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Author(s): Sonia Rosenfeldt & Beatriz G. Galati
Source: Annales Botanici Fennici, 48(3):263-271. 2011.
Published By: Finnish Zoological and Botanical Publishing Board
DOI: http://dx.doi.org/10.5735/085.048.0307
URL: http://www.bioone.org/doi/full/10.5735/085.048.0307

Ann. Bot. Fennici 48: 263–271 ISSN 0003-3847 (print) ISSN 1797-2442 (online)
Helsinki 30 June 2011

Megasporogenesis and megagametophyte development in ten species of *Oxalis*

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Received 3 Nov. 2009, revised version received 23 Feb. 2010, accepted 2 Mar. 2010

Rosenfeldt, S. & Galati, B. G. 2011: Megasporogenesis and megagametophyte development in ten species of *Oxalis*. — *Ann. Bot. Fennici* 48: 263–271.

Ovule morphology, megasporogenesis and megagametogenesis of ten species of *Oxalis* were examined. The ovule is anatropous, bitegmic and the integuments form a zig-zag micropyle. The type of female gametophyte development is variable in the studied species. The majority of them have a monosporic Polygonum-type female gametophyte. Two of the studied species show binucleate dyad members, so they have a bisporic Allium-type female gametophyte. Only *O. articulata* follows tetrasporic Adoxa-type development of the megagametophyte. This is the first report of a tetrasporic female gametophyte in Oxalidaceae. The endosperm is nuclear, and development of the embryo corresponds to the Solanad type.

Introduction

Studies of the ovule and female gametophyte development in Oxalidaceae are rare (Johri *et al*. 1992). The majority of the studied species have an anatropous, bitegmic and tenuinucelate ovule. However, a crassinucellate-type ovule has been reported from *Averrhoa bilimbi* and *A. carambola* (Thathachar 1942, Boesewinkel 1985). Herr and Dowd (1968) and Herr (1972) described the ovule of *Oxalis corniculata* as hemianatropous.

The ontogeny of the female gametophyte has been described for most species as belonging to the Polygonum type, whereas the tetrads of megaspores can present a linear or T disposition. Nevertheless, *O. corniculata* shows several unusual features. Cytokinesis is produced normally after meiosis I, but it is usually absent after meiosis II. The result of this is the formation of binucleate dyad members, and the development of the female gametophyte follows the Allium type. Less frequently, nonsynchronous cytokinesis occurs first in the chalaza and then in the micropylar dyad cell to produce four megaspores usually in a linear arrangement. A monosporic, Polygonum-type female gametophyte develops from the chalazal megaspore (Herr & Dowd 1968, Herr 1972). Herr (1972) studied the possible division planes of the micropylar and chalazal nuclei during the first stages of the megagametophyte development and the possible orientation of the cells in the egg apparatus.

Knowledge of the ovule and female gametophyte in Oxalidaceae is scanty, so we decided to study the megasporogenesis, megagametogenesis, and the ovule ontogeny of ten species of

Oxalis. The aim is to increase basic embryological knowledge of the family and to provide information of taxonomic and systematic value.

Material and methods

The species studied were collected from natural populations growing in the field. Voucher specimens of the species studied, listed in an alphabetic order, are deposited in Darwinion Institute Herbarium (SI), San Isidro, Buenos Aires Province, Argentina.

Oxalis articulata. Argentina. Ciudad Autónoma de Buenos Aires. Ciudad Universitaria. Nuñez. 10/2000. *Rosenfeldt S.1*.

Oxalis conorrhiza. Argentina. Córdoba. Colón. Río Ceballos. 1/2003. *Rosenfledt, S. 4.;* Argentina. Ciudad Autónoma de Buenos Aires. Ciudad Universitaria. 2/2004. *Rosenfeldt, S. 8*.

Oxalis corniculata var. *corniculata*. Argentina. Ciudad Autónoma de Buenos Aires. Ciudad Universitaria. 10/ 2003. *Rosenfeldt, S. 10*.

Oxalis floribunda. Argentina. Entre Ríos. Concepción. 11/2003. *Rosenfeldt, S. 12*.

Oxalis hispidula. Argentina. Ciudad Autónoma de Buenos Aires. Ciudad Universitaria. Nuñez. 5/2001. *Rosenfeldt S. 2*.

Oxalis lasiopetala. Argentina. Entre Ríos. Concordia. Parque San Carlos. 11/2003. *Rosenfeldt, S. 6*.

Oxalis niederleinii. Argentina. Entre Ríos. Concordia. Parque San Carlos. 11/2004. *Rosenfeldt, S. 9*.; Entre Ríos. Concordia. 11/2002. *Rosenfeldt S. 3*.

Oxalis paludosa. Argentina. Entre Ríos. Concordia. Parque San Carlos. 11/2003. *Rosenfeldt, S. 7*.

Oxalis perdicaria. Argentina. Buenos Aires. Zárate-Campana. 4/2006. *Rosenfeldt, S. 14*.

Oxalis refracta. Argentina. Buenos Aires. Isla Martín García. 12/2005. *Rosenfeldt, S. 11*.

Flowers in different stages of development were fixed in FAA (formalin–alcohol–acetic acid), dehydrated in an ascendant alcohol series, transferred to xilene and then embedded in paraffin wax. Sections 8–10 μm thick were cut following standard botanical micro-techniques. The sections were stained in a safranin–fast green combination (D'Ambrogio 1986) and the slides were mounted in a synthetic resin.

Cellular chemical substances were detected using several reagents on histological sections. Lipids and cutine were localized with Sudan IV and starch with lugol. The material was viewed with a Wild M20 light microscope and photographed with a Nikon Labophot.

Results

Ovule

The ovule is anatropous, bitegmic and the micropyle is formed by both integuments. The endostome and the exostome are slightly displaced, forming a slightly zigzag pattern (Fig. 1A). Both integuments are 3–5-cell-layer thick (Fig. 2G). The origin of the inner integument is exclusively dermal, whereas the outer integument is formed by dermal and hypodermal cells (Figs. 1B and C, 2A). The inner integument is initiated first, and appears as a ring of cells in the base of the nucellus. The outer integument develops later, but by its more rapid growth, it extends beyond the inner integument (Fig. 1A–C).

A conspicuous archesporial cell is differentiated in the nucellus prior to or as the inner integument is initiated. Rarely, two archesporial cells differentiate. This cell does not divide mitotically and acts directly as megaspore mother cell covered only by the nucellar epidermis. Thus, the ovule is tenuinucellate (Figs. 1B and C, 2A).

The nucellar epidermis differs notably from the rest of the ovule cells due to the cutinized walls of these cells in most of the studied species (Figs. 1C and 2D). As megagametogenesis proceeds and with the increase in size of the female gametophyte, cells of the micropylar nucellar epidermis are compressed (Fig. 2E). Finally, at the stage of the 4- or 8-nucleate female gametophyte, the nucellar epidermis breaks at the micropilar end, and the developing megagametophyte becomes exposed (Figs. 1D and 2F). The nucellar cells in contact with the female gametophyte from the chalazal pole towards the middle region are cutinized in the mature ovule. Only in *O. corniculata*, *O. conorrhiza*, and *O. paludosa* the cells of the nucellar epidermis are not cutinized. In these three species, the epidermal cells disappear rapidly, and the young female gametophyte stays in direct contact with the internal epidermis of the inner integument, which shows cells with conspicuous nuclei and a dense cytoplasm. Therefore, this epidermis acts as an endothelium.

Megasporogenesis

The megaspore mother cell divides meiotically giving rise to a linear tetrad of megaspores in *O. niederleinii*, *O. floribunda*, *O. lasiopetala*, *O. perdicaria* and *O. conorrhiza* (Figs. 2B and C, 3A).

In *O. refracta*, the tetrad has a T disposition (Fig. 3B). In *O. paludosa* and *O. hispidula*, only triads occur (Figs. 2D and 3C), whereas in *O. corniculata* only dyads are formed (Fig. 3D). In *O. paludosa* and *O. hispidula*, the chalazal dyad member, and in *O. refracta* and *O. corniculata* the chalazal megaspore, produce the female gametophyte, whereas others become aborted. In *O. articulata*, the meiosis I without cytokinesis produces directly a binucleate female gametophyte (Fig. 3E and F). This enlarges notably and develops a large central vacuole. Meiosis II, without cytokinesis, gives rise to a 4-nucleate, tetrasporic female gametophyte.

In some cases, binucleate dyad members are produced in *O. perdicaria*, where the micropylar one aborts rapidly before completing its development.

Megagametogenesis

After three karyokineses in the functional megaspore, an 8-nucleate female gametophyte is formed, where the nuclei are arranged in two quartets, one micropylar and the other chalazal. Separation of the quartets results from formation of a large vacuole after the first mitotic division, which pushes each nucleus towards the micropylar and chalazal poles of the female gametophyte (Fig. 4A). The volume of the female gametophyte increases notably, and cytokinesis produces an heptacellular, 8-nucleate female gametophyte (Fig. 4B).

The egg apparatus is formed by the egg cell and the two synergids. The nuclei of the synergids are positioned toward the micropylar pole by chalazal vacuoles (Figs. 4C–F and 5B). Only *O. perdicaria* has synergids with nuclei polarized towards the chalazal end by micropylar vacuoles (Figs. 4B and 5A). The synergids are hooked at the micropylar pole in *O. lasiopetala*, *O. floribunda*, *O. conorhiza* and *O. articulata* (Figs. 4C and D, 5D). In *O. perdicaria* and *O. hispidula*, the synergids become longer and slimmer at the micropylar pole, and in some cases they extend into the micropylar channel (Fig. 4B).

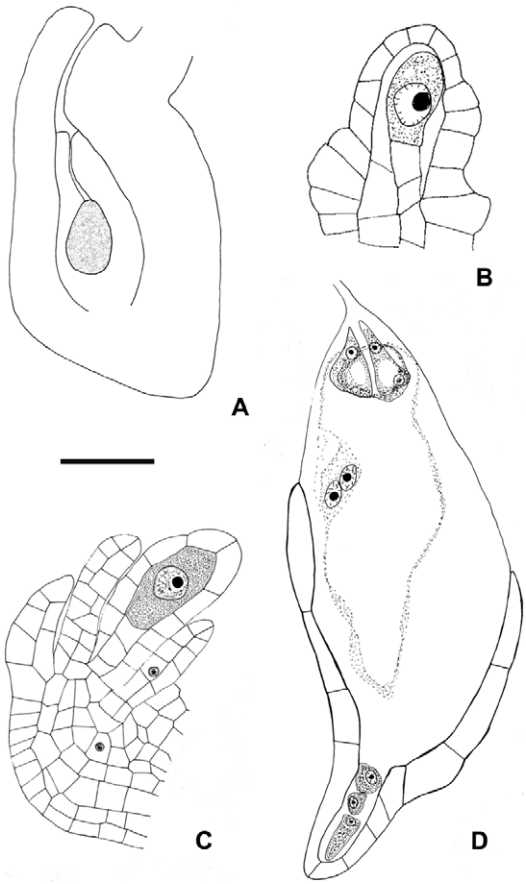

Fig. 1. Ovule ontogeny. — **A**: Longitudinal section of the ovule in *Oxalis floribunda* at the anthesis stage. — **B** and **C**: Tenuinucellate ovule primordium with the inner integument developing. — **B**: *O. corniculata*. — **C**: *O. niederleinii*. — **D**: Young seven-celled female gametophyte in *Oxalis refracta*. Scale bars: **A**: 69.4 μm, **B**: 32 μm, **C**: 17.5 μm, **D**: 25 μm.

The egg nucleus is positioned at the chalazal end of the cell below a large micropylar vacuole (Fig. 5C). The central cell occupies the largest portion of the mature megagametophyte (Fig. 4E and F). This cell is very vacuolated and contains two polar nuclei at opposite poles of the young mature female gametophyte (Fig. 4B). They fuse (Fig. 5E) to form a secondary nucleus located near the egg apparatus in the mature female gametophyte (Fig. 5C).

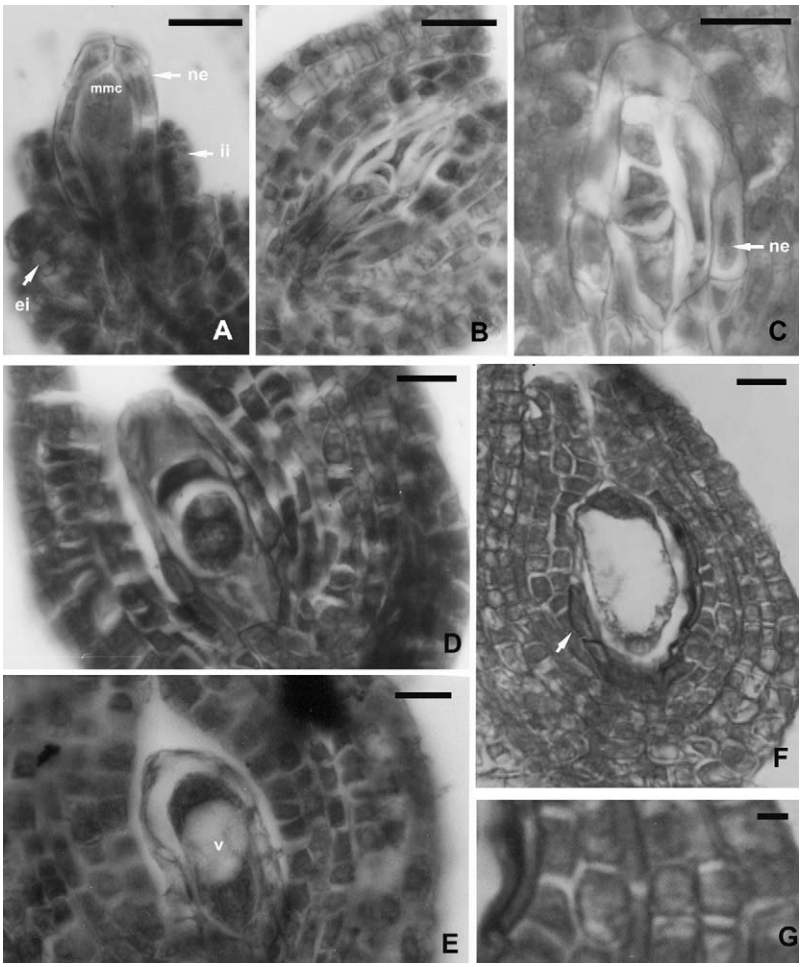

Fig. 2. Megasporogenesis. — **A** and **B**: *Oxalis niederleinii*. **A**: Tenuinucelate ovular primordium with the inner integument developing. **B**: Tetrade of megaspore. **C**: *O. conorhiza*. Tetrad of megaspores. — **D** and **E**: *O. hispidula*. **D**: Tenuinucelate ovular primordium with triad of megaspores. **E**: Binucleate female gametophyte. — **F** and **G**: *O. perdicaria*. **F**: Binucleate female gametophyte. **G**: Detail of the ovule integuments. mmc = megaspore mother cell, ii = inner integument, ei = external integument, ne = nucellar epidermis, v = vacuole. Scale bars: **A** and **C**: 1.5 *μ*m; **B**, **D** and **E**: 1 *μ*m; **F**: 2 *μ*m; **G**: 0.5 *μ*m.

The antipodals are small cells with few vacuoles in the cytoplasm. Those of *O. lasiopetala*, *O. floribunda* and *O. articulata* are so ephemeral as not present in young mature female gametophytes. In *O. refracta* and *O. niederleinii*, the antipodals are located linearly in a strait caecum in the chalazal end of the megagametophyte (Fig. 4E and F). In the latter species, only two antipodals occur in some megagametophytes, but one of them is binucleate (Fig. 4F).

Fertilization, endospermogenesis and embryogenesis

The pollen tube penetrates into one of the synergids where both sperms are released. One male gamete fuses with the egg cell to form the zygote. The fusion of the second male gamete with the secondary nucleus results in the primary endosperm cell. The nucleus of this cell divides repeatedly without cytokinesis, and the nuclei produced are located in the peripheral cytoplasm around a large central vacuole (Fig. 6A). A transverse division of the zygote produces a highly vacuolated basal cell and a small apical cell with dense cytoplasm (Fig. 6B).

The first division of the apical cell is also transverse. Then, both daughter cells divide longitudinally, producing a pro-embryo of five cells, four cells derived from the apical cell, and one cell that corresponds to the future suspensor (Fig. 6C and D).

The formation of the endosperm cell walls begins at the globular or heart-shaped embryo stage. At the mature embryo stage, the

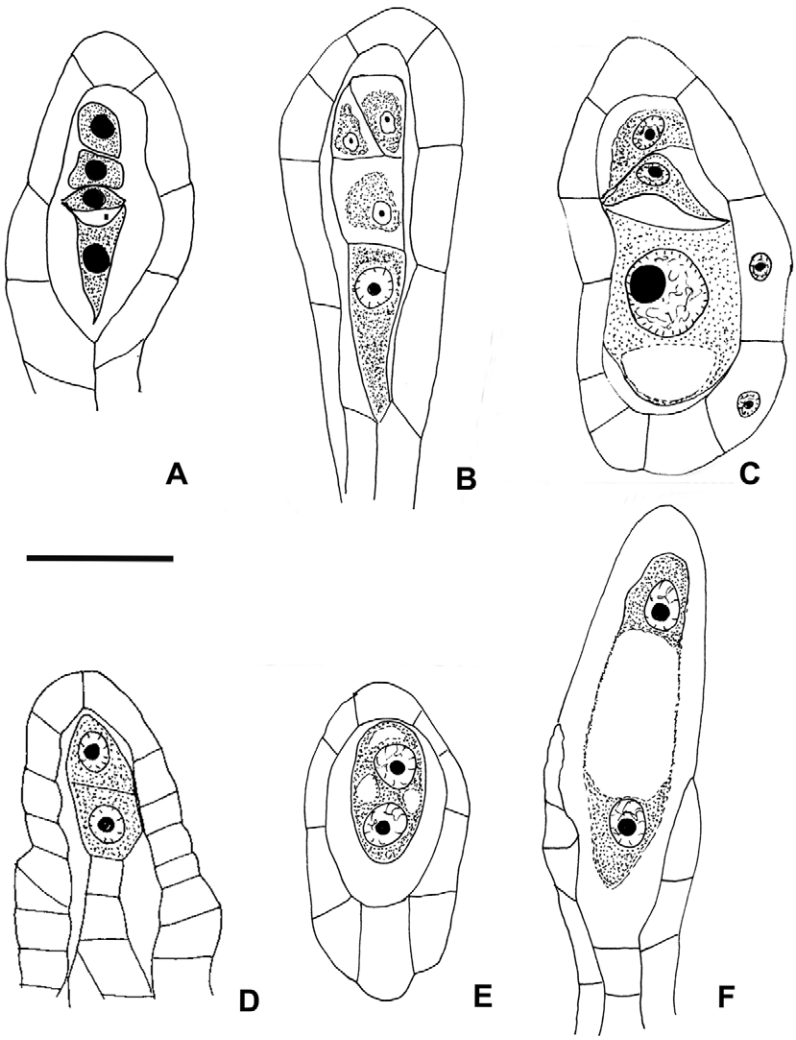

Fig. 3. Megasporogenesis. — **A**: *Oxalis conorhiza*, linear tetrad of megaspores. — **B**: *O. refracta*, megaspores tetrad with T disposition. — **C**: *O. hispidula*, two megaspores and a dyad member. — **D**: *O. corniculata*, dyad. — **E**–**F**: *O. articulata*, binucleate female gametophyte. Scale bars: **A**: 20 μm; **B** and **C**: 30 μm; **D** and **E**: 33 μm.

endosperm development is complete and its cells accumulate abundant starch grains and lipids.

Discussion

In the species of *Oxalis* studied in this research, the archesporial cell differentiates in the primordial ovule before the integuments start to develop, as reported by Herr and Dowd (1968) for *O. corniculata*.

Megagametogenesis was investigated in ten *Oxalis* species. *Oxalis corniculata* is the only species of the genus previously investigated. As reported by Hammond (1908), three or four megaspores are formed in this species, whereas according to Herr and Dowd (1968), the megaspore mother cell divides by meiosis to produce a dyad. Usually the micropylar dyad member aborts and the chalazal member undergoes meiosis II so to form a bisporic, binucleate female gametophyte. Less frequently, meiosis II in both dyad members produces a linear tetrad of megaspores, the chalazal member of which produces the female gametophyte. Thathachar (1942) reported monosporic female gametophyte development from the chalazal megaspore in *Averrhoa carambola* and *A. bilimbi*, and bisporic development in *Biophytum sensitivum*.

The majority of the species studied here (*O. niederleinii*, *O. floribunda*, *O. lasiopetala*, *O. perdicaria*, *O. conorrhiza*, *O. refracta*) form tet-

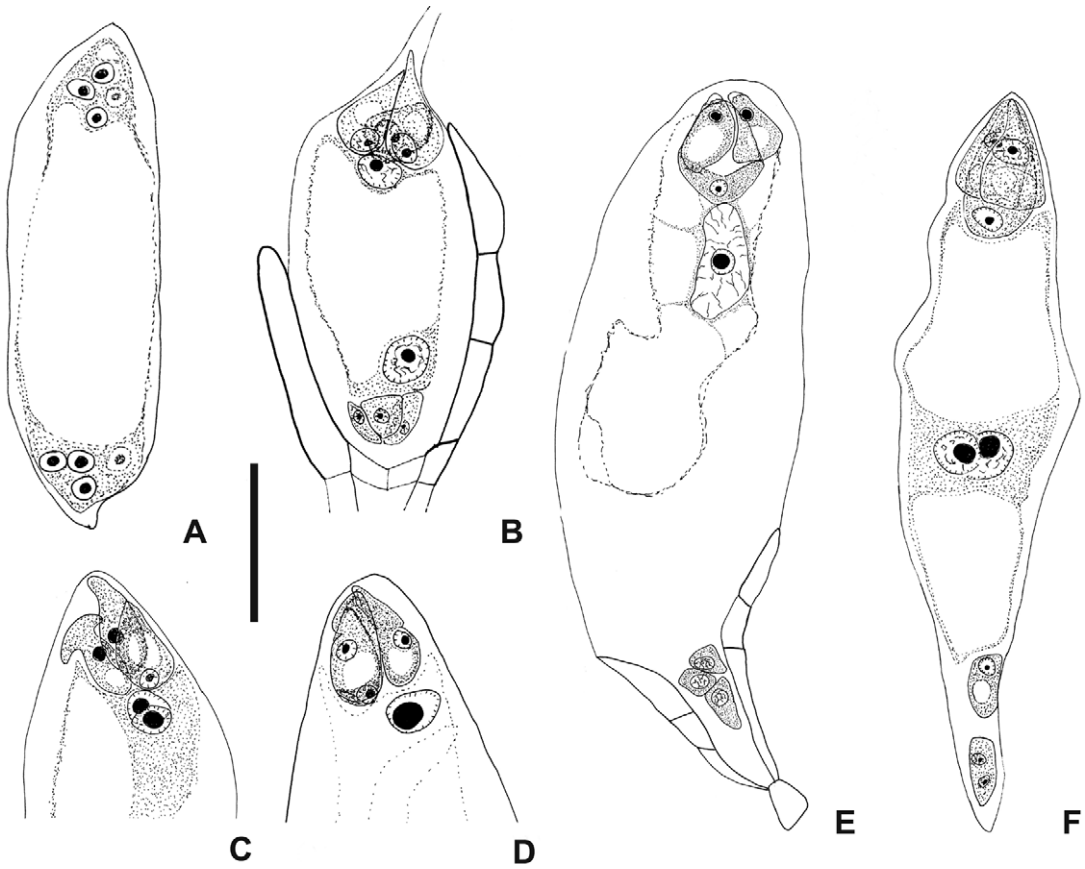

Fig. 4. Megagametophytes. — **A**: Eight-nucleate female gametophyte in *Oxalis lasiopetala*. — **B**: Young seven-cellular embryo sac in *O. perdicaria*. — **C** and **D**: Detail of the micropylar portion of the mature female gametophyte showing synergids with hooks. **C**: *O. lasiopetala*. **D**: *O. floribunda*. — **E** and **F**: Mature female gametophyte. **E**: *O. refracta*. **F**: *O. niederleinii*. Scale bars: **A**: 25 μm, **B**: 20.8 μm, **C**: 15.5 μm, **D**: 29.4 μm, **E**: 16.6 μm, **F**: 11.5 μm.

rads of megaspores where the three micropylar ones abort and the chalazal one develops into the female gametophyte. However, in *O. paludosa* and *O. hispidula*, meiosis II in the chalazal dyad member produces two megaspores. Meiosis II in the micropylar dyad member is interrupted by abortion of the cell. In *O. corniculata*, we observed that the chalazal member of the dyad produces a bisporic female gametophyte. Monosporic development was not found. Two binucleate dyad members were found only occasionally in *O. perdicaria*. Moreover, the formation of a tetrasporic female gametophyte was observed only in one of the ten species investigated, *viz*., *O. articulata*.

Oxalis niederleinii, *O. floribunda*, *O. lasiopetala*, *O. perdicaria*, *O. conorrhiza*, *O. refracta*, *O. paludosa* and *O. hispidula* follow a monosporic, Polygonum-type development.

Oxalis corniculata forms a binucleate chalazal dyad member, a bisporic, Allium-type female gametophyte. Although most ovules of *O. perdicaria* follow the Polygonum-type development, occasionally a bisporic, Allium-type female gametophyte is formed. Herr and Dowd (1968) found in *O. corniculata* both monosporic and bisporic development, with the monosporic Polygonum-type less frequent. The presence of two types of female gametophyte development, monosporic and bisporic, within the same species is relatively rare (Maheshwari 1955). However, similar cases in other families of angiosperms were reported by Chopra and Agarwal (1960) for *Benincasa cerifera* (Cucurbita-

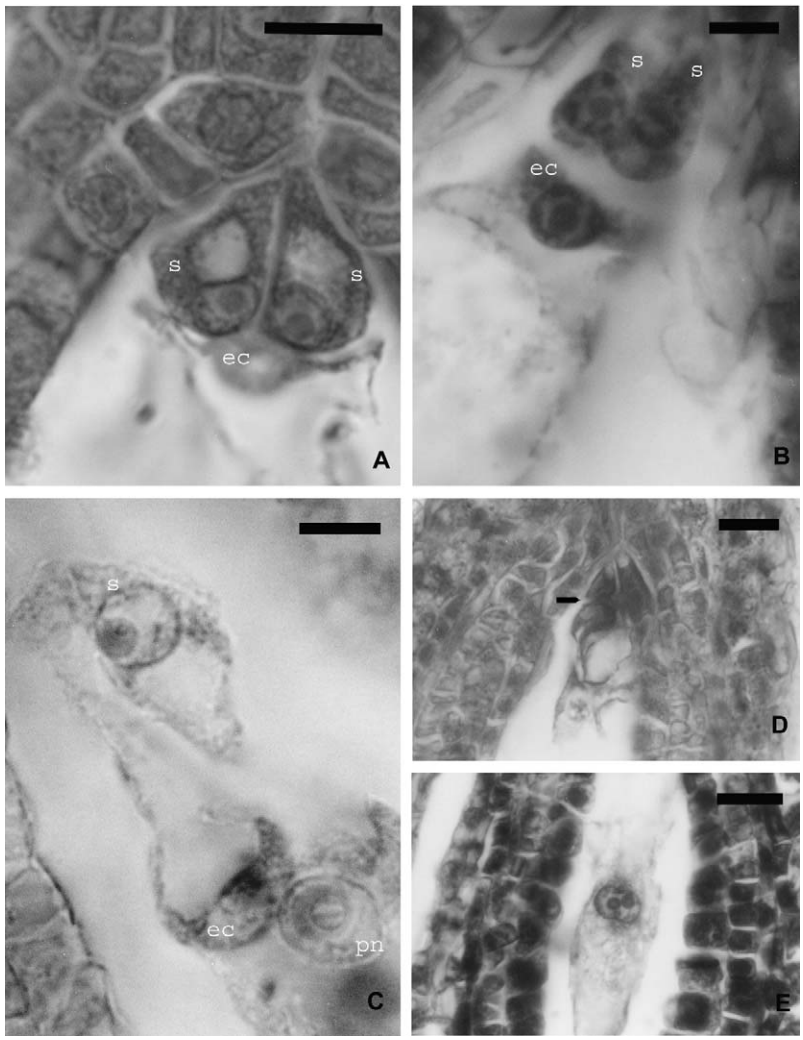

Fig. 5. Mature megagametophyte. — **A** and **B**: Detail of the egg apparatus showing the egg cell and the two synergids. — **A**: *Oxalis perdicaria.* — **B**: *O. niederleinii.* — **C**: Detail of egg apparatus and polar nuclei in *O. hispidula*. — **D**: Detail of egg apparatus. The arrow shows the synergid hook in *O. conorhiza*. — **E**: Detail of polar nuclei in *O. niederleinii.* s = synergid, ec = egg cell, pn = polar nuclei. — Scale bars: **A**: 2 *μ*m; **B** and **C**: 0.25 *μ*m; **D** and **E**: 0.5 *μ*m.

ceae), and by Johri and Vasil (1956) for *Ehretia laevis* (Boraginaceae). The presence of bisporic female gametophytes appears independently in several angiosperm taxa and requires further investigation (Herr & Dowd 1968).

Oxalis articulata follows tetrasporic, Adoxa-type development of the megagametophyte, and this is the only report of tetrasporic development for Oxalidaceae.

The morphology of the megagametophyte is similar in all the studied species. However, some of them are distinguished by the synergid morphology. *Oxalis lasiopetala*, *O. floribunda*, *O. conorhiza* and *O. articulata* have hooked synergids; *O. perdicaria* and *O. hispidula* have synergids thinner at the mycropilar end and partially extended into the micropylar channel.

In *O. lasiopetala*, *O. floribunda* and *O. articulata*, the antipodal cells degenerate earlier than in the other species, and in *O. refracta* and *O. niederleinii* these cells are located linearly in a strait caecum in the chalazal end of the female gametophyte.

Although morphological differences in the megagametohyte of the *Oxalis* species were found by us, they do not contribute to the sectional subdivision of *Oxalis* (Lourteig 2000). All the species studied in this work have a nuclear-type endosperm as reported for other Oxalidaceae by Thathachar (1942).

The development of the embryo corresponds to the Solanad type, because the first division

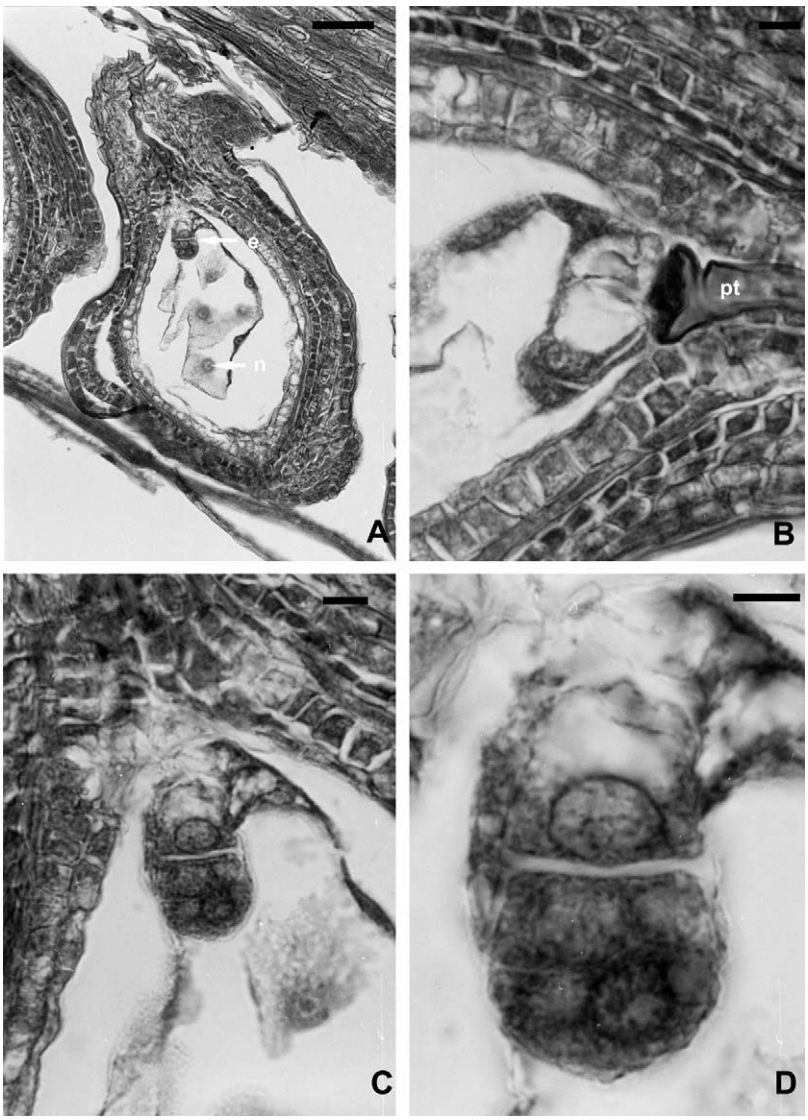

Fig. 6. Endospermogenesis and embryogeny in *Oxalis perdicaria*. — **A**: Longitudinal section of the ovule with nuclear endosperm and five-cellular embryo. — **B**: Detail of bi-celled pro-embryo and persistent pollen tube. — **C** and **D**: Five-celled pro-embryo. **D**: Detail of **C**. e = pro-embryo, n = nuclei, pt = pollen tube. — Scale bars: **A**: 4 μm, **B** and **C**: 1 μm, **D**: 0.5 μm.

of the apical cell is transverse and the basal cell gives rise to the suspensor. According to Narayana (1962), the development of the embryo in *Biophytum intermedium* and *O. pubescens* follows the Asterad type, since the first division of the apical cell is longitudinal. This feature does not occur in the species we studied here.

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