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DEVELOPMENTAL MORPHOLOGY AND CYTOLOGY OF THE YOUNG MAIZE EMBRYO (ZEA MAYS L.)

A. A. M. VAN LAMMEREN

Department of Plant Cytology and Morphology, Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands

SUMMARY

Young embryos of maize were analyzed with light and electron microscopical techniques to determine the sequence of morphological, anatomical and cytological events resulting in the onset and initial development of the embryo axis and the scutellum.

The proembryo stage ends at 5 days after pollination (DAP). The radial symmetry of the proembryo changes into a bilateral symmetry, probably caused by the excentric position of the embryo apex within the endosperm. Scutellum formation starts at the apex and posterior side of the embryo proper and is characterized cytologically by different types of cell growth and multiplication in epidermis and mesophyl. From anatomical observations it is concluded that the coleoptile arises as a protuberance of the scutellum.

Shoot meristem formation initiates in the protoderm at the anterior side of the embryo and is characterized cytologically by decreasing cell sizes, by vacuolation and by an increase of cytoplasm. The lateral orientation of the shoot meristem is likely caused by the failure of a second cotyledon to develop.

Root meristem formation is somewhat retarded. Only vacuolized cells at the base of the embryo proper were detected at 5 DAP but at 7 to 8 DAP the root meristem was identified as a group of dividing cells with few vacuoles and much cytoplasm. The meristem is not located at the exterior of the embryo but at the base of the embryo proper, in direct line with the suspensor. The coleorhiza is formed adjacent to the seminal root. Its exogenous character was not established.

The new axis from root to shoot meristem deviates from the axis of the proembryo as a result of the lateral position of the shoot meristem. Lateral outgrowth of the shoot is, however, restricted by mechanical forces from outside and growth of tissues inside the embryo.

In the determination of the location of the meristems next to morphological, also physiological and genetical factors are important. Metabolic gradients and physiological sinks are established already before the zygote divides and expression of polarity is present in all cormophyta.

1. INTRODUCTION

The sexual reproduction of maize has been the object of extensive genetical, physiological and structural research (SPRAGUE 1977, SHERIDAN 1982 and JOHRI 1984). Embryogenesis, which is a part of this process, starts with fertilization and includes both proembryo formation as well as development of the embryo proper. Questions related to developmental morphology made it necessary to visualize differentiation of the embryo with the aid of optical techniques. Many early workers studied fertilization and embryogenesis in maize by light microscopy (LM) (e.g. MILLER 1919; WEATHERWAX 1919, RANDOLPH 1936 and KIESSELBACH 1949). More recently, electron microscopical (EM) techniques have been applied by DIBOLL (1964, 1968), CHEBOTARU (1970), VAN LAMMEREN (1981, 1986) and SCHEL et al. (1984). Recent work on the embryo region of other grass

caryopses has been that of NORSTOG (1972) on barley and of SMART & O'BRIEN (1983) on wheat.

Morphological data on the embryogenesis of maize were reported by AVERY (1930), who compared maize with several other monocots, and by RANDOLPH (1936), who gives a detailed account of the developmental history of the caryopsis (see also SASS 1977). The first phase of maize embryo development is characterized by the formation of a proembryo in which the epidermis and the apical meristems of the root and the shoot are not yet differentiated.

Cytological studies of proembryo formation in maize have been reported recently (VAN LAMMEREN 1981, VAN LAMMEREN & SCHEL 1983). During the second phase of embryo development a scutellum and two apical meristems differentiate. Examinations on this phase, particularly those concerned with the organization of the system of primary meristems (protoderm, procambium and ground meristem) and of the apical meristem are rather limited in number in monocots and dicots (ARNOTT 1962, BUELL 1952, MILLER & WETMORE 1945, REEVE 1948, SHAH 1982, 1983 and VAN LAMMEREN & KIEFT 1983). In particular, the cytological aspects of primary meristem formation and scutellum development in maize, have only been investigated incidentally. However, the subsequent development of the maize shoot has not only been studied in embryos (AVERY 1930, RANDOLPH 1936, ABBE & STEIN 1954, CLOWES 1978b) but in seedlings as well (AVERY, 1930, ABBE et al. 1951. LEDIN 1954, CLOWES 1978b). ABBE et al. (1951) reported on the cytological aspects of cell proliferation in the shoot meristem and CLOWES (1978b) determined mitotic frequencies in the region of the embryo axis. The root growth in maize seedlings was investigated by, amongst others, CLOWES & JUNIPER (1964) and CLOWES (1978a) but little attention was paid to the initial development of the seminal root in the developing embryo. Moreover, a report on meristem initiation in which fine structure is combined with LM observations lacks, although it is felt to be of great value to compare the histogenesis in somatic embryos with the in vivo development (VASIL et al. 1985). The cytology of meristem formation in vivo was only scarcely studied in detail with EM techniques (VAN LAMMEREN & KIEFT 1983) and the following questions can still be posed. What determines the location of meristem formation? How do the developmental patterns of cells in a meristem proceed?

Therefore the present report focusses upon the morphological and fine structural changes during the onset of primary meristem formation to analyse the sequence of events taking place during this early phase of embryogenesis. The maize inbred line A188 is used because of its favourable response in experimental conditions for embryogenesis in vitro (GREEN & PHILLIPS 1975). Especially the cytology of the formation of the root and shoot meristems and the resulting embryo axis are analysed using light and electron microscopical techniques.

2. MATERIALS AND METHODS

Maize plants of strain A188 (kindly provided by Dr. C. E. Green, University

of Minnesota, St. Paul, USA) were grown under greenhouse conditions i.e. 16 hrs in the light at 23 °C and 8 hrs in the dark at 18 °C in a relative humidity of 70–90%. Sampling times varied from 5 up to 15 days after controlled pollination (DAP). For the cytological studies, ovaries were dissected and from each a thick sagittal section containing the median part of the embryo was fixed for 2 hrs in a solution of 5% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.0) containing 0.1% $CaCl_2$. The sections were rinsed in the buffer and post-fixed with 1% OsO_4 in buffer for 2 hrs. Alternatively, they were fixed with a saturated, aqueous solution of $KMnO_4$ for 5 to 10 min., all at room temperature. Preparations were rinsed thoroughly in water and dehydrated in a graded series of ethanol. They were transferred to propylene oxid and embedded in Epon. Semi-thin and thin sagittal sections of the median part of the embryo were made using a LKB ultramicrotome equiped with glass and diamond knives respectively. Semithiñ sections (2 µm) were used for LM photography and 'camera lucida' drawings. Cell sizes were quantified with a Kontron MOP-30 by measuring cell areas on photos of semi-thin section. Thin sections were optionally poststained with uranyl acetate and lead citrate and observed with a Philips 301 EM at 60 kV.

For scanning electron microscopy (SEM), intact embryos were dissected from the caryopses and treated with various fixatives. The adapted method which was eventually used for embryo fixation, is given in the results. After dehydration in a graded series of ethanol, the ethanol was substituted by amylacetate and the embryos were critical point dried in a "Balzers Union" equipment, coated with gold in a "Polaron E 5100" sputter coater and examined in a JEOL ISM-35C scanning electron microscope.

3. RESULTS

3.1. Preparation of maize embryos for SEM

In order to study the embryo development by SEM, procedures for specimen preparation were evaluated (see also VAN LAMMEREN & MARTI 1983). Observa-

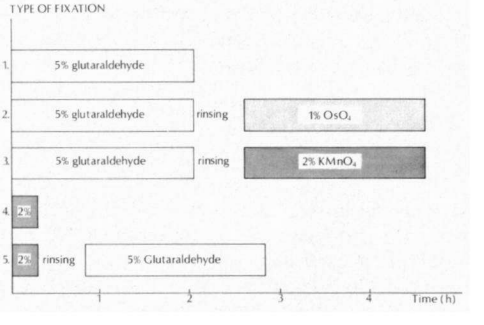

Fig. 1. Survey of 5 fixation procedures for scanning electron microscopical observation of immature embryos of *Zea mays* L. For additional information see Materials and Methods.

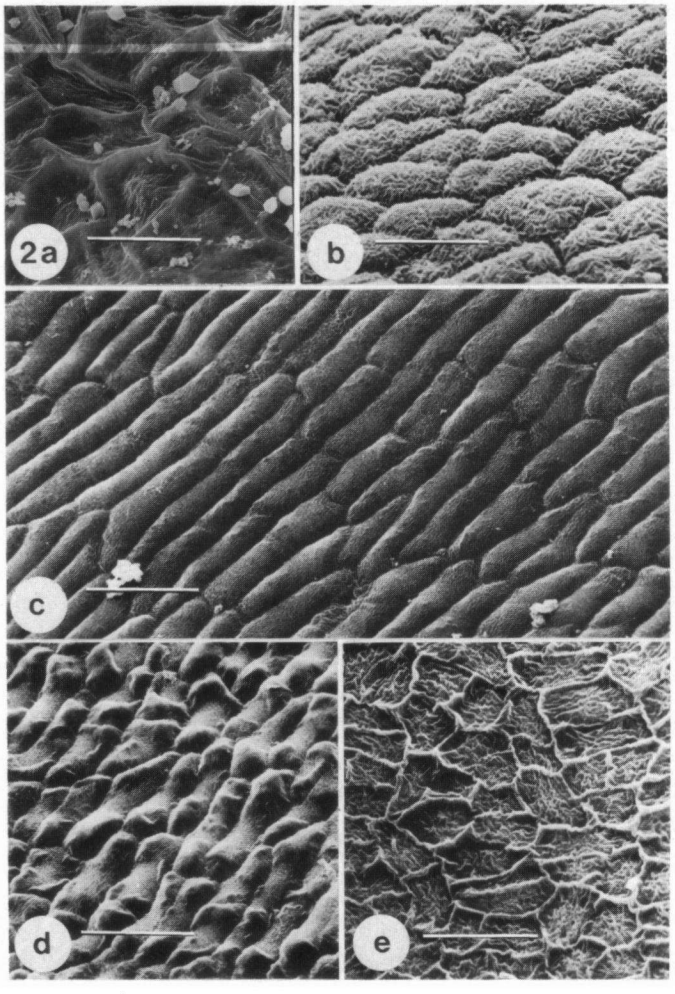

Fig. 2. Epidermal cells of maize embryos seen after the application of 5 different types of fixation. The micrographes a, b, c, d and e show the results of fixation types 1, 2, 3, 4 and 5 respectively (for fixation types 1–5, see *fig. 1*). Note the good quality of surface preservation with fixation type 3 (glutaraldehyde-$KMnO_4$). The bars represent 10 μm.

Abbreviations in the figures: C = coleoptile, CA = calyptra, CG = calyptrogen, CPD = critical point drying, cw = cell wall, cy = cytoplasm, D = dictyosome, DAP = days after pollination, EA = embryo axis, ER = endoplasmic reticulum, LP = leaf primordium (1, 2, 3), M = mitochondrion, N = nucleus, P = plastid, PB = peribleme, PD = plasmodesma, PL = plerome, PRD = protoderm, PS = polysome, RER = rough endoplasmic reticulum, RM = root meristem, S = starch, SC = scutellum, SM = shoot meristem, SN = scutellar node, SU = suspensor, V = vacuole, VE = vesicle.

tions on fresh material with SEM coincided with rapid distortion of the tissue and contamination of the electron microscope. The initial shape, however, appeared to be quite natural. Special attention was then paid to the effect of fixation on the shape of the epidermal cells. The various fixation procedures which were tested are shown in *fig. 1*. *Fig. 2* represents epidermal cells of maize embryos seen after various types of fixation. Fixation type 3 with glutaraldehyde and $KMnO_4$ gave best results with respect to the preservation of the cell shape (*fig. 2c*). Artificial shrinkage of the embryos was, however, caused by processing and was quantified (*fig. 3*). With fixation type 3 the critical point drying and especially the duration of the decompression phase appeared to influence the size of the embryos markedly. When a slow decompression was applied, shrinkage could be minimized, but not prevented. Best results were obtained with glutaraldehyde-$KMnO_4$ fixation and therefore this procedure was selected for the present study.

3.2. Morphology of the developing embryo proper

The morphological aspects of the transition of the proembryo to the embryo proper are shown in *fig. 4*. Proembryo formation took about five days in which a club-shaped embryo with a long cylindrical suspensor and a hemispherical apex reached a length of about 270 μm. The apex of the proembryo had a diameter of 100 μm. From about 5 DAP onward it gave rise to the embryo proper the side of which directed towards the ovary wall is called the anterior side.

The initial growth at the apex of the proembryo results in a trowel-shaped cotyledon, called the scutellum, on the epidermis of which the cell pattern indicates the direction of cell elongation (*figs. 4b* arrow and *5a1* arrow). Already at 6 DAP one can distinguish the region of the shoot meristem which is initiated

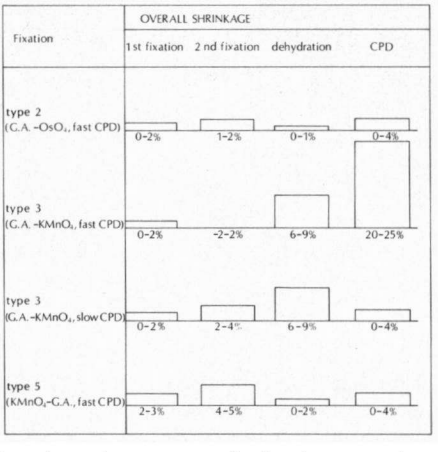

Fig. 3. Shrinkage of whole maize embryos as a result of various procedures of fixation, dehydration and critical point drying (CPD). For the types of fixation, see *fig. 1*. Fast CPD includes a gradual decompression from 77 to 1 bar in 12 min. Slow CPD includes a gradual pressure decrease from 77 to 65 bar in 30 min. followed by a decompression to 1 bar in 7 min.

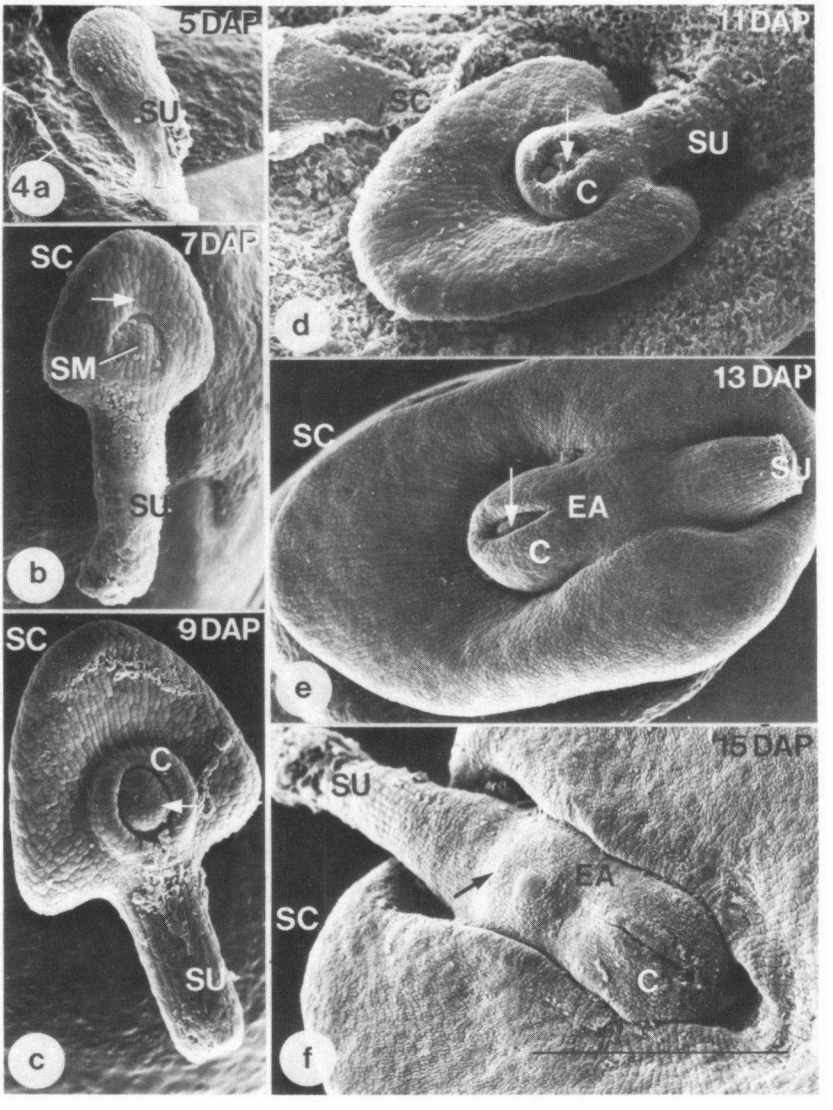

Fig. 4. Morphology of the developing maize embryos from 5 up to 15 days after pollination (DAP). The adaxial side of the scutellum is faced in every picture. The development of the proembryo (a) to a well differentiated embryo (f) is marked by the initiation (b) and rapid enlargement of the scutellum and the concommitant exogenous formation of the shoot meristem. Note the differentiation of the coleoptile from 7 DAP and the formation of the first leaf primordium from 9 DAP (c, d and e, white arrows). Details of b, c and e are given in *figs. 5b1*, *5c1* and *6b* respectively. The bar represents 100 μm for all pictures.

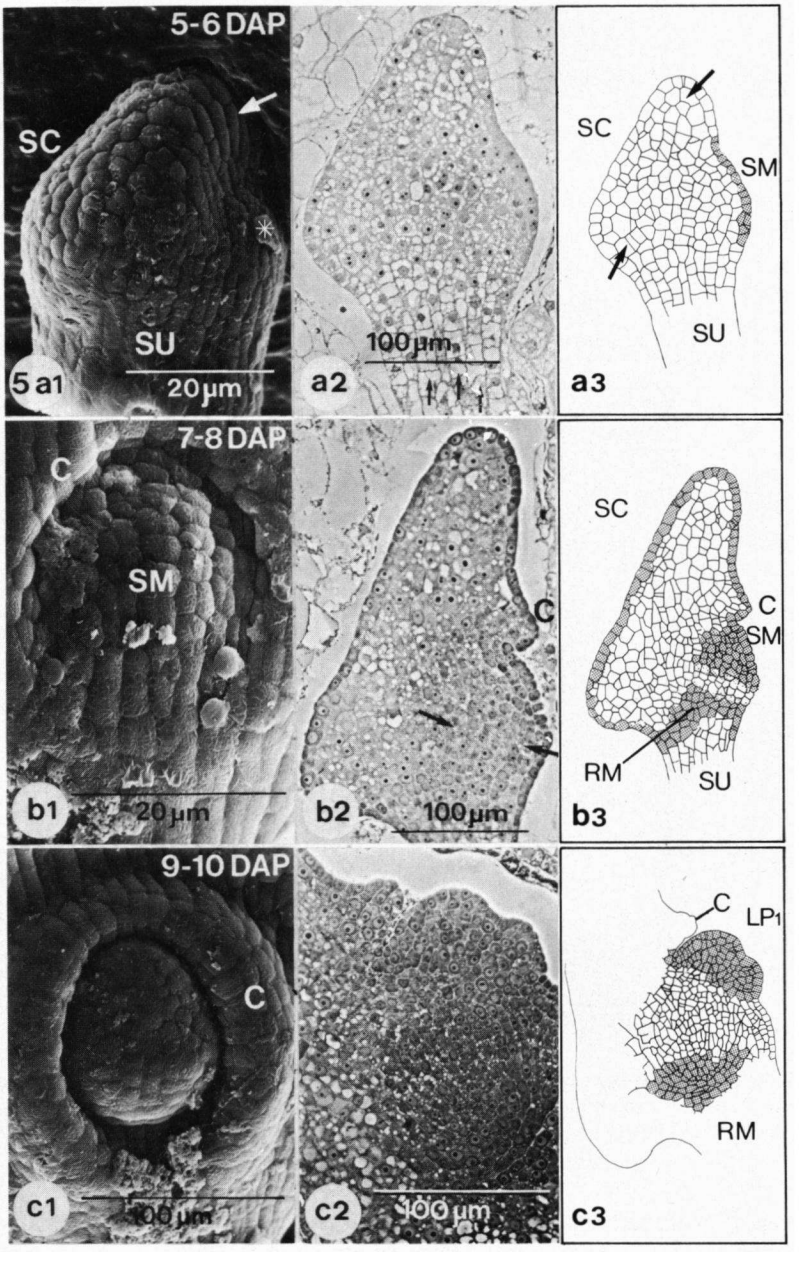

Fig. 5. The developing embryo proper of *Zea mays* L. from 5 to 10 days after pollination. The SEM pictures show the appearance of the shoot meristem. In the longitudinal sections the anterior side of the scutellum is directed to the right. Note the development of the shoot meristem and coleoptile in b and the appearance of the root meristem in c. The stippled areas in the drawings accentuate the regions where cells have a meristematic appearance.

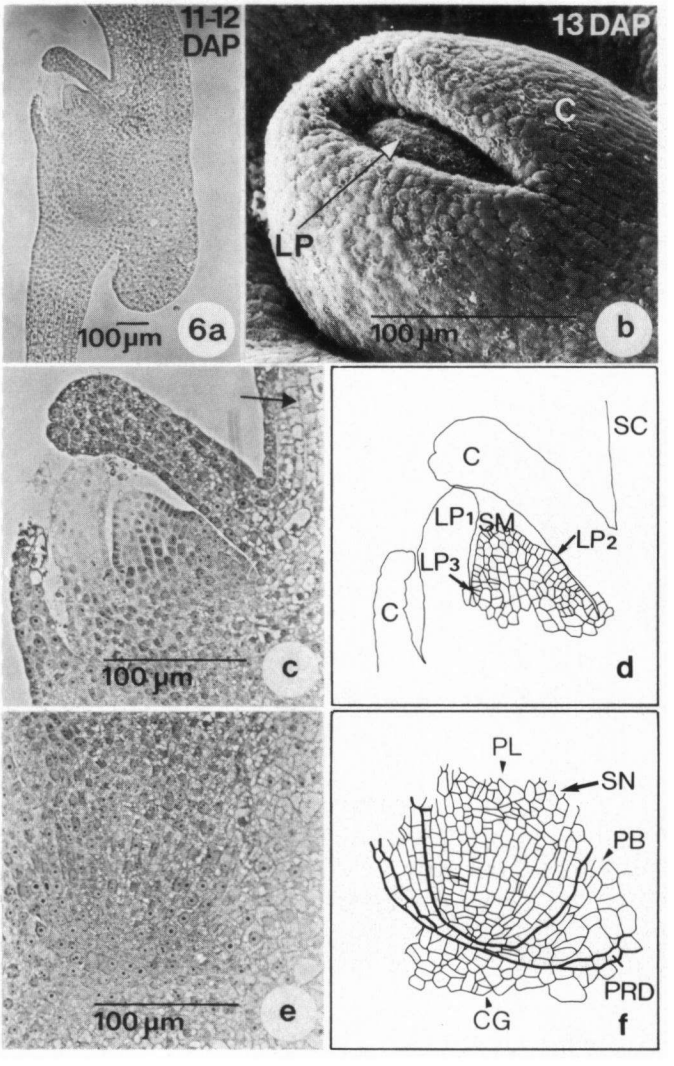

Fig. 6. Longitudinal sections and drawings of a maize embryo at 11 to 12 DAP show the anatomical differentiation of the shoot and the root meristems. The SEM micrograph presents the face view of the shoot of a 13 DAP embryo which is, however, in the same stage of development.

as a small protuberance consisting of relatively small cells at the anterior side near the apex of the embryo (*figs. 4b* and *5a1*, asterisk). Enlargment and elongation of the scutellum continues and the promeristem of the shoot is clearly demarcated by a furrow at 7 DAP (*figs. 4b* and *5b1*). Because of that furrow the shoot meristem will get a hemispherical shape as is shown in *fig. 9a*. From 9 up to 12 DAP the scutellum still enlarges and the coleoptile which is generated

as a ridge of tissue surrounding the shoot meristem envelopes the whole meristem eventually (see *figs. 4 c–e* and *fig. 6b*). Starting at 13 DAP and clearly visible at 15 DAP the lateral sides of the scutellum enlarge and partly overgrow and enclose the embryo axis (*fig. 4e, f*).

At about 9 DAP the shoot meristem itself initiates the development of the first leaf primordium at circa 40 μm from its tip (*fig. 4c*, arrow). In the following period of development the first leaf overgrows the apex of the shoot meristem (*fig. 4d, e*, arrows). The leaf itself is then enclosed by the differentiated coleoptile.

The root meristem is not located at the exterior of the embryo and hence it can not be detected morphologically. The embryo axis, consisting of the two meristems and the tissue in between, elongates and can be discerned from about 13 DAP. The root meristem lies in direct line with the suspensor and it can be noted as a small swelling at the anterior side as indicated in *fig. 4f*, arrow.

3.3. Cytology of the developing embryo proper

The transition of the proembryo to the embryo proper is shown in *fig. 5* and *6* and *table 1* with respect to overall shape and size of the embryo and to location, size and contents of the composing cells. The development of the scutellum, its coleoptile and the embryo axis will be treated hereafter.

Elongation of the suspensor is the dominating feature of proembryo formation. Sub-apical cells divide once or more and elongate, creating regular rows of cells in which new cell walls are perpendicularly arranged to the long axis of the suspensor. However, at a length of about 500 μm the proembryo changes shape. The growth of the suspensor decreases considerably and only some cells in the upper part of the suspensor still divide once or more. Then, the spherical apex of the club-shaped proembryo transforms into the embryo proper by the formation of the trowel-shaped scutellum primordium and the promeristem of the shoot of an embryo at 6–7 DAP.

3.3.1. Development of scutellum and coleoptile

Scutellum formation is initiated at the abaxial side of the hemispherical apex

Table 1. Cell sizes in scutellum and apical meristems of maize embryos at various stages of development. The cell areas are expressed in μm^2. The standard deviations are high because many cells are not sectioned at their maximal width and length. Number of counted cells is 10–25 for each value. (1) anterior side of scutellum, (2) posterior side of scutellum, (3) peribleme, (4) plerome.

Stage (DAP)	Scutellum apex		Shoot Meristem		Root Meristem
	Epdermis	Mesophyl	Protoderm	Inner cells	
5– 6	175 ± 77	253 ± 61	81 ± 15	149 ± 25	156 ± 56
7– 8	97 ± 36	139 ± 63	57 ± 19	56 ± 25	82 ± 27
9–10	67 ± 40 (1) 147 ± 55 (2)	380 ± 146	41 ± 16	52 ± 25	62 ± 27
11–12	69 ± 7 (1) 155 ± 62 (2)	342 ± 80	38 ± 11	60 ± 23	111 ± 32 (3) 94 ± 49 (4)

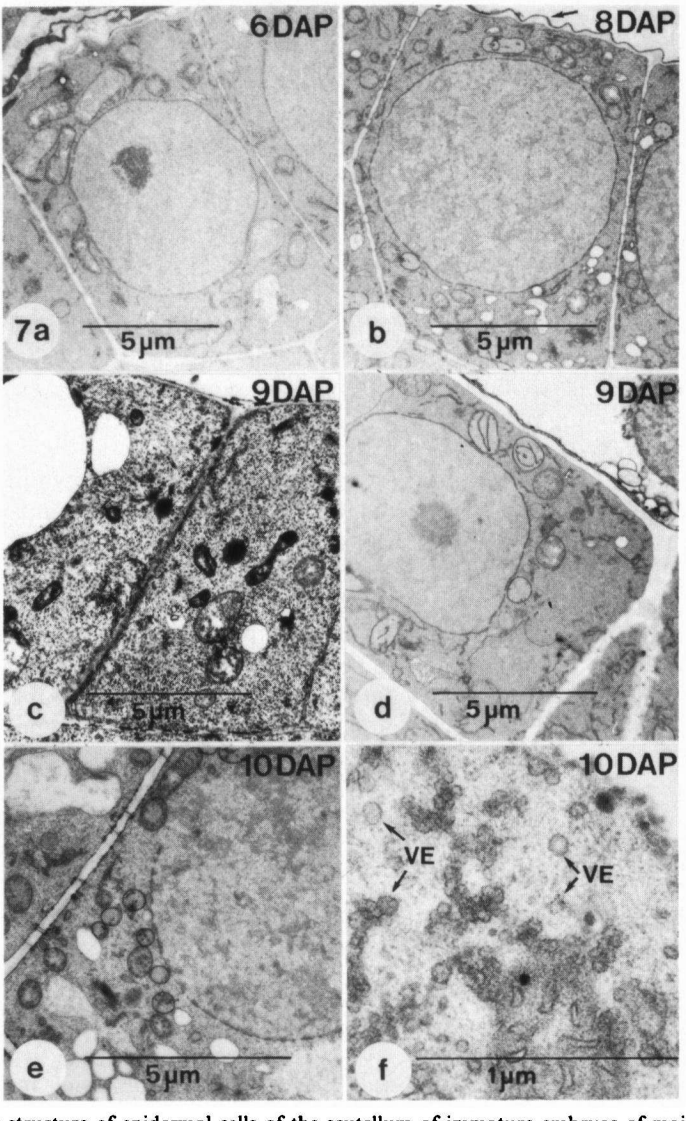

Fig. 7. Fine structure of epidermal cells of the scutellum of immature embryos of maize at 6, 8, 9 and 10 days after pollination. Spherical nuclei occupy a large part of the cell volume. Fixation with $KMnO_4$ reveals the existence of various membranous organelles (a, b, d, e, f) whereas numerous ribosomes are detected after OsO_4 fixation (c). a, b, c, e are cells at the anterior side, d up to f are cells at the posterior side.

of the proembryo. Already at 6 DAP three zones were distinguished anatomically in the young scutellum; the adaxial protoderm, the mesophyl and the abaxial protoderm. The protoderm is the primary meristematic tissue that give rise to the epidermis. The *figs. 5a2* and *5a3* show the scutellum primordium in a

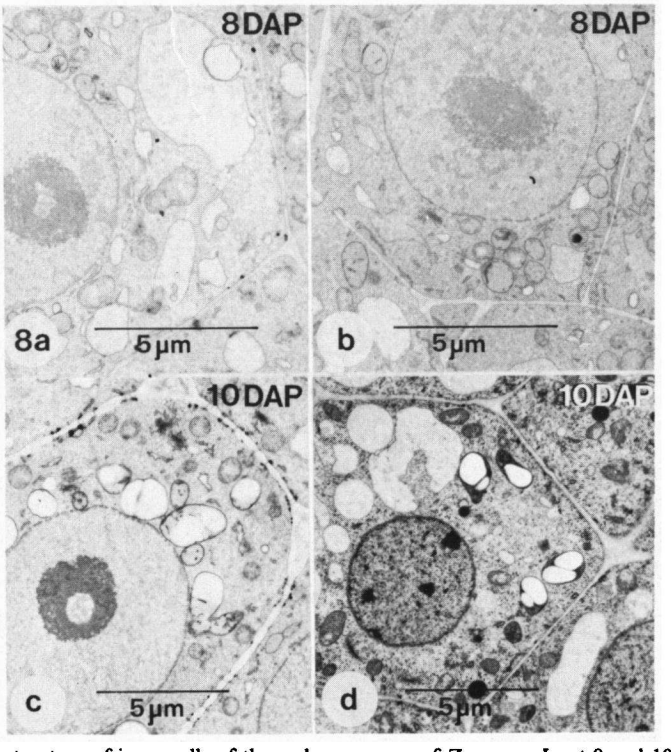

Fig. 8. Fine structure of inner cells of the embryo proper of *Zea mays* L. at 8 and 10 days after pollination after glutaraldehyde-$KMnO_4$ (a, b, c) or glutaraldehyde-OsO_4 (d) fixation. Cells a, c and d are located in between the root and shoot meristem. Cell b takes part of the root meristem. Note the presence of amyloplasts at 10 DAP.

median section at this stage. The stippled area accentuates the region where cells have a meristematic appearance. In the protoderm anticlinal planes of cell division were detected preferentially at this stage. As compared with the region of the shoot meristem, the initial enlargement of the scutellum apex is caused by cell stretching rather than by an increase of cell number (see *fig. 5a3* arrows). The subsequent elongation of the scutellum, which can clearly be seen at 8 DAP (*fig. 5b*), is, however, mainly caused by growth and multiplication of cells in the upper part of the scutellum. The new cell walls are often perpendicular to the long axis of the scutellum and during further development the processes of cell division and cell elongation resulted in regular rows of cells in the epidermis and mesophyl (see *fig. 6c* arrow).

Epidermal and mesophyl cells appeared to differ in structure from the onset of development. Some epidermal cells of the developing embryo are shown in *fig. 7*. From 6 DAP onward the greater part of the cell volume of these relatively small cells is occupied by a large nucleus and the cytoplasm. The nucleus has a central position and the other organelles are spread over the cytoplasm. Mito-

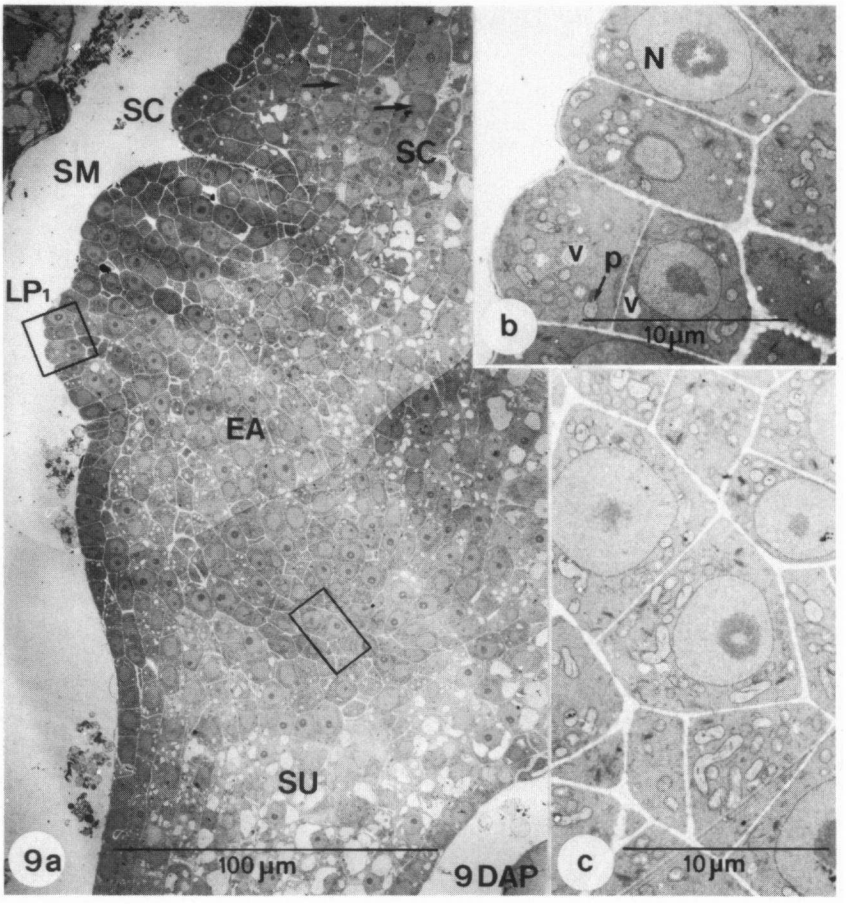

Fig. 9. Meristem formation in the embryo proper of *Zea mays* L. at 9 DAP. The root meristem develops endogenous at the base of the embryo proper in direct line with the suspensor. The shoot meristem develops at the outside with an angle of about 40 degrees to the long axis of the embryo proper. Note the high degree of vacuolation in suspensor, scutellum and embryo axis. The meristematic cells of the leaf primordium (inset b) have less vacuoles and the cells of the developing root meristem (inset c) contain few vacuoles, too. Note the periclinal division in the protoderm.

chondria and plastids have comparable sizes and can be spherical or elongated. Starch was not found in these cells. Few vacuoles and some strands of RER are scattered throughout the cytoplasm but polysomes occurred frequently as shown in OsO_4-fixated material (*fig. 7c*). Only few cytological changes were observed in the subsequent days. A cuticle was already formed at 8 DAP (*fig. 7b*, arrow) and at 10 DAP, epidermal cells slightly increased the number of dictyosome vesicles (*fig. 7f*). Most mesophyl cells are larger than the epidermal cells (*table 1*). They tend to vacuolize in the apical and abaxial zones of the scutellum (see *fig. 5b2*) and they form regular rows in the elongating part of the scutellum as indicated by arrows in *fig. 9a*.

Near the shoot meristem and in direct line with the suspensor, cells are relatively small and contain only few vacuoles. These cells will take part in the formation of the embryo axis (*figs. 5b2* and *9a*) and do not belong to the scutellum.

Coleoptile formation started at 7 DAP in the epidermal and subepidermal scutellum cells which border the shoot meristem (*fig. 5b*). The epidermal cells divided anticlinally and subepidermal cells divided in a restricted zone and formed new cell walls perpendicular to the direction of cell growth. At 9 DAP the division and enlargement in distinct directions gave rise to a ridge of tissue, the coleoptile primordium (*figs. 5c* and *9a*). The coleoptile developed fast and at 12 DAP it was an elongated, leafy structure that partly enveloped the shoot meristem and the developing primary leaves (*fig. 6*). Its epidermis was still connected with the scutellum epidermis and its mesophyl, which was 3 to 4 cell layers thick, was linked up with the subepidermal scutellum cells.

3.3.2. Development of the embryo axis

When the trowel-shaped scutellum primorium is formed at 5 to 6 DAP there is no random distribution of cell divisions in the embryo proper. Most mitoses were found in and near the protoderm at the anterior side at about 100 μm from the apex as is indicated with the stippled area in *fig. 5a3*. This appears to be the location where the shoot meristem will differentiate. A typical cell of that region is depicted in *fig. 7a*. Its ultrastructure points to an organization which is common for a meristem. The epidermal cells had large nuclei, small proplastids which never accumulated starch, only few vacuoles and thin cell walls. The subepidermal cells in that region were larger (*table 1*), contained many more vacuoles and were about isodiametric (*fig. 5a2*).

At 7 DAP the location of the shoot meristem was marked by the indentation and the coleoptile formation (*fig. 5b*). The epidermal cells of the meristem region still had the meristematic appearance like in the protoderm of the proembryo. In the proembryo, however, the outer cells of the apical region divided periclinally as well as anticlinally but as soon as the coleoptile primordium was formed, the epidermal cells of the meristem gave rise to a cell layer in which periclinal divisions were not encountered anymore. Subepidermal cells dedifferentiated from 6–8 DAP. The amount of cytoplasm increased, cells divided and formed smaller cells (*table 1*) which took part in the formation of the shoot meristem.

In direct line with the suspensor a group of cells at the base of the embryo proper dedifferentiated, too (*fig. 5b*). These cells are the forerunners of the root meristem. Initially they were large (*table 1*), isodiametric and contained many vacuoles. Thereafter the vacuoles disappear and the cells divide once or more. In the cells close to the suspensor, the orientation of the new cell walls is mainly parallel to the axis of that suspensor. From these relatively large cells the calyptra develops. Cells which are adjacent to them at the side of the embryo proper are smaller and will give rise to the other constituents of the root meristem (*fig. 5c*). A typical cell of the root meristem at 8 DAP is shown in *fig. 8b*. The regions of the shoot meristem and the root meristem are separated by a zone of cells which have a higher degree of vacuolation (*figs. 5b2*, arrows and *8a*) as compared

to the cells of the meristems. This zone will form the scutellar node of the embryo axis (see *fig. 6f*).

At 9 DAP the shoot meristem was hemispherical and it consisted of many small epidermal and subepidermal cells (*table 1*) which still had large nuclei, much cytoplasm but hardly any vacuoles. The first leaf primoridum is formed at its anterior side (see *figs. 5c* and *9*). The ultrastructure of some epidermal cells of the primordium is shown in *fig. 9b*. Except for the tendency of increasing vacuolation there is no striking difference in appearance of these cells and the protoderm cells of the shoot meristem. However, periclinal divisions in the outer cells, as depicted in *fig. 9b*, were only detected in leaf primordia and not in the protoderm of the meristem. In the zone between the meristems the vacuolized cells clearly enlarged and elongated by which the distance between the meristems increased. The cells of the middle zone of the embryo axis accumulated starch and contained many ribosomes (*fig. 8c, d*). Moreover, cells which were formed by the root meristem, elongated, too, and thus contributed to the growth of the embryo axis as well (*fig. 9a*). At 9 DAP one can not yet clearly distinguish the various histogenes within the root meristem, because the differentiation is not that far already (see *fig. 9a* and *c*).

The developmental stage of the embryo axis at 12 DAP is shown in *fig. 6*. The shoot meristem is enveloped by the coleoptile. The first leaf primordium (LP_1) had elongated and a second leaf primordium (LP_2) had generated at the opposite side of the meristem. The location of the third leaf primordium (LP_3) is marked in *fig. 6d*. The shape of the shoot meristem has changed from hemispherical to pointed but the ultrastructure of its cells is comparable to that in the previous stages. Now the root meristem is composed of well distinguisable tissues as is indicated in *fig. 6f*. One tier of meristematic cells forms the peribleme. A central group of cells forms the plerome (see *fig. 6f*) and a cluster of cells forms the root cap. Some root formation was observed too. Root formation at 13 DAP is based on an increase of cell size (*table 1*) and cell number.

During the development from 6 up to 13 DAP the orientation of the shoot meristem changed. With reference to the future root meristem or the axis of the suspensor, the shoot meristem was found at an angle of 90° at 5 DAP. Gradually this angle changed to about 105° at 8 DAP and to about 145° at 10 and 11 DAP. The change of angle is caused by several factors. The wall of the caryopsis exerts a mechanical force on the embryo. This restricts the lateral development when the embryo axis widens. The development of the primary leaf (LP_1) restricts the direction of growth of the shoot meristem as well and when the scutellar node develops, the elongation and multiplication of cells at the anterior side of the embryo axis is accelerated as compared to the opposite side.

4. DISCUSSION

In this report the developmental stages of embryos are related to the number of days after pollination (DAP). The real age of the embryos is about one day

less because that is the time the programic phase takes. Comparing embryos of different ages is only valid when the embryo-bearing plants are grown under identical conditions. Physical conditions such as humidity, temperature, light intensity and duration of irradiation strongly influence the rate of embryogenesis. The greenhouse conditions applied in these experiments probably caused the faster embryo development as compared to the descriptions of AVERY (1930), RANDOPH (1936), KIESSELBACH (1949) and ABBE & STEIN 1954) who studied embryogenesis under field conditions.

4.1. Development of bilateral symmetry and of the single cotyledon

During the differentiation of the embryo proper we questioned what determines the changes of radial symmetry to bilateral symmetry and what determines the locations of the apical meristems and the scutellum. In a study of grass embryogenesis SOUÈGES (1924) found evidence that the origin of organs in Poaceae could be assigned to specific tiers in the young embryo. Like RANDOLPH (1936) we were not able to distinguish such specific tiers in the embryo of maize whatever stage we observed.

In a previous report it was noticed that during the elongation of the proembryo there is a curving growth of the proembryo when it protrudes the endosperm at 4 and 5 DAP (VAN LAMMEREN & SCHEL 1983). During this developmental stage the apex of the proembryo nears the endosperm epidermis. Because of its new position in the endosperm the embryo apex is encompassed by endosperm cells that are different among themselves: small epidermal cells are found at the anterior side of the embryo apex and larger inner cells covered the rest of the embryo apex. With reference to the club-shaped proembryo, which itself has a radial symmetry, the endosperm environment has a bilateral symmetry by which that embryo is influenced directly. Exogenous influences like metabolites, minerals, osmotic values or electric potentials (RYCZKOWSKI et al. 1985) might stimulate the embryo to develop a bilateral symmetry which is first expressed by the location of the developing cotyledon that develops into the scutellum.

If a strict bilateral symmetry in the embryo proper exists, the scutellum primordium can only be initiated in the median plane of the embryo which coincides with the median plane of the ovule. Thus a two-ranked leaf sequence can develop. In fact, in many monocots and dicots, e.g. *Triticum, Capsella* and *Ricinus,* the attachment of the cotyledons on the embryo axis lies in the median plane of the ovule as well. The epiblast which is found in several monocots is then interpreted as the second but rudimentary cotyledon (see NEGBI & KOLLER 1962). In maize there is no epiblast and the remaining cotyl, the scutellum, is appressed to the inner endosperm from which it will be supplied with nutrients. In dicots the shoot meristem is located at the apex of the embryo and the two cotyledons differentiate lateral. When the cotyledons elongate, the differentiation and growth in the shoot meristem is greatly retarded. In monocots, scutellum formation precedes meristem activation as well. The single cotyledon in

monocotyledons sometimes appears terminal and the apical meristem lateral. This situation was observed in maize, too.

The relation of the single cotyledon of monocotyledons to the apex of the embryo is discussed by ESAU (1965, 1977) as a matter of controversy (see also BROWN 1960 for references). According to one view the cotyledon is terminal in origin, the shoot apex is lateral, and the whole plant is a sympodium of lateral shoots (SOUÈGES 1954 and GUIGNARD 1975). Other authors consider the terminal positions of the cotyledons to be only apparent: the lateral position of the apical meristem results from its displacement by the cotyledon (HACCIUS 1952 and BAUDE 1956). Evidence from dicotyledons which develop only one cotyledon supports the view that the cotyledon is a lateral structure. It has been suggested that several species of the monocotyledon *Dioscorea* have two cotyledons, one of which has an absorbing function and remains within the endosperm while the other emerges and functions as a leaf (LAWTON & LAWTON 1967). In the embryo of wheat there is still a rudiment of the second cotyl and one finds the shoot meristem in between that epiblast and the scutellum. It might be argued that the longitudinal symmetry, which is found in proembryos of monocots and dicots and in embryos of dicots, is disturbed in monocots because of the absence or rudimentary development of the second cotyledon. So the lateral position of the apex is caused by the unbalance in development of the two cotyledons. This is already expressed during early embryo development in the lateral location of the shoot meristem due to development and enlargement of only one cotyledon. It is remarkable, however unexplained, that the ontogenesis of the ovary, the ovule and the embryo all lead to bilateral symmetry. With respect to the spikelet axis, the adaxial side of the ovary grows faster resulting in a lateral position of the silk. In the ovule the unequal growth results in the semi-anatropous position. Even the megagametophyte sometimes grows towards the abaxial side as well, and in the embryo the lateral position of the meristem and the scutellum enlargement point to the same phenomena.

There has been much controversy about the status of the coleoptile. Some interpret the coleoptile as the first leaf (GUIGNARD 1961), and BROWN (1960) assumes that the coleoptile is new aqcuisition without homologues in other embryos. We regard the coleoptile as a protuberance of the scutellum, the scutellar sheath, rather than that it would be a product of the apical meristem as was suggested by amongst others PANKOW & VON GUTTENBERG (1957).

4.2. Development of the embryo axis

The formation of the shoot meristem starts soon after the development of the protoderm which is generated by periclinal divisions in the upper part of the proembryo. Protoderm formation is often regarded as the termination of the proembryo stage (see ESAU 1977) and at the anterior side of the proembryo it gradually passes into shoot meristem formation. The first cytological features of the shoot meristem formation in epidermal and subepidermal cells are the enlargement of the nucleus, the increase in amount of cytoplasm and dictyosomes, and the decrease of vacuolation. Subsequently the cells divide in deter-

mined directions e.g. the anticlinal divisions in the epidermal cells which form the tunica. CLOWES (1978b) studied the development of the shoot apex of maize by stathmokinesis and DNA labeling to find rates of cell proliferation in the regions of the developing shoot apex. It was found that the tunica which contains about half the cells in the apex, contributes a decreasing fraction of the total cell production as the embryo ages. This finding is in agreement with our observations that meristem formation first means a substantial enlargement of the meristem by an increase in cell number in corpus and tunica. Thereafter the meristem enlarged only slowly, it became pointed and cells with a meristematic appearance were predominantly found at its base where leaf primordia were formed. Between the developing meristems of the root and the shoot of 7 DAP embryos we observed cells which neither belong to the meristems nor to the scutellum. They form a procambial plate which is held to be the first node of the young plant (WARDLAW 1955). Because of their specific cytological differentiation we suggest that they already function in the transport of metabolites in an early phase of embryogenesis.

The development of primary roots in lower vascular plants is initiated exogenously in the protoderm. The radicula is a strictly lateral organ with respect to the main vertical axis but its place and time of development is variable (FOSTER & GIFFORD 1959 and VON GUTTENBERG 1964). In dicotyledons, primary root development is exogenously, too. A dermacalyptrogen, which forms the rhizodermis and the calyptra, develops directly from the protoderm of the proembryo. Most monocotyledons have a real radicula, as well, because it was inititated in the protoderm of the proembryo. They have, however, a secundary dermatogen which is descended from the root peribleme. In the Gramineae the seminal root is not a primary root. The extant root meristem develops endogenously as is regular for adventitious roots. The coleorhiza is often considered to be the rudiment of that primary root (VON GUTTENBERG 1964) but we could not establish the exogenous character of the coleorhiza.

In most seed plants the first root is found at a fixed location in the basal part of the embryo proper, in line with the suspensor. With respect to that fixed root position, VON GUTTENBERG (1964) argues that embryos of seed plants are bipolar without exception. The basipolar position of the root might facilitate germination. The identical position of the adventitious root in the Gramineae embryo strengthens this explanation. MCCALL (1934) stresses that the positional relationships of organs in adult plants and in embryos are essentially similar. The axial polarity from shoot apex to root apex often coincides with the polarity of the proembryo. Thus it is important to understand the polarity development in proembryos. Like in the majority of the archegoniate plants, the apex of the young seed plant embryo faces inwardly towards the gametophytic tissue of the embryo sac and away from the neck of the archegonium. This is called endoscopic polarity (FOSTER & GIFFORD 1959). In the proembryo of maize it is already established before the zygote divides (VAN LAMMEREN 1986) and probably it is maintained by metabolic gradients during proembryo development. According to amongst others WARDLAW (1968) and SCHEL et al. (1984) it may reasona-

bly inferred from visual evidence that nutrients are mainly taken up by the tissues in the basal region of the proembryo and are translocated to the apex. Cells in the apex are specially active, divide repeatedly and so constitute a "physiological sink" to which a general flow of metabolic materials is directed.

The influence of the nutrition flow towards and within the embryo proper can be considered as an important morphogenic factor. As long as the suspensor elongates, it brings the embryo proper in intimate contact with the endosperm. Soon the anterior side of the embryo proper is but covered by a persisting endosperm epidermis whereas the developing scutellum is in contact with the degenerating inner endosperm cells. Hence the uptake of nutrients will be highest at the posterior or scutellar side and nutrients will be transported to the "physiological sinks". During further development the scutellum enlarges fast at the cost of the endosperm at its posterior side. Moreover the uptake by and transport of nutrients in the embryo proper can be deduced from the cytological features observed such as the vacuolation and elongation of the future transport elements in the scutellum and in the embryo axis, and the accumulation of starch and the differentiation of vascular elements in several regions.

The cause of the precise location of root meristem formation on the transition region of the embryo proper and suspensor remains to be elucidated. In most embryos of seed plants the bipolarity of the proembryo remains unchanged in the embryo proper because of the basipolar location of the root meristem. Growth regulators already generated in the shoot apex at the proembryo stage and transported basipetally might evoke the very initiation.

In conclusion it can be stated that there is an ordered sequence of events resulting in the initiation and activation of the meristems and the development of the scutellum. Vacuolation and starch accumulation are early cellular features of differentiation whereas an increase of cytoplasm a decrease of cell size and vacuolation precede meristem formation. The endosperm environment of the embryo can initiate the shift to the bilateral symmetry. The failure of the second cotyledon to develop, causes an apparent change of the orientation of the shoot meristem. The coleoptile and the coleorhiza are not considered to be parts generated by the eventual shoot and root meristems. The former is considered to be a part of the scutellum, the latter probably the rudimentary radicula.

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