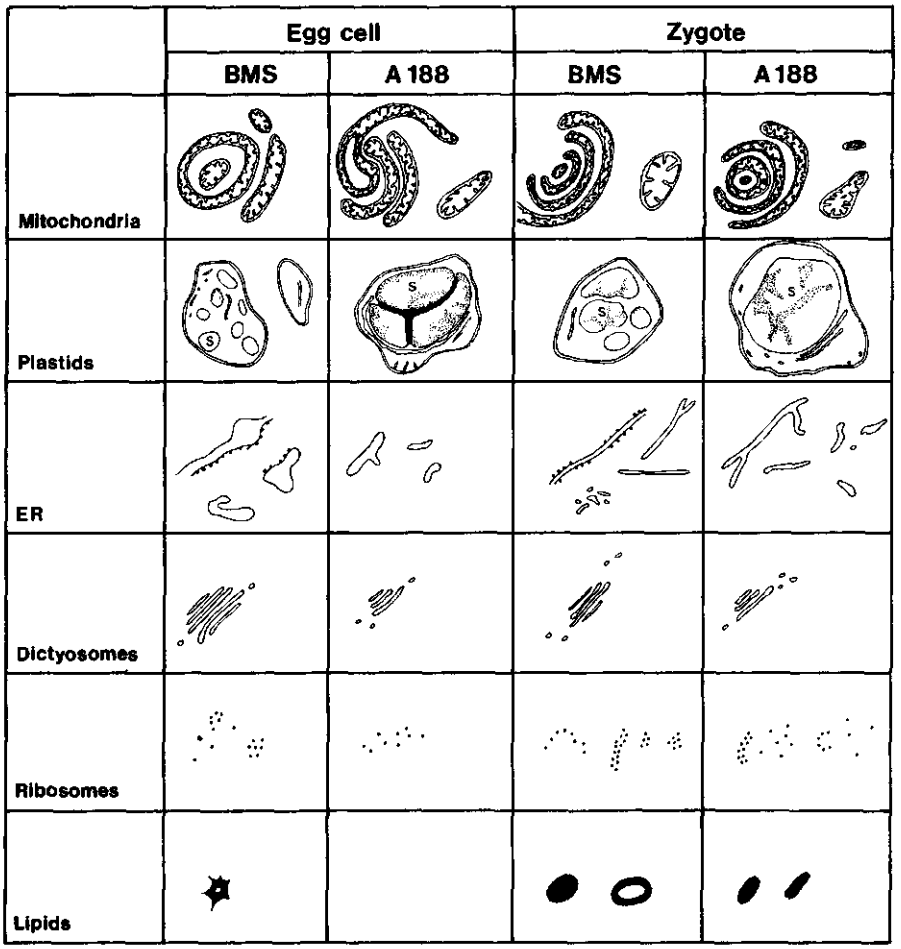

DIAGRAM 1. Schematic representation of organelles in the egg cell and zygote of *Zea mays*, strain BMS and A188.

SINGH and MALIK (1976) propose that metabolites, such as carbohydrates, are also passed on to the filiform apparatus which serves as an entry for metabolites towards the embryo sac. In both strains of *Zea* only one pollen tube entered each ovule. Multiple pollen tube entry into the ovule has been observed in *Triticum* (CHANDRA and BHATNAGAR, 1974), in a remote hybridization of *Triticum* (BATYGINA, 1966) and in *Oryza* (CHO, 1956). The entry of only one maize pollen tube in the nucellus might be explained by the relative short period of time which is necessary for the growth of that pollen tube through the thin layer of tissue from the nucellar cuticle to the degenerated synergid (distance: 20–40 μm; estimated time 1,5–3 min.). As soon as the synergid is penetrated and probably

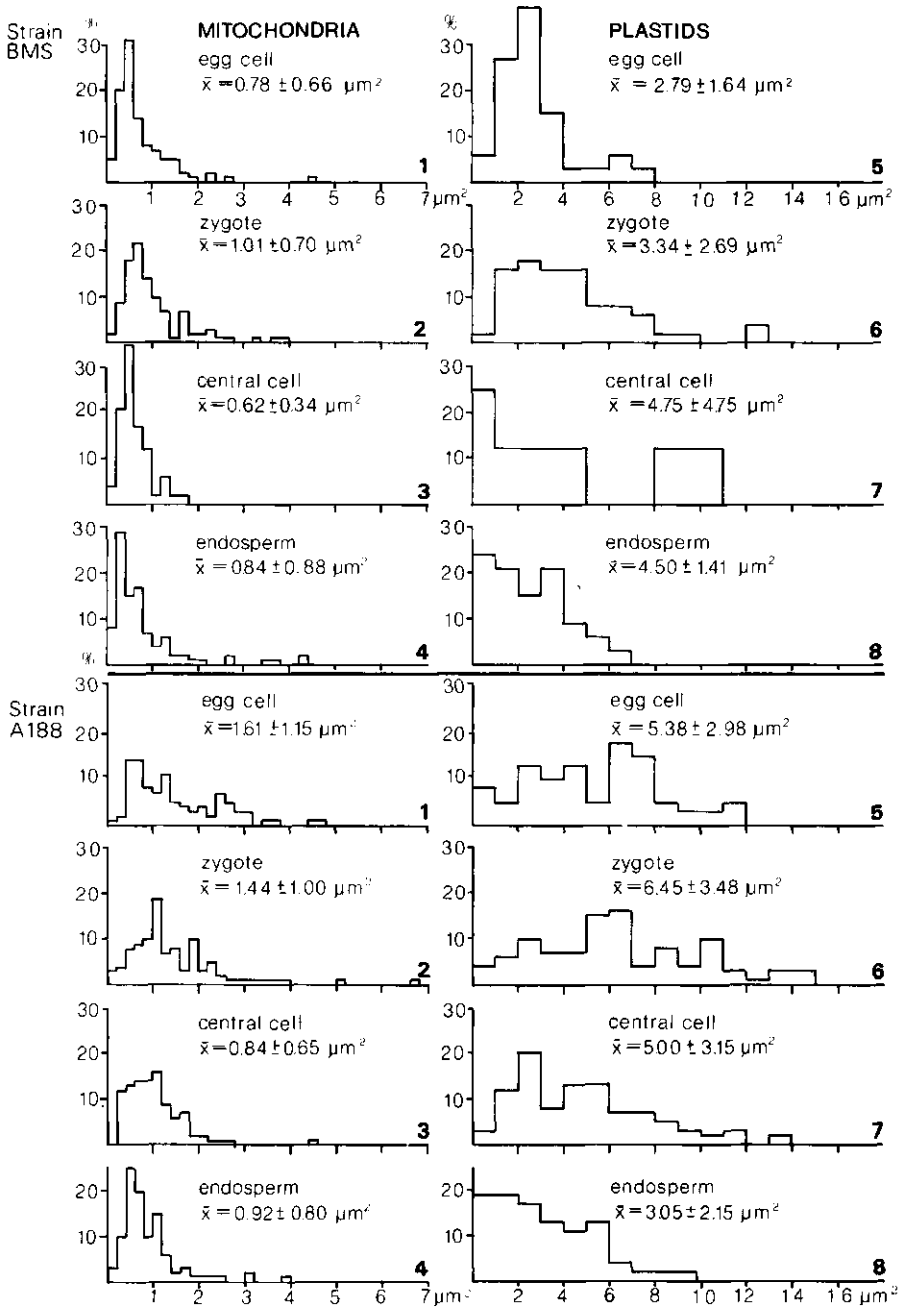

DIAGRAM 2. Size distributions, calculated average sizes and standard deviations of cut areas of mitochondria and plastids in the egg cell–zygote and central cell–endosperm of *Zea mays*, strain BMS and A188. Each histogram is based on measurements in five different areas of cytoplasm.
When organelle surfaces are measured on thin sections one should realize that the organelles can be cut at their maximal diameter and that they can be grazed. Therefore the average sizes do not present real organelle sizes and they coincide with high standard deviations.

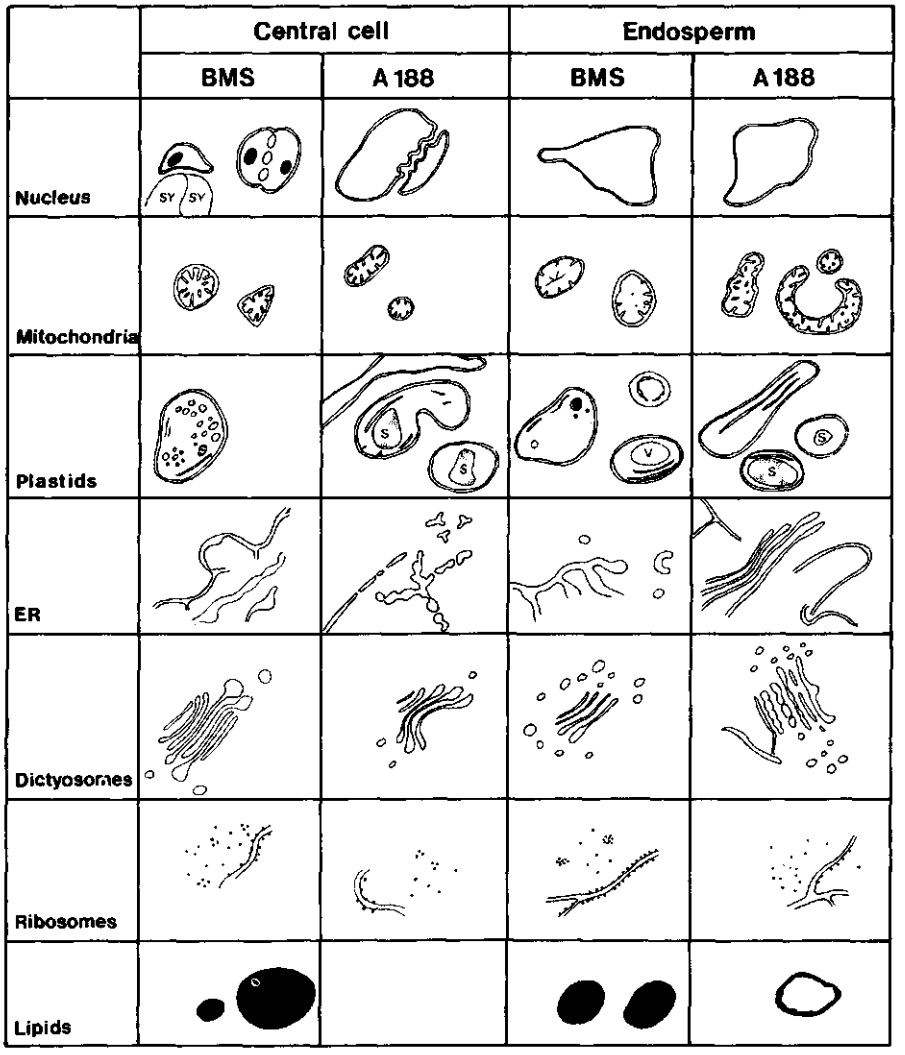

DIAGRAM 3. Schematic representation of organelles in the central cell and endosperm of *Zea mays*, strain BMS and A188.

during nucellus penetration the chemoattraction might disappear and no more pollen tubes will enter the ovule.

When the pollen tube discharges its contents into a synergid, that synergid can be degenerated to some extent as has been observed in various monocot species such as *Sorghum* (VAZART, 1955), *Panicum* (NARANAYANASWAMI, 1955) and *Hordeum* (CASS and JENSEN, 1970). Synergid degeneration has, however, not been observed with *Oryza* (CHO, 1956) and *Triticum* (BHATNAGAR and

CHANDRA, 1975) in which the pollen tube enters the embryo sac between one synergid and the egg cell. With *Hordeum* the degeneration of a synergid starts after pollination but before fertilization (CASS and JENSEN, 1970). In that case the degeneration of the synergid is thought to be necessary for the entry of the pollen tube through that synergid into the embryo sac. The nondegenerated synergid could have a function in nourishing the zygote and the endosperm as was suggested by JENSEN (1964) for *Gossypium*. MAZE and LIN (1975) also suppose that each of the two synergids has specific functions. The synergid which will be penetrated controls the pollen tube growth or the transfer of pollen tube growth directing substances out of that synergid while the persistent synergid and its filiform apparatus function in the transfer of material into the megagametophyte. Synergids might indeed be considered as transfer cells in the sense described by PATE and GUNNING (1972) because the synthesis of the filiform apparatus implies an increase of the plasma membrane area. WILMS (1981) suggested that enzymes are secreted by the synergids because middle lamellae of nucellus cells start to dissolute near the filiform apparatus when the ovule becomes receptive, a phenomenon which was observed in the present study as well. In *Plumbago* there are no synergids in the embryo sac. Here, the egg cell develops filiform cell wall protrusions which are likely to function in pollen tube attraction (RUSSELL, 1982). In relation to pollen tube attraction BROOKS (1963) discusses the function of electrical potentials in physiologically polarized activities. As these potentials require ionic gradients the filiform apparatus might be the source of available carbohydrates for conversion to acids. These acids could function in pollen tube attraction and in fertilization and they could also be involved in the development of the polarity of the embryo.

When the pollen tube grows in the pistel of maize it penetrates only intercellularly until the degenerated synergid is reached and when that synergid is considered not to be a living cell, the pollen tube never enters the symplasts of both the sporophyte and the megagametophyte. In maize the latter two never make contact through plasmodesmata. In the developing caryopsis the plasmodesmata disappear during the development of the megaspore mother cell (DIBOLL and LARSON, 1966) and later on during megasporogenesis, when the three nonfunctional megaspores are degenerated (RUSSELL, 1979). Afterwards, the embryo sac, the developing endosperm and the embryo appear to have exclusively apoplasmic communication with the nucellus, the placento–chalazal region and endosperm respectively (SCHEL et al., 1984). All these phenomena stress the importance of the transmembranal transport of organic and anorganic compounds and minerals and thus the dependence of the heterotrophic gametophytes, embryos and endosperm on the sporophyte.

The growth of the pollen tube through the micropyle and the nucellus is different for the two strains of maize being more straight and regular with strain BMS. This phenomenon can be explained by the existence of only one micropylar opening and a more regular arrangement of the cells between the embryo sac and the micropyle in BMS. This arrangement leads to a continuous gradient in attraction from the micropyle straight to the filiform apparatus. When the

pollen tube reaches the embryo sac at the base of the filiform apparatus it penetrates the cell wall or middle lamellae between the two synergids. This cell wall still has some filiform projections in the micropylar region but at the site where the projections are absent the pollen tube penetrates the degenerated cell. Then its growth ceases and it opens directly in a subterminal pore. The choise to penetrate the degenerated synergid is similar to that in other species such as *Torenia* (VAN DER PLUIM, 1964), *Capsella* (SCHULZ and JENSEN, 1968a,b), *Petunia* (VAN WENT, 1970) and *Hordeum* (CASS and JENSEN, 1970) and might be caused by the excretion of attraction substances by this cell. With respect to attraction calcium is assumed to be the chemotropic agent because it appears to be important in the direction of growth of pollen tubes (MASCARENHAS and MACHLIS, 1964; JENSEN, 1965; REISS and HERTH, 1982; PICTON and STEER, 1983).

The attraction stimulates the pollen tube to penetrate the very base of the filiform apparatus. This might be caused by the high concentration of attractants which are accumulated in the filiform structures of the cell wall. At the top of the synergid the cell wall is incomplete or absent and so attraction will diminish when the tube grows on. It should also be realized that the pronounced polar distribution of mitochondria, dictyosomes and plastids in the synergids can influence the pollen tube growth (see DIBOLL, 1968).

When the pollen tube has discharged its cytoplasm into the synergid, its cytoplasm still appears to be intact. Like in *Cucumis* (VAN WENT et al., 1985) the very active mitochondria and dictyosomes and the profiles of ER give evidence of a high metabolic activity. Soon afterwards, the cytoplasm degenerates; osmiophilic droplets appear, organelles disorganize and electron transparant areas arise. It has been suggested that the latter contain neutral and acid polysaccharid components (DIBOLL, 1968). The function of the pollen tube residue is not yet known, but RNA has been demonstrated in it and also proteins rich in lysine, arginin and histidin; the osmiophilic droplets probably represent lipids (DIBOLL, 1968).

Egg cell–zygote

The overall structures of the egg cell cytoplasms of the two strains, BMS and A188, show similarities in many aspects such as the location of the cytoplasm, the distribution of the organelles and vacuoles in the cells, the frequency of occurrence and the occupation rates of most organelles and, the structural changes initiated by the fertilization. The most remarkable indication for fertilization of maize egg cells is the translocation of the cytoplasm and nucleus from the micropylar side of the cell towards the antipodal side. This reversal of cytoplasmic polarity has been reported for *Zea mays* strain BMS (VAN LAMMEREN, 1981) and *Papaver nudicaule* (OLSON and CASS, 1981). In the zygote, the polarity is now expressed by the dominating presence of vacuoles at the micropylar side and a concentration of cytoplasm surrounding the nucleus at the antipodal side as was also observed in *Capsella* (SCHULZ and JENSEN, 1968a,b). The development of that polarity resulting in a two-celled embryo with a small apical and a large basal cell is widely distributed but not obligatory (SIVARAMAKRISHNA,

1978; JOHRI, 1984). In contrast to many dicots the position of the cell plate resulting from the first zygotic mitosis is not well predictable in the Gramineae family (see WARDLAW, 1955; JOHRI, 1984). Both the cell size and the orientation of the new cell wall may variate. The change in the compartmentation of the zygote results in the polarity. Whether this polarity is developed by an intensive formation of vacuoles at the micropylar side of the cell or by an active translocation of the cytoplasm and nucleus towards the antipodal side remains to be elucidated. The translocation always coincides with the sideward movement of the cytoplasm and nucleus of the fertilized central cell. The existence of a correlation of these phenomena has, however, not been stated up to now. Whether microtubules are involved in the translocation has not been observed because they have only been detected in the zygote during mitosis.

The overall structures of the cytoplasms of the egg cell and zygote indicate a high potency of energy supply. The egg cell is in a state of relatively low activity first. After fertilization, however, the synthesis capacity of the zygote increases as can be deduced from the increasing amount of RER and the higher density of polysomes and dictyosomes (see Table I). Indeed, this cell needs the equipment for high activities such as cell division and increased production of cytoplasm. SINGH and MALIK (1976) observed very feeble enzyme activity in ovules of *Zea mays* and strong reactions for several enzymes prior to, during and after fertilization indicating tempory enhancement of metabolic activity. Studies using radioactive nucleic acid and amino acid tracers show that many animal egg cells are also in a state of metabolic inhibition at maturity (MONROY, 1965). The observation that egg cells of maize are in rest and not meristematic corresponds to observations on the barley egg cell (BUTTROSE, 1963) and to several observations on the maize egg cell (VAZART 1958; DIBOLL and LARSON, 1966). In wheat, equal cytological and cytochemical changes like an increased concentration of starch grains around the nucleus, an increased vacuolation, and a decrease of RNA are regarded as manifestations of life activity at a low level (HU, 1964). With maize, however, mitochondria in the egg cell are well differentiated bearing many cristae. This indicates the potential for the high metabolic rates generally associated with post-fertilization activity (RUNNSTRÖM et al., 1959). Their clustered location points to high and local demands for ATP in both the egg cell and the zygote. After fertilization the increase in size of the mitochondria and plastids in strain A188 corresponds to the observations of DIBOLL (1968) and this can indeed be a sign of the increased metabolic activity in the zygote. At the time of the first mitosis of the zygote, however, the decrease in size and the increase in number of mitochondria suggest that the division of these organelles precedes mitosis. Protein synthesis is restricted to ribosomes and ER and since protein synthesis is ATP dependent, the occurrence of many ribosomes adjacent to the mitochondria supports the suggestion that there is either a high potential capacity or a high rate of protein synthesis. Starch containing plastids were detected in both the egg cells and zygotes of BMS and A188. The cytoplasm of BMS and A188 egg cells can, however, be distinguished because the plastids of BMS are smaller and occur in significantly higher fre-

quencies and occupation rates. Moreover, the plastids of the starchy inbred line A188 contain more starch and larger starch grains in the egg cell and zygote than in the sweet corn BMS. After fertilization there are more yet smaller grains in a plastid and the total amount of starch has decreased, which is related to the increased cell metabolism.

Central cell–endosperm

The composition of the cytoplasm of the central cell in BMS is comparable to that in A188. The two strains can be distinguished by the differences in the structure of the central cell plastids. Black Mexican Sweet corn plastids contain many small starch grains whereas A188 plastids only contain one or a few large starch grains each, which might be related to the starchy nature of this inbred line. Microscopical observations revealed the majority of the central cell cytoplasm to lie characteristically on top of the egg apparatus in both BMS and A188. This position favours an efficient nutrition of the egg cell and zygote. It also permits a fast karyogamy with the polar nuclei. The sequence of the fusion of polar and gamete nuclei appears not to be strictly ordered. MILLER (1911) first noticed a gamete fusion with one polar nucleus and then a fusion with the other nucleus as was also shown in barley (LUXOVA, 1967) and oat (BONNET, 1961) whereas RANDOLPH (1936) has reported a simultaneous fusion of the polar nuclei together with the male gamete nucleus.

The abundance of organelles in the cytoplasm of the central cell and the continuity of the ER with the nuclear membrane (c.f. DIBOLL and LARSON, 1966) indicate an intensive metabolism or at least on a high potency of cell activities such as protein synthesis, secretion and ATP production. Fertilization evokes an increase in cellular activity which is equal for both BMS and A188 as can be deduced from the comparable increase in complexity or size of organelles such as polysomes, RER, dictyosomes and mitochondria. Indeed, these young endosperm cells synthesize cytoplasm and induce mitosis frequently in both inbred lines. The enlargement of the mitochondria coincides with a decrease in occupation rates. The former phenomenon points at higher ATP production, the latter is probably caused by the synthesis of cytoplasm. An increase of enzyme activity was demonstrated histochemically in the cytoplasm of the fertilized central cell by SINGH and MALIK (1976). The central cell and the endosperm might be the most important nutrient resources for the egg cell and zygote respectively. The location of the cytoplasm and the extremely long profiles of ER, aligning the egg cell and zygote indicate that.

The young endosperm is supplied by nutrients coming from the antipodals and nucellus cells (SINGH and MALIK, 1976). There is no cytoplasmic contact with the nongametophytic nucellus cells and thus the uptake of all nutrients must proceed through the cell walls and the cell membranes. The wall papillae in the apical pockets might function in that uptake of metabolites in the young endosperm and indeed soon after fertilization the majority of the endosperm cytoplasm is found there. Metabolites coming from the antipodals may also be essential to the early stages of endosperm development in grasses (BRINK

and COOPER, 1944). The papillate cell walls of the antipodals are probably functional in the movement of large amounts of nutritive materials from adjacent tissues. SCHNEPF (1964) demonstrated that cells which emerge secretory products have papillate cell walls, too. The cytoplasm of the antipodals exhibits a characteristic increase in organelle number and apparent activity after fertilization as was demonstrated both histochemically (SINGH and MALIK, 1976) and structurally (DIBOLL and LARSON, 1968).

In all, it appears that the cytological development in the cells of the megagametophytes show many similarities in the two varieties of *Zea mays*, both before and after fertilization. Principally some remarkable differences in the structure of the ovule and the position of the embryo sac characterize the two inbred lines. Up to now the functions of all the cell types of the megagametophyte can not be determined unambiguously. The subcellular organization, however, clearly indicates the most probable functions. Synergids and micropylar nucellus cells function in pollen tube attraction. The central cell and endosperm nourish the egg cell and young embryo respectively. The antipodals function in the synthesis and secretion of organic compounds made from precursors coming from the nucellus. Before fertilization the cells of the megagametophyte exhibit signs of moderate metabolic activity. This explains the various stages of degeneration of one synergid at the moment of fertilization. After fertilization the metabolic activity increases.

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Abbreviations in the figures

A = antipodals; CA = carpel; CC = central cell; CH = chromosome; CU = cuticle; CW = cell wall; CY = cytoplasm; DSY = degenerated synergid; D = dictyosome; EC = egg cell; EN = endosperm; ER = endoplasmic reticulum; ES = embryo sac; FA = filiform apparatus; II = inner integument; IS = intercellular space; L = lipid; M = mitochondrion; MP = micropyle; N = nucleus; NUC = nucellus; OI = outer integument; P = plastid; PD = plasmodesma; PN = polar nucleus; PSY = persistent synergid; PS = polysomes; PT = pollen tube; RER = rough endoplasmic reticulum; S = starch; SH = stigmatic hair; SI = silk; SY = synergid; V = vacuole; VE = vesicles; WP = wall protrusions; Z = zygote.

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