ACTA BIOLOGICA CRACOVIENSIA Series Botanica 46: 121–132, 2004

SEED DEVELOPMENT IN *SOLANUM MURICATUM* AITON

JOANNA KOPCIŃSKA[1], BARBARA ŁOTOCKA[1], KATARZYNA KOWALCZYK[2], AND JOLANTA KOBRYŃ[2]

[1]*Department of Botany, Warsaw Agricultural University, ul. Nowoursynowska 159, 02-776 Warsaw, Poland*
[2]*Department of Vegetable and Medicinal Plants, Warsaw Agricultural University, ul. Nowoursynowska 166, 02-787 Warsaw, Poland*

Received September 15, 2003; revision accepted March 8, 2004

In *Solanum muricatum* Aiton the development of cellular endosperm preceded the divisions of the zygote, and 5 days after pollination (dap) it consisted of several cells. The mature endosperm accumulated lipid and protein bodies. The separation and secretion zone on the embryo/endosperm interface was noted at 20 dap. The first division of the zygote occurred at 7 dap. The apical cell of the 2-celled proembryo produced the embryo proper, and the basal one gave rise to the suspensor, the central part of the root cap (columella) and the connecting layer of the embryonic root meristem. Four-celled proembryos were observed with the cells in linear arrangement at 8 dap. The first globular embryos were found at 16 dap, heart-stage embryos at 26 dap, torpedo-shaped embryos at 30 dap, and circinate embryos at 33 dap. After 56 dap no further changes were observed in the anatomical structure of the embryos. The testa of mature seeds was composed of the outer epidermis of the integument and a layer of obliterated parenchyma and endothelium.

Key words: Solanaceae, embryo, endosperm, pepino, seed, testa.

INTRODUCTION

Solanum muricatum Aiton – pepino (or *pepino dulce* to distinguish it from cucumber to which the Spanish name *pepino* is also applied) – is an Andean herbaceous crop (Anderson et al., 1996). It is a cultigen which has more than one origin, most likely one from *Solanum tabanoense* and the other from *S. caripense* (Anderson et al., 1996). This crop received little attention outside of its ancestral home until relatively recently. Because of increasing interest in diversifying market crops, the pepino has become available in many other tropical and temperate regions of the world. Pepino is an alternative greenhouse crop (Prohens et al., 2000) and has a high yield potential, as yields up to 150 kg ha^{-1} have been recorded. Many clones of pepino set parthenocarpic fruit under a wide range of environmental conditions (Ruiz and Nuez, 2000; Prohens and Nuez, 2001). This provides a high yield of good market-quality fruits, but no seed for generative reproduction or breeding, especially when grown in greenhouses.

The reproductive system obviously has important implications in dealing with lack of seed set. Successful seed set requires adequate micro- and macrosporogenesis and successive development of male and female gametophytes, then pollen release, pollination, pollen germination, pollen tube growth, and ovule fertilization. However, no developmental disturbances which could prevent seed set have been observed in the development of male or female gametophytes and pollen germination after artificial pollination (Kopcińska et al., 2002a,b). Since further stages in seed development might be blocked, the present study investigates endosperm, embryo and testa development.

PL ISSN 0001-5296
© Polish Academy of Sciences, Cracow 2004

TABLE 1. Summary of embryo development in pepino (*Solanum muricatum* Aiton)

First stage of formation of embryo segmentation	
Proembryo comprising two cells in two tiers	ac (gives rise to l and l') bc (gives rise to m and ci)
Second stage of formation of embryo segmentation	
Proembryo comprising four cells in linear arrangement	l (gives rise to the shoot) l' (gives rise to h and h') m (gives rise to d and f) ci (gives rise to n and n')
Third stage of formation of embryo segmentation	
Linear proembryo comprising six cells and quadrant + octant	l (gives rise to shoot) l' (gives rise to h and h') d (gives rise to d and d') f (adds to suspensor) n (adds to suspensor) n' (adds to suspensor)
Fourth stage of formation of embryo segmentation	
Early globular embryo	Derivatives of l (development of shoot) Derivatives of h Derivatives of h' d (gives rise to d and d') Derivatives of f (development of suspensor) Derivatives of n (development of suspensor) Derivatives of n' (development of suspensor)
Fifth stage of formation of embryo segmentation	
Globular to fully formed embryo	Derivatives of l (development of shoot and cotyledons) Derivatives of h (development of hypocotyl) Derivatives of h' (development of hypocotyl and root) d (connecting layer) d' (central part of root cap) Derivatives of f (suspensor) Derivatives of n (suspensor) Derivatives of n' (suspensor)

MATERIALS AND METHODS

For the present study a clone of pepino (clone 6) originating from Israel was chosen. The conditions of plant growth and all preparative and microscopic procedures were as described by Kopcińska et al. (2002a).

To ensure setting of seed for investigation, flowers were hand-pollinated (without actual emasculation) with the pollen from clone 1. As found in preliminary observations, this clone yielded considerable amounts of pollen relatively independently of ambient temperature. Also, its pollen was relatively viable and germinated easily on the stigmas of clone 6 (Kopcińska et al., 2002a). Pollination took place at 9–12 h, as at about 14 h the flowers started to close. In any particular inflorescence, 1–4 flowers that opened on the same day were pollinated: flower buds and flowers that had opened earlier were cut off. After a suitable number of developing fruits was noted, the plants were decapitated.

The samples of fertilized ovules or placenta with developing seeds were collected daily 1–10 days after pollination (dap), then every second day till 30 dap, then every third day till 51 dap, then every fifth day till 66 dap, and at 75 and 85 dap. This made a schedule of 32 arbitrarily planned intervals. For the last terms, seeds were collected from ripe fruits. Till 7 dap, surface-cut ovaries were fixed, then whole placentas (8–10 dap), fragments of placentas with developing seeds (10–14 dap) and finally developing seeds. In older seeds (from 33 dap) the testa was cut parallel to its surface to ensure better penetration of fixative.

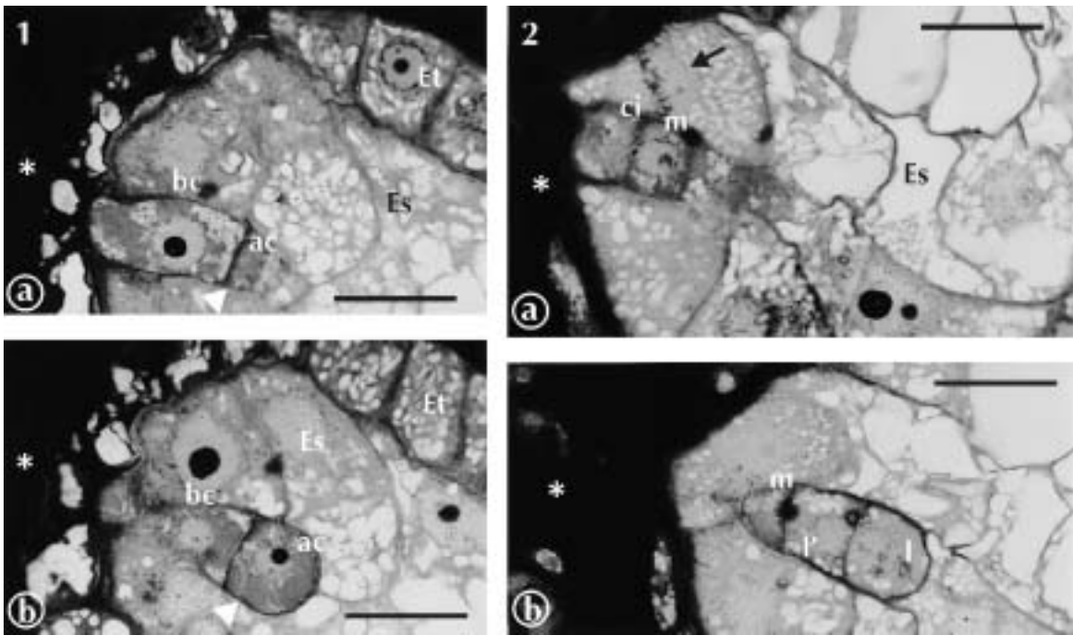

Fig. 1. Two-celled proembryo in successive sections (**a,b**). ac – apical cell; bc – basal cell; Es – endosperm; Et – endothelium; rosette – degraded and crushed cells of integument parenchyma; arrowhead – cell wall separating ac and bc cells. Bar = 22 µm. **Fig. 2.** Four-celled proembryo in successive sections (**a,b**). l, l' – apical cell derivatives; ci, m – basal cell derivatives; Es – endosperm; rosette – degraded and crushed cells of integument parenchyma; arrow – prophase in endosperm cell. Bar = 22 µm.

RESULTS

The first event leading to seed formation was discernible microscopically at 4 dap. At this term, differentiation of endothelium was visible in the ovules (not shown). The endothelium originated from the cells of the inner epidermis of the integument. The cells elongated anticlinally and became vacuolated. Their nuclei were bigger than in the other integument cells. In the integument parenchyma at the endothelium, the middle lamellae of the anticlinal walls expanded, and after pollination the parenchyma itself began gradual disintegration starting from its inner layers. Already at this term the parenchymatous cells adjoining the endothelium (1–2 layers) were degraded and crushed.

The divisions of the fertilized central cell (primary endosperm cell), that is, the development of endosperm (of the cellular type), commenced earlier than the divisions of the diploid zygote. Five days after pollination, 4-celled endosperm was found in the developing seeds (not shown). The first two divisions were perpendicular to the embryo sac axis. Later the cells divided in different planes. At this time the cells of the outer integumentary parenchyma divided periclinally, thus balancing the loss of parenchyma cells at the endothelium. In the placenta, close to fertilized ovules, cell divisions were observed in the epidermis and the subepidermal parenchyma. Starting from this term, significant increases in amounts of starch were noted in the placenta cells.

At 7 dap the first 2-celled proembryos were found (Fig. 1a,b). The first division of the zygote was transverse, yielding an apical cell (ac) and a basal cell (bc). The (bc) cell was anchored in the endothelium. The endosperm was multicelled already, and its cells surrounding the proembryo were visibly less vacuolated than the other endosperm cells. In the endothelial cells the vacuole underwent fragmentation into numerous small ones. The zone of degraded integument parenchyma cells expanded, while cell divisions were still visible in its outer layers. The cells of the integument's outer epidermis became strongly vacuolated and elongated anticlinally.

Ten days after pollination the first 4-celled proembryos were found (Fig. 2a,b). The apical and basal cells divided transversely, giving rise to linearly arranged (l and l') cells originating from (ac), as well as (m) and (ci) cells, originating from (cb). In the endosperm cells next to the proembryo, the vacuoles underwent fragmentation into numerous small

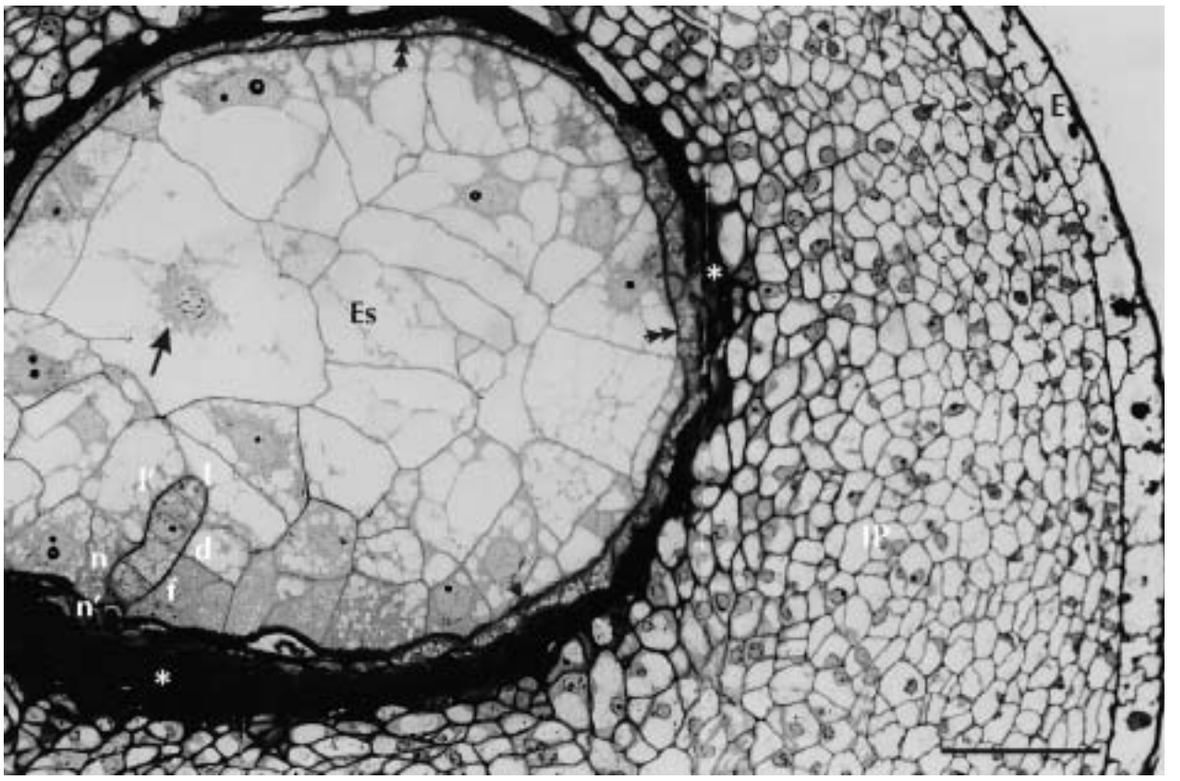

Fig. 3. Six-celled proembryo in developing seed. l, l', – apical cell derivatives; d, f – derivatives of m cell; n, n' – derivatives of ci cell; IP – integument parenchyma; rosette – degraded and crushed cells of integument parenchyma; arrow – prophase in endosperm; double arrowheads – endothelium. Bar = 55.6 μm.

ones. The developing seeds were surrounded up to half their height with proliferating placenta.

At 12 dap the first 6-celled proembryos were observed (Fig. 3). The cells of the proembryo divided transversely into (d) and (f) cells derived from the (m) cell, and (n) and (n') cells derived from the (ci) cell. Endothelial cells became extended tangentially. In the integumentary parenchyma, the striated arrangement of its cells disappeared and dividing cells were no longer found. The anticlinal cell walls in the outer epidermis became folded.

At 16 dap the proembryo was in the quadrant or octant stage with a 10-celled suspensor (Fig. 4a–c). All the suspensor cells were of similar size. In the endosperm cells, numerous strongly osmiophilic lipidic globules were observed. In the vicinity of the proembryo they appeared somewhat earlier, while the last to produce them were the surface layers of the endosperm. In the micropylar part of the developing seed, close to the suspensor, the amount of accumulated degraded cells of integument-derived parenchyma was especially large. The nondegraded yet integumentary parenchyma cells were enlarged and became vacuolated. The anticlinal elongation of outer epidermal cells was noticeable.

In the seeds collected 18–20 dap, early globular embryos were found with epidermal initials present in the (l) tier, as well as within derivatives of the (l') tier (Fig. 5). In the (l) tier, the cells adjoining the dermatogen divided anticlinally versus the epidermis initials. In the (l') tier, divisions occurred that gave rise to (h) and (h') tiers. Successively, in tiers (h) and (h'), cell divisions produced periblem and plerom initials. In the suspensor cells, relatively large but not numerous starch grains appeared. No differences in the amount of lipidic globules in the inner endosperm were observed anymore; its surface layer still contained only sparse numbers of them. In the parenchyma of the integument, that is, the differentiating testa, loosening of the middle lamellae was still observed next to the strongly flattened endothelium.

Numerous divisions occurred in the more advanced globular embryos observed 20–24 dap (Fig.

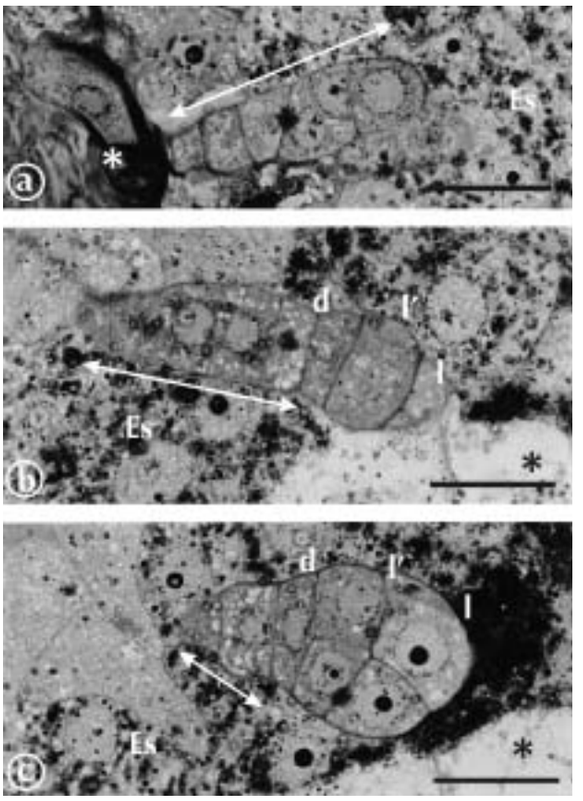

Fig. 4. Octant stage of proembryo in successive sections (**a,b,c**). l, l', – apical cell derivatives; d – derivatives of m cell; Es – endosperm; rosette – degraded and crushed cells of integument parenchyma; two-headed arrow – suspensor; asterisk – endosperm cells with fewer lipid bodies. Bar = 22 µm.

6). The divisions were anticlinal in the dermatogen of the (l), (h) and (h') tiers, and longitudinal or transverse in the (l) tier as well as in the periblem and plerom of the (h) and (h') tiers. In the (d) tier, divisions occurred that gave rise to the formation of another two tiers of derivative cells: the inner tier (d) comprising a only few cells, and the (d') tier which completely surrounded the cells of the (d) tier. In the endosperm, the zone of separation and secretion (ZSS) became visible by light microscopy, surrounding the more advanced globular embryos. Between the embryo and embryo-adjoining endosperm cells a homogenous matrix appeared. Transmission electron microscopy (TEM) revealed the matrix to be a thick layer of secondary cell wall accumulated by endosperm cells (Fig. 7a,b). The layer of secondary cell wall was composed of fine osmiophilic fibrils and differed ultrastructurally from the primary cell wall or middle lamella. Deposits of strongly osmiophilic substance were observed within the secondary wall

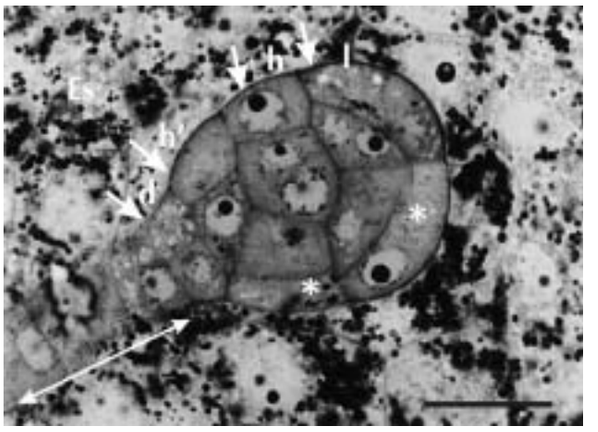

Fig. 5. Early globular stage of immature embryo. l – apical cell derivatives, h, h' – derivatives of l' cell; d – derivatives of m cell; two-headed arrow – suspensor; asterisks – dermatogen cells; arrows – cell walls delimiting segments of embryo cells; Es – endosperm. Bar = 22 µm.

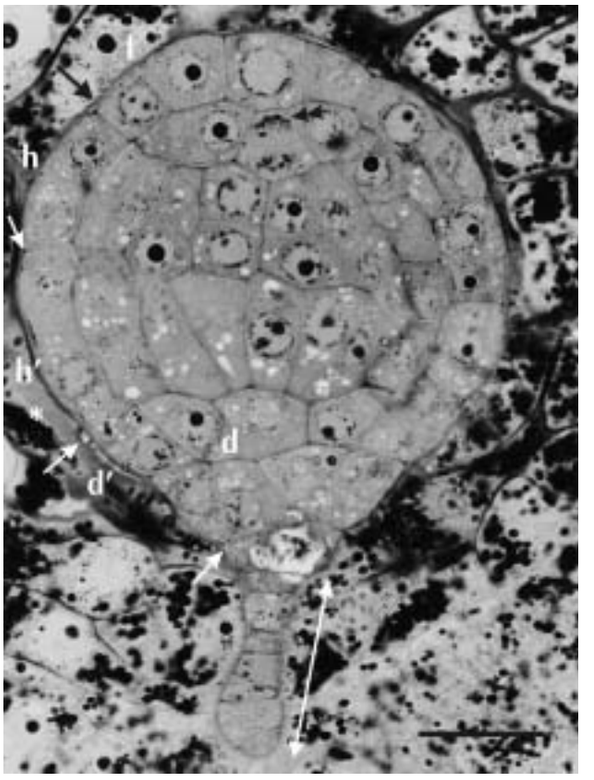

Fig. 6. Immature embryo at the globular stage. l – apical cell derivatives; h, h' – derivatives of l' cells; d – derivatives of m cell; d' – derivatives of d cells; two-headed arrow – suspensor; asterisk – the zone of separation and secretion; double arrowhead – cytokinesis in l tier; arrows – cell walls delimiting segments of embryo cells. Bar = 22 µm.

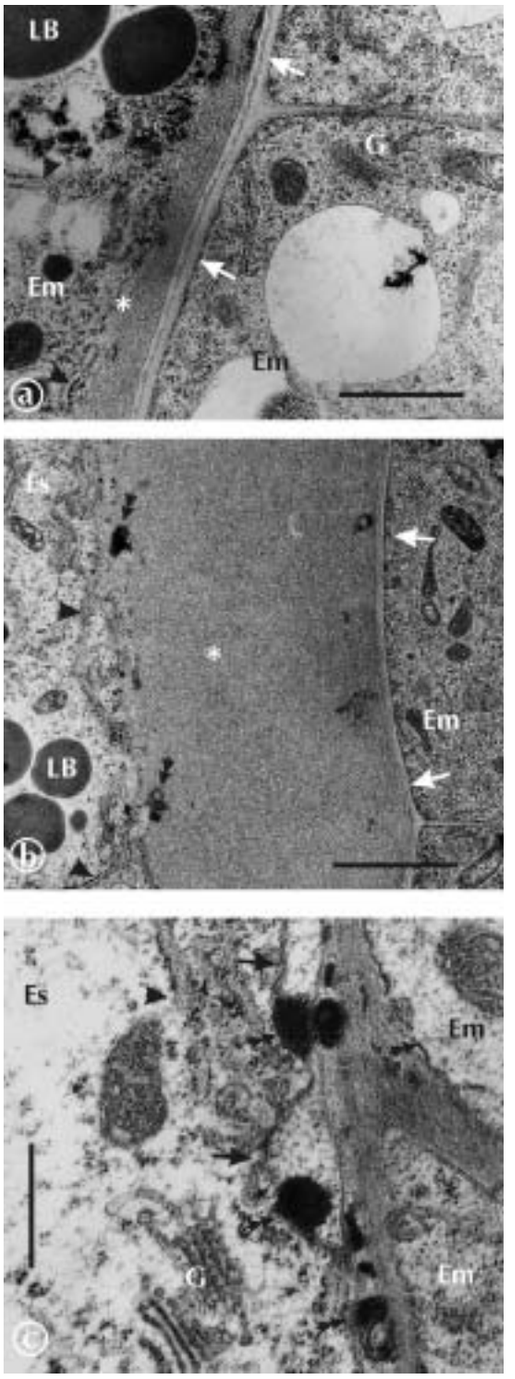

Fig. 7. Formation of zone of separation and secretion in developing seeds (**a,b,c**). Em – embryo; Es – endosperm; G – Golgi bodies; LB – lipid bodies; rosettes – layer of secondary cell wall accumulated by endosperm cells; white arrows – middle lamella; double arrowheads – osmiophilic deposits; arrowheads – rough endoplasmic reticulum; black arrows – plasma membrane. Bar in (a) = 1.4 μm, in (b) = 2.8 μm, in (c) = 0.7 μm.

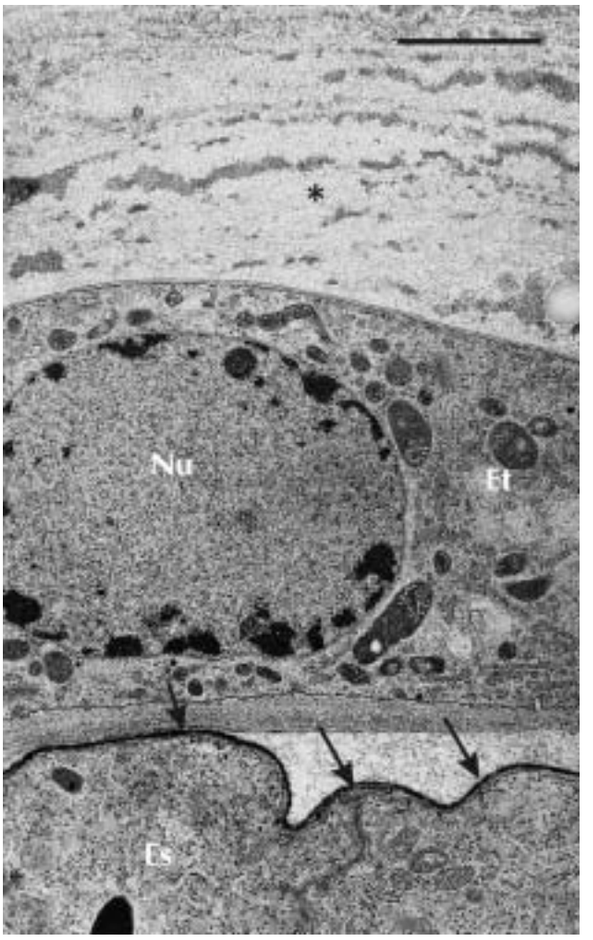

Fig. 8. Boundary of endosperm and testa in developing seed. Es – endosperm; Et – endothelium; Nu – cell nucleus; rosette – degraded and crushed cells of integument parenchyma; arrows – cuticle on outer surface of endosperm. Bar = 2.8 μm.

and in the periplasmic space (Fig. 7b,c). Presumably the secondary wall was of the mucilaginous type. Successive separation of cells was observed in this part of the endosperm, but no degeneration of the protoplasts accompanied it. On the contrary, the cells were filled with dense cytoplasm, and a specific trait of these cells was the proliferation of rough endoplasmic reticulum. The ZSS elongated further into the endosperm in the seeds containing globular embryos, thus determining the direction of embryo growth in the next stages of its development. At this term an osmiophilic cuticle was observed on the outer surface of the uniformly thin-walled endosperm, facing the still-functional epithelial cells (Fig. 8). The number of parenchyma layers decreased in the differentiating testa. The cells of its outer epider-

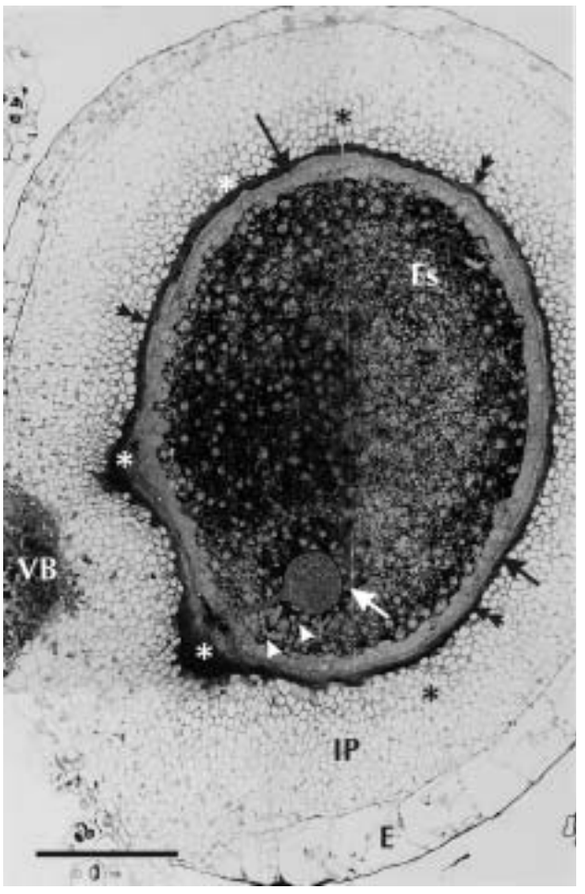

Fig. 9. Globular immature embryo in developing seed. E – epidermis; Es – endosperm; IP – integument parenchyma; VB – vascular bundle in funiculus; white rosettes – degraded and crushed cells of integument parenchyma; black rosettes – zone of dissolving middle lamellae between parenchyma cells of developing testa; double arrowheads – flattened layer of endothelium; black arrows – endosperm surface layer with fewer lipid bodies; white arrow – globular embryo; arrowheads – suspensor. Bar = 111 μm.

mis were very large and strongly vacuolated; they contained large nuclei and amyloplasts, and their anticlinal walls were folded (Fig. 9).

In seeds fixed 28 dap, intermediary embryos with a flattened (l) tier were observed (not shown).

Heart-shaped embryos were found in seeds collected 26–30 dap (Fig. 10a). Such embryos featured extensive growth of the cells derived from tiers (h) and (h'). This growth resulted in the formation of hypocotyl and root, with clearly delineated procambial cells which were slimmer and less vacuolated than pro-cortical and pro-epidermal cells. Also visible was the connecting layer of the root originating from the (d) tier, as well as a bilayered root cap originating from the (d') tier. Developing cotyledons as well as the cells of the shoot apical meristem were visible in the (l) tier. No significant changes were observed within the endosperm, except for elongation of the ZSS. The testa contained just a few layers of parenchyma cells, and the layer of degraded parenchyma remnants was wider (Fig. 10a). The cells of the outer epidermis still contained large amyloplasts, and during fixation the outer cell wall released much mucilage which was visible in the sections (Fig. 10b). The endothelial cells were still present.

In seeds fixed 30–36 dap, torpedo-like embryos with a significantly elongated hypocotyl and cotyledons were found (Fig. 11). Numerous lipid bodies appeared in embryos of this stage, especially in the hypocotylar cortex. Fewer lipids were accumulated in the procambium, and they appeared later in cotyledons. No changes in endosperm or testa were observed.

At 33 dap the first U-shaped embryos were found, and at 39 dap the still-elongating embryo became circinate due to elongation of the cotyledons (Fig. 12). The shoot apical meristem was flat and built of four cell layers, and even in embryos from mature seeds the tunica and corpus were not distinguishable from one another (Fig. 13a). The root apical meristem was fully organized, with clearly delineated cells of the connecting layer, plerom, periblem and dermatocalyptrogen (Fig. 13b). The root cap in its apical part was built of seven cell layers. Analysis of embryo segmentation indicated that the central part of the root cap originated from the (d') tier, and its lateral parts from the (h') tier. Even in mature seeds the suspensor was still present, and its cells did not exhibit symptoms of degeneration in light microscopy.

Large protein bodies with heterogeneous content occurred in the fully formed embryo (Fig. 14). The bodies were most numerous in the hypocotylar cortex, less numerous in the root and cotyledons, and fewest in the procambium and shoot apical meristem.

Parallel to the development of the circinate embryo, the ZSS was still enlarged within the endosperm. Thus the embryo was embedded in a homogenous matrix that separated it from the solid endosperm tissue. Separate endosperm cells were observed within this matrix. Separate cells were also seen in the space between the cotyledons and at the shoot apical meristem (Fig. 13a). The expansion of the ZSS was completed when the seeds reached maturity, and at this stage the zone surrounded the whole embryo. After 33–36 dap, lipid bodies accumu-

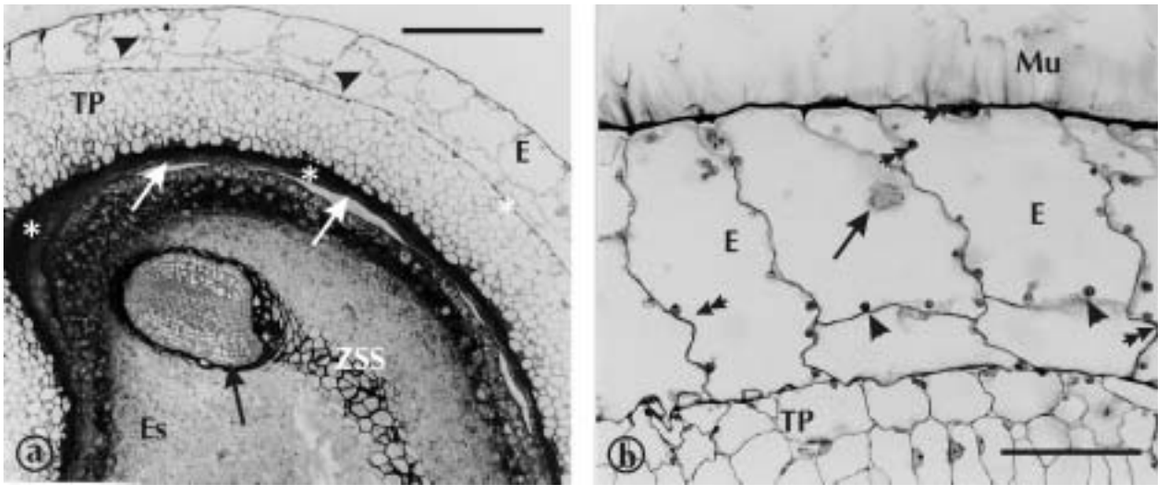

Fig. 10. (**a**) Heart-shaped embryo in developing seed. E – epidermis; Es – endosperm; TP – parenchymatous layer of developing testa; ZSS – zone of separation and secretion; white rosettes – degraded and crushed cells of integument parenchyma; white arrows – flattened layer of endothelium; black arrow – fragment of cotyledon; arrowheads – folded cell walls in testa epidermis. Bar = 222 μm, (**b**) Mucilage on surface of epidermis in developing testa. E – epidermis; Mu – mucilage; TP – parenchymatous layer of developing testa; double arrowheads – folded cell walls in testa epidermis; arrowheads – amyloplasts; arrow – cell nucleus. Bar = 55.6 μm.

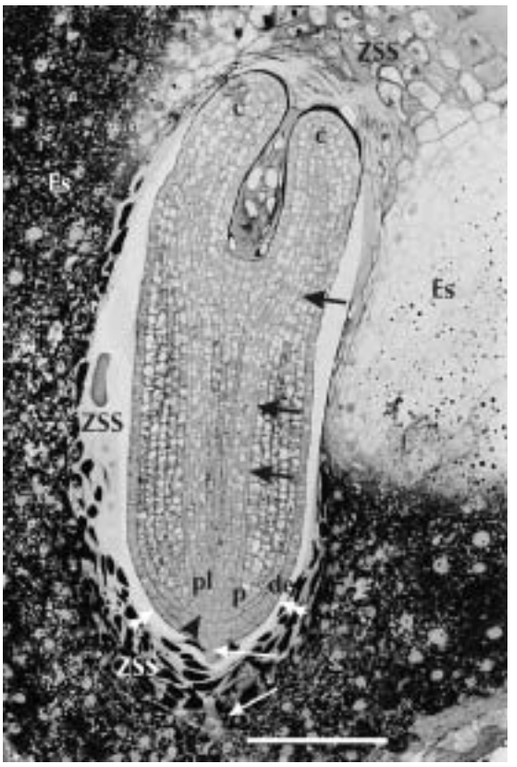

Fig. 11. Early torpedo-shaped embryo. C – cotyledon; Es – endosperm; ZSS – zone of separation and secretion; de – dermatocalyptrogen; p – periblem; pl – plerom; black arrows – procambium; white arrows – suspensor; double arrowheads – cell walls delimiting root cap; arrowhead – connecting layer within root apical meristem. Bar = 111 μm.

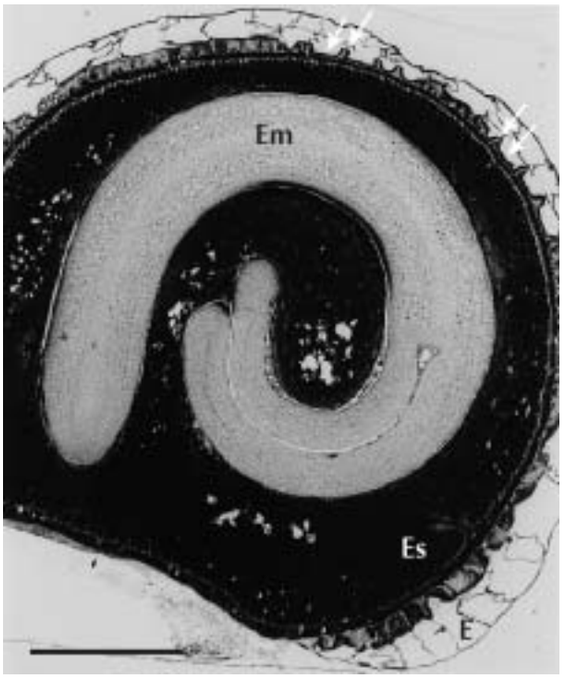

Fig. 12. Circinate embryo. E – epidermis; Em – embryo; Es – endosperm; arrows – thickenings in cell walls in testa epidermis. Bar = 444 μm.

lated in the surface endosperm cells. At the same time, the endosperm cell walls facing the remnants of the endothelium were thickened significantly (Fig. 15), and their outermost layer was lignified.

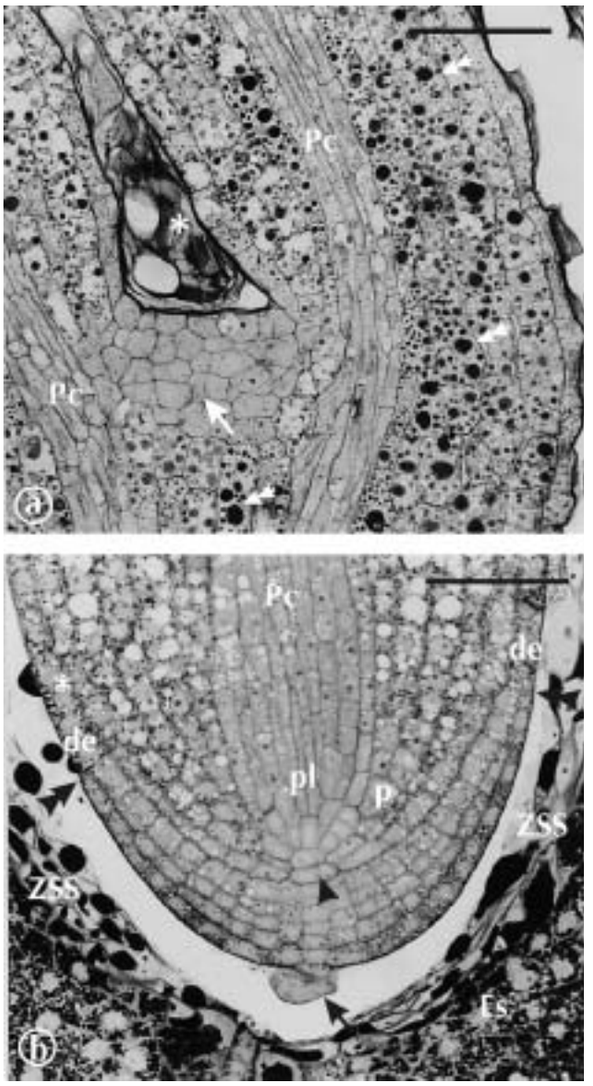

Fig. 13. (**a**) Shoot apical meristem. Pc – procambium; white arrow – cells of apical meristem; asterisk – separate endosperm cells belonging to zone of separation and secretion; double arrowheads – protein bodies. Bar = 55.6 μm, (**b**) Root apical meristem. Es – endosperm; Pc – procambium; ZSS – zone of separation and secretion; de – dermatocalyptrogen; p – periblem; pl – plerom; black arrow – suspensor, arrowhead – cells of connecting layer within apical meristem; double arrowheads – cell walls delimiting root cap. Bar = 55.6 μm.

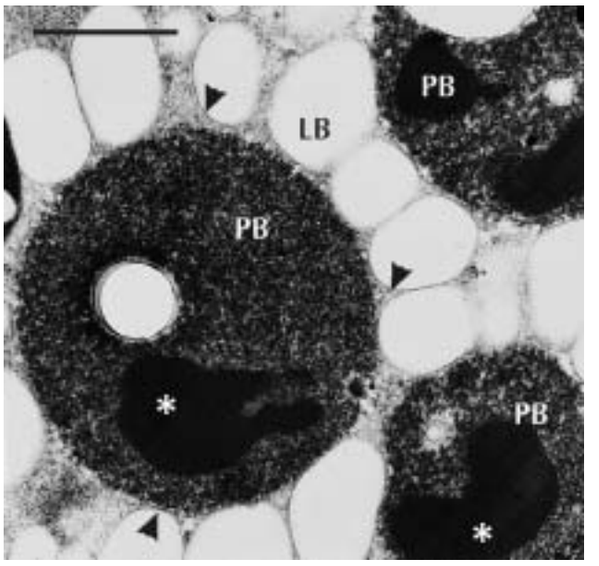

Fig. 14. Ultrastructure of cotyledons in mature embryo. LB – lipid bodies; PB – protein bodies; rosette – osmiophilic inclusions within protein bodies, arrowheads – membranes of lipid bodies. Bar = 1.6 μm.

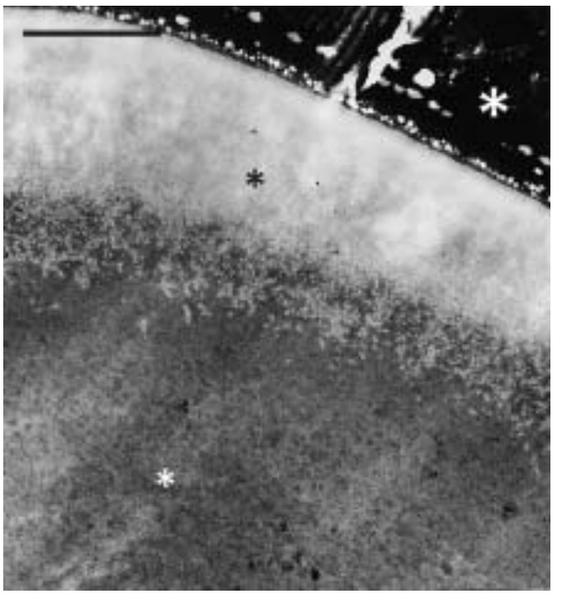

Fig. 15. Thickened cell wall in surface layer of endosperm cells facing remnants of degraded endothelium (asterisk). Black rosette – lignified layer of cell wall, white rosette – osmiophilic layer of cell wall. Bar = 4.17 μm.

During development of the circinate embryo, significant changes occurred in the testa structure. Whereas at 42–45 dap the flattened endothelium was still visible (Fig. 16a), it disappeared at about 48–52 dap. Till this time all integumentary parenchymatous layers were completely degraded and obliterated. Thus, in the mature seed the testa comprised exclusively outer epidermis and crushed remnants of the other tissues of the ovule integument. While the embryo developed into the circinate stage,

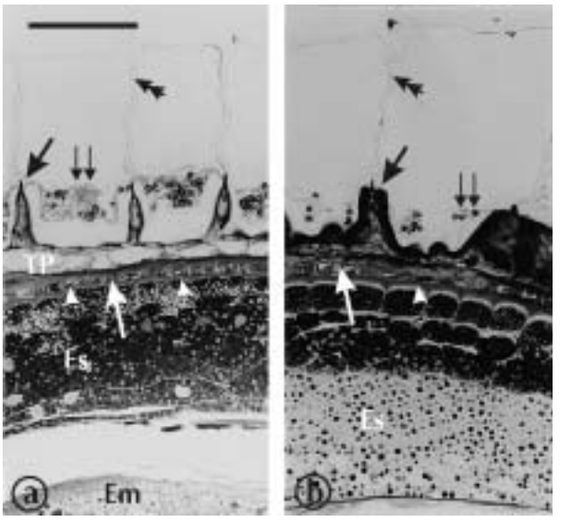

Fig. 16. Fragments of testa in maturing seeds (**a,b**). Em – embryo; Es – endosperm; TP – parenchymatous layer of developing testa; double arrowheads – separated anticlinal cell walls in epidermal cells; black arrows – cell wall thickenings; double arrows – protoplasts of epidermal cells; white arrows – flattened and degrading endothelial cells; arrowheads – thickened outer cell walls of endosperm. Bar = 111 μm.

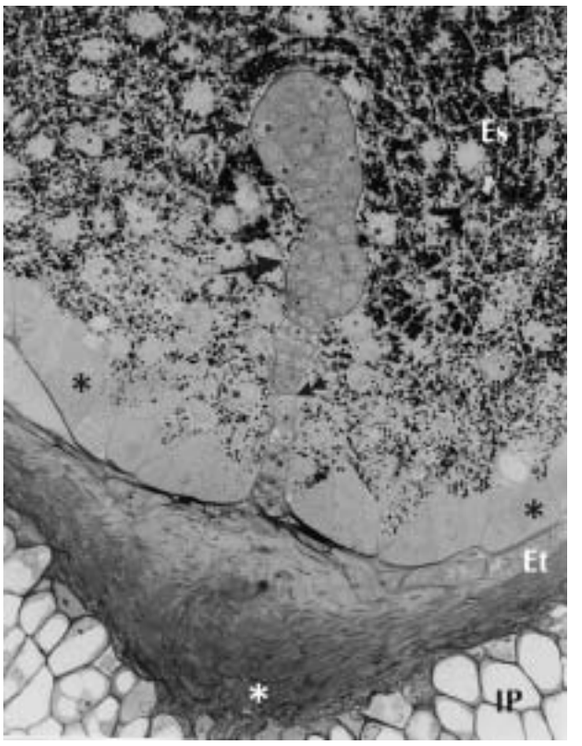

Fig. 17. Deformed embryo (arrows) in seed. Es – endosperm; Et – endothelium; IP – integument parenchyma; rosette – degraded and crushed cells of integument parenchyma; asterisks – endosperm surface layer with fewer lipid bodies; double arrowhead – suspensor. Bar = 55.6 μm.

in the testa epidermal cells the secondary cell wall began to form and the cytoplasm became polarized. The cell nuclei and most of the cytoplasm shifted towards the inner wall, and more amyloplasts were observed. The secondary cell wall was built nonuniformly: a thick layer was deposited on the inner wall and partway up the anticlinal walls. Next, the nonthickened parts of the anticlinal walls elongated and the middle lamellae were digested, resulting in partial separation of neighboring cells (Fig. 16a,b). In the mature seeds, the thickened and striated secondary wall reached only 1/4–1/5 way up the anticlinal walls. No cytoplasm was observed at this stage. The hilum was not covered with epidermis, of course, and in this part of the seed surface the derivative cells of the ovular chalaza and integumentary parenchyma were retained.

In some seeds at the globular embryo stage, disturbances in embryo development were evident. Deformed embryos (Fig. 17) or additional ones were present, usually much smaller. On the basis of the collected documentation, no definite conclusion could be drawn as to how they developed. Occasionally some seeds did not contain an embryo. These seeds were of typical size and were filled with endosperm.

DISCUSSION

In pepino the development of the embryo was identified as of the Solanad type. The apical cell gave rise to the shoot apex with cotyledons, hypocotyl and root, while the basal cell produced the connecting layer, root cap and suspensor. Development of the pepino embryo proceeded according to the outline shown in Table 1.

In the family Solanaceae, embryogenesis is usually of the Solanad type (Mohan Ram and Kamini, 1964; Erdelská, 1985; Johri et al., 1992). The process was described in detail for *Solanum phureja* (Dnyansagar and Cooper, 1960), and the course of embryo development in this plant generally agreed with that observed in pepino in this work, but with a difference in early embryo development. In pepino, the (l) cell divided later than the (m) and (ci) cells, resulting in a linear 6-celled proembryo. In *S. phureja* the quadrant proembryo was 6-celled due to longitudinal divisions of the (l) and (l') cells. However,

subsequent divisions masked this difference between pepino and *S. phureja*.

In pepino, the endosperm was cellular ab initio, which is a general feature of Solanaceae, although plants with nuclear or helobial endosperm have also been described in this family (Johri et al., 1992). In pepino the first division of the fertilized central cell was transverse, as was the division of its first derivatives, as in *Solanum macranthum* (Mohan, 1970a). However, the next divisions of endosperm initials occurred differently in pepino and *S. macranthum*.

In pepino the cells of the developing endosperm were initially vacuolated, with the vacuolation quickly diminishing and with the accumulation of large amounts of lipids. A distinct trait of the differentiation of the endosperm layer facing the endothelium was the formation of strongly thickened cell walls at the final stages of seed development and a significant delay in the accumulation of lipids, which appeared here as late as in the maturing seed. Similarly, in the development of endosperm in *S. phureja* (Dnyansagar and Cooper, 1960) and *Nicotiana tabacum* (Erdelská, 1985), various differentiation pathways were observed in the surface and inner endosperm layers.

The Solanaceae are not uniform with regard to the compounds stored in the endosperm – in *Withania somnifera*, mostly lipids and starch are accumulated (Mohan Ram and Kamini, 1964), in *Datura* mostly proteins, and starch in *S. phureja* and *Petunia nyctaginiflora* (Johri et al., 1992). Lipid and protein bodies were observed only in mature seeds of pepino (in both endosperm and embryo); the protein bodies had heterogeneous content of matrix, globoids and irregular inclusions, presumably of proteinaceous type. During development of the pepino endosperm, at 20–24 dap the formation of the ZSS was noted around the embryo. At the ultrastructural level the process was similar to the one described in *Solanum nigrum* (Briggs, 1993, 1996), starting with deposition of the secondary cell wall in endosperm cells and successive loosening of all wall layers in the vicinity of the embryo. In older papers this process was regarded as a symptom of cell disintegration and autolysis (Beamish, 1955; Lee and Cooper, 1958; Dnyansagar and Cooper, 1960; Erdelská, 1985). However, in *Solanum nigrum* it has been shown (Briggs, 1993, 1996) that the zone did not appear as a result of disintegration, but rather separation of endosperm cells accompanied by secretion of lipidic-carbohydrateous material. Separated cells of the zone remained alive, and created a pathway for the embryo to grow through the cellular endosperm, without damage to the embryo or endosperm itself. Some features of endosperm cell ultrastructure within this zone (temporary disappearance of lipidic globules and the presence of glyoxysomes) led to the supposition that the cells mobilized some amount of their storage compounds during periods of decreased photosynthate import, thus ensuring unperturbed embryo development. Lester and Kang (1998) reached similar conclusions.

We observed profound anatomical changes during transformation of the ovule integument into the seed testa in pepino. The integument of differentiated ovules comprised an outer epidermis, 5–7 parenchyma layers and an inner epidermis (Kopcińska et al., 2002b), the latter differentiating into the endothelium, which is a common phenomenon in Solanaceae (Cooper, 1931; Lee and Cooper, 1958; Mohan, 1970a,b; Erdelská, 1985). As we noted, during pepino seed development the number of parenchymatous layers increased initially, but then the parenchyma disappeared completely due to gradual lysis of the cells adjacent to the endothelium. The endothelial cells, initially enlarged and elongated radially, gradually flattened and eventually lysed. Thus, in the mature testa of pepino seeds the only tissue that retained cellular structure was the outer epidermis. Its cells produced irregular thickenings of the inner walls and basal parts of the anticlinal walls. Nonthickened parts of the anticlinal walls underwent separation but not disintegration. The course of testa development in *S. phureja* was similar (Dnyansagar and Cooper, 1960), but in this plant the outer cell walls of the outer epidermis disintegrated, and the thickenings on the radial walls produced the effect of 'hairs' on the seed surface. In contrast to pepino, in *S. phureja* the endothelium did not disappear but formed the inner epidermis of the testa. As in pepino and *S. phureja*, disintegration of the parenchyma was also observed in *S. macranthum* (Mohan, 1970a) and *Solanum demissum* (Beamish, 1955), implying that the disintegrating parenchyma initially could be a source of nutrients for the young developing endosperm and embryo.

Under conditions of controlled pollination, normal development of pepino seeds was observed in the experiment reported here. Only a small fraction of seeds exhibited abnormal or additional embryos, and seeds were devoid of an embryo only occasionally. Thus, to ensure seed set under greenhouse cultivation, deposition of pollen on the stigma seems to be of crucial importance.

ACKNOWLEDGEMENTS

The authors thank Dr. Teresa Tykarska for critical discussion of the results and Ewa Znojek and Alicja Żuchowska for expert technical assistance. The work was performed with financial support from the Polish State Committee for Scientific Research (KBN), grant no. 5 P06C 00415.

REFERENCES

ANDERSON GJ, JANSEN RK, and KIM Y-D. 1996. The origin and relationships of the pepino, *Solanum muricatum* (Solanaceae): DNA restriction fragment evidence. *Economic Botany* 50: 369–380.

BEAMISH KI. 1955. Seed failure following hybridization between the hexaploid *Solanum demissum* and four diploid *Solanum* species. *American Journal of Botany* 42: 297–304.

BRIGGS CL. 1993. Endosperm development in *Solanum nigrum* L. Formation of the zone of separation and secretion. *Annals of Botany* 72: 303–313.

BRIGGS CL. 1996. An ultrastructural study of the embryo/endosperm interface in the developing seeds of *Solanum nigrum* L. Zygote to mid torpedo stage. *Annals of Botany* 78: 295–304.

COOPER DC. 1931. Macrosporogenesis and the development of the macrogametophyte of *Lycopersicon esculentum*. *American Journal of Botany* 18: 739–751.

DNYANSAGAR VR, and COOPER DC. 1960. Development of the seed of *Solanum phureja*. *American Journal of Botany* 47: 176–186.

ERDELSKÁ O. 1985. Dynamics of the development of embryo and endosperm I. (*Papaver somniferum*, *Nicotiana tabacum*, *Jasione montana*). *Biologia (Bratislava)* 40: 17–30.

JOHRI BM, AMBEGAOKAR KB, and SRIVASTAVA PS. 1992. *Comparative embryology of angiosperms*. Springer-Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong, Barcelona, Budapest.

KOPCIŃSKA J, ŁOTOCKA B, KOWALCZYK K, and KOBRYŃ J. 2002a. Flower Organs of *Solanum muricatum* Aiton. I. Perianth and Stamen. *Acta Biologica Cracoviensia Series Botanica* 44: 125–134.

KOPCIŃSKA J, ŁOTOCKA B, KOWALCZYK K, and KOBRYŃ J. 2002b. Flower Organs of *Solanum muricatum* Aiton. II. Pistil. *Acta Biologica Cracoviensia Series Botanica* 44: 135–143.

LEE JH, and COOPER DC, 1958. Seed development following hybridization between diploid *Solanum* species from Mexico, central and south America. *American Journal of Botany* 45: 104–110.

LESTER RN, and KANG JH. 1998. Embryo and endosperm function and failure in *Solanum* species and hybrids. *Annals of Botany* 82: 445–453.

MOHAN RAM HY, and KAMINI I. 1964. Embryology and fruit development in *Withania somnifera* Dunal. *Phytomorphology* 14: 574–587.

MOHAN K. 1970a. Morphological studies in *Solanaceae*. V. Embryology as well as structure and development of seed of *Solanum macranthum* Dun. *Agra University Journal of Research (Science)* 19: 55–66.

MOHAN K. 1970b. Morphological studies in *Solanaceae*. VI. The development of ovule and embryo sac in *Solanum khasianum* Clarke. *Agra University Journal of Research (Science)* 19: 39–44.

PROHENS J, RUIZ JJ, and NUEZ F. 2000. Growing cycles for a new crop, the pepino, in the Spanish Mediterranean. *Acta Horticulturae* 523: 53–60.

PROHENS J, and NUEZ F. 2001. The effects of parthenocarpy on pepino (*Solanum muricatum*) yield and fruit quality. *Journal of Horticultural Science and Biotechnology* 76: 101–106.

RUIZ JJ, and NUEZ F. 2000. High temperatures and parthenocarpic fruit set: Misunderstandings about the pepino breeding system. *Journal of Horticultural Science and Biotechnology* 75: 161–166.