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Developmental Stages of Ovule and Megagametophyte in *Chenopodium botrys* L. (Chenopodiaceae)

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Abstract: The ovule ontogenesis and the megasporogenesis stages in *Chenopodium botrys* L. were studied with light microscopy. Flowers and young pods were removed from natural plants and fixed in FAA 70, stored in 70% ethanol, embedded in paraffin, and sectioned at 7 µm with a microtome. Staining was carried out with Hematoxylin and Eosin and developmental stages of ovule were studied. The results of this research showed that ovule development, including megasporogenesis and initial stages of megagametogenesis, occurred while flowers were still in bud. In *C. botrys* the female gametophyte has a monosporic origin and the developmental pattern exhibited by this species is referred to as the polygonum type. Development of ovule starts with the formation of a primordium. In this primordium, an archesporial cell produces a megaspore mother cell, which undergoes meiosis, forming a linear tetrad. The micropylar cell is a functional megaspore that survives and will function in megagametophyte development. The mature gametophyte is composed of 7 cells: 1 secondary nucleus, 2 synergids, 1 egg cell, and 3 antipodal cells.

Key Words: *Chenopodium botrys*, embryo sac, megagametogenesis, megasporogenesis, ovule development, Chenopodiaceae

Introduction

Chenopodiaceae family is a member of Centrospermae, which covers about 100 genera and 1500 species (Datta, 2003). The members of this family are distributed chiefly in Australia, the Karro (South Africa), the Red Sea shores, the South-West Caspian coast, Central Asia, and the salt steppes of East Asia. Most of the Chenopodiaceae are halophytes, adapted to grow in salty or alkaline soil. Since this necessitates the reduction of transpiration, these plants show xerophytic characters (Singh & Jain, 1999).

In Iran, Chenopodiaceae is represented by 41 genera, one important of which in Iran is *Chenopodium* L. (Assadii, 2001). This genus is characterised by its distinctive reduced succulent leaves, spike-like compound inflorescences, comprised of paired cymules of tiny flowers that are sessile within succulent free bracts. These flowers have 5 free perianth lobes, 5 stamens, a single, unilocular, and superior ovary; 1 bitegmic, crassinucellar, and campylotropous ovule with a basal solitary (Zhu et al., 2003; Shepherd et al., 2005). In the genus *Chenopodium*, like other members of Centrospermae, the whole seed is occupied by a long curved embryo, except for a central mass of perisperm (Siew Young et al., 1975).

There exist comprehensive studies involving members of this family on the embryo sac (Fischer, 1880; Dahlgren, 1916; Maheshwari, 1950, 1963; Davis, 1966), on endosperm (Hegelmaier, 1885), and on seed coats (Netolitzky, 1926).

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Other studies dealing specifically with Chenopodiaceae also emphasize these phases of seed study, only briefly describing the extensive embryo work has been done (Soueges, 1920; Marion, 1932; Ernest et al., 1961; Prego et al., 1998).

The study of the development of the embryo in Chenopodiaceae is suggested, because although many studies have described the megagametogenesis and megasporogenesis in Angiosperms, few of Chenopodiaceae have been studied from a viewpoint of the embryo development, and also Chenopodiaceae is a taxonomically difficult group, largely due to the lack of diagnostic characters available; identification of the embryological characters in this family can be useful for determining taxonomic relationship between its members (Marion, 1932; Shepherd et al., 2005). Moreover, the flowers appear as the stem elongates, mature seeds may be found on the same branch unfertilized ovules. For this reason, selection of embryo in successive stages of development is easily made (Marion, 1932).

The objective of this work was to determine the developmental stages of ovule and embryo sac in *Chenopodium botrys* L. Based on our knowledge, this is the first report about ovule and megagametophyte development in *C. botrys.*

Materials and Methods

Plant material

Young flowers and pods at different sizes were collected from natural habitats. Inflorescence material for the characterization of stages of reproductive development was collected in the summer of 2007 from Hame Kasi (Hamedan, Iran). A voucher specimen was placed in Bu-Ali Sina Herbarium (BASUH, 1037), Botany Department at the same university.

Cytological studies

The flowers and buds were fixed in FAA 70 (formaldehyde, glacial acetic acid, and 70% ethanol, 5:5:90 v/v), (Johansen, 1940), stored in 70% ethanol. Specimens were embedded in paraffin. The blocks were sectioned at 7 µm with a Leitz 1512 microtome (Germany). Staining was carried out with Hematoxylin-Eosin according to the protocol suggested by Meyer (Yeung, 1984).

Several sections were studied under a light microscope, Zeiss Axiostar Plus (Germany), for each embryonic sac and ovule developmental stage. Developmental stages that were examined in this research work were according to Maheshwari's criteria (1963) that are including ovular primordial, archeosporial cell, megaspore cell, dyad and tetrad cells, mitotic division in coenocytic embryo sac, and mature embryo sac. At least 20 samples were studied for each above mentioned stages of reproductive developmental characters.

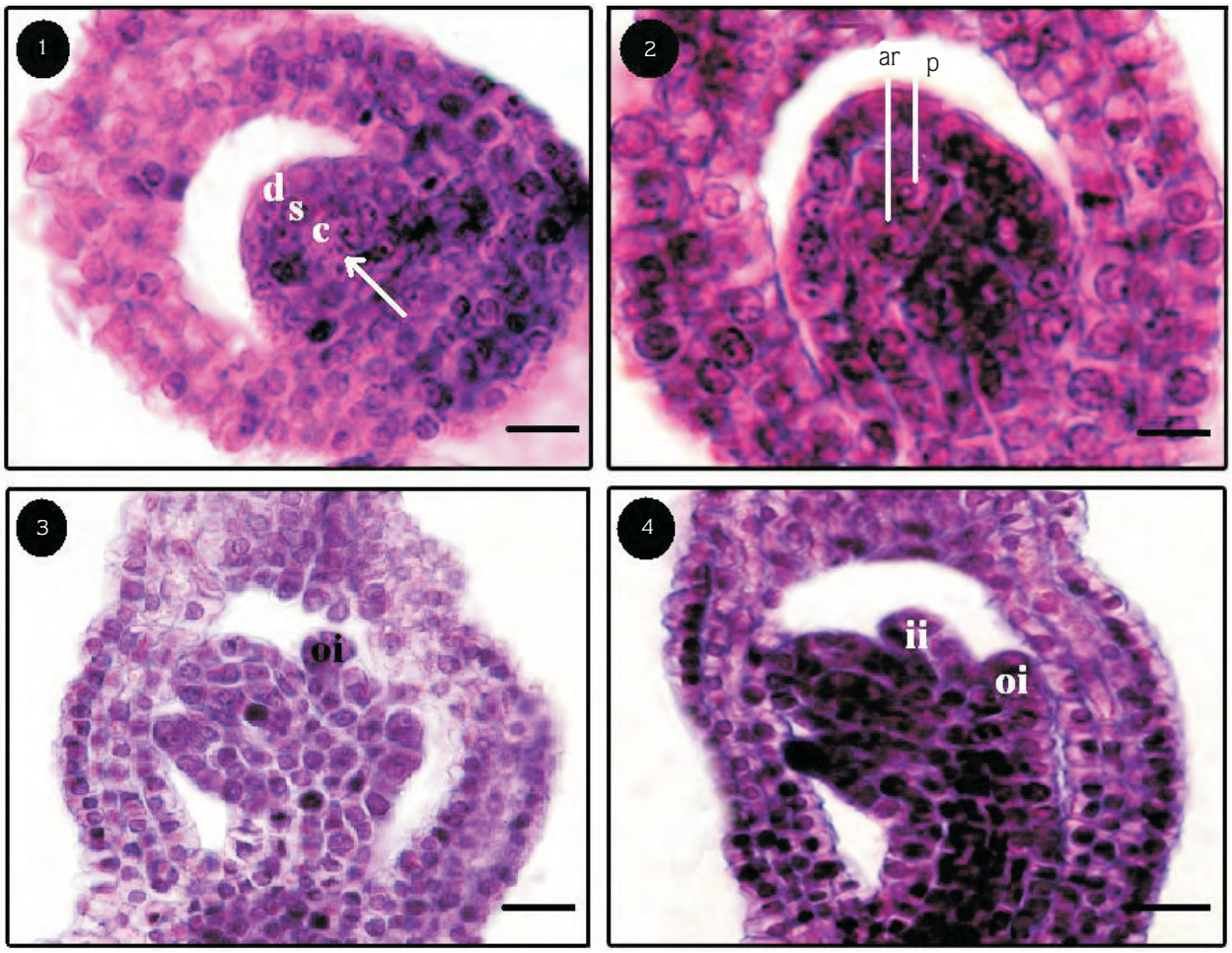
Results

Ovule development was investigated in *Chenopodium botrys.* Female gametophyte development, including megasporogenesis and initial stages of megagametogenesis, occurred while flowers were still in bud. The carpel is already closed when the first primordium appears (Figure 1). Ovule initiation is basipetal and starts with mitotic activity in meristematic regions organized in 3 layers: dermal (nucellar epidermis), subdermal, and central (Figure 1). In general, one of hypodermal cells enlarges and functions as an archesporium. In fact, this cell differentiates immediately below the nucellar epidermis. The initial archesporial cell is distinguished from the other subdermal cells, because it presents a larger volume, dense cytoplasm, and distinct nucleolus. This cell divides periclinally to give rise to the primary parietal cell outerly and the archesporial cell proper innerly (Figure 2).

The primary parietal cell undergoes periclinal, anticlinal, and/or oblique divisions, contributing to form nucellar parietal layers. Simultaneously with the division of the initial archesporial cells, 2 integuments initiate from divisions of dermal cells. The inner integument develops asymmetrically, rising from the distal primordium flank (Figure 3).

The outer one differentiates simultaneously as a ring around nucellus (Figure 4) but in some samples differentiation of outer integument occurs sooner than the inner one.

Following this stage, the archesporial cell proper grows and produces a megaspore mother cell (megasporocyte). With the production of this cell, megasporogenesis begins. As a result of this process megaspores are formed. The megaspore mother cell is a large cell, with many small vacuoles. In this stage, 2 integuments of ovule are well defined, both of which have 2 layers of cells (Figure 5).



Figures 1-4. Photomicrography of longitudinal section of the carpel.

1. Ovule primordium initiation with 3-zonate organisation: dermal, subdermal, and central (6500×).
2. Parietal cell that produces nucellar parietal layers and archesporial cell proper in subdermal position that produces megasporocyte (6500×).
3. Dermal origin of the integuments, outer integument (4000×).
4. The outer and inner integuments have initiated the formation of the inner integument; the integuments develops asymmetrically and differentiation of outer integument occurs sooner than the inner one (5100×).

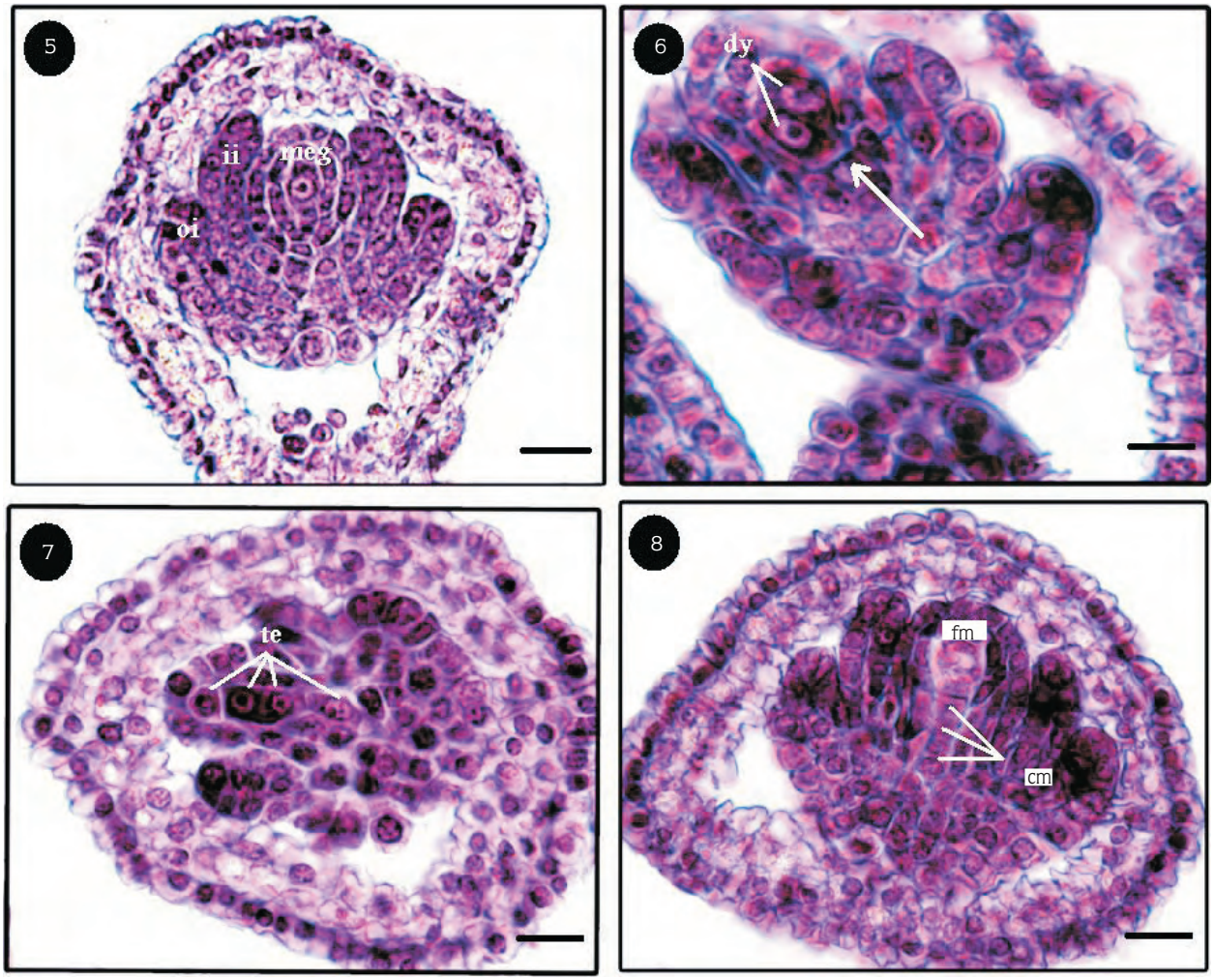
d = dermal layer; s = subdermal layer; c = central layer; p = primary parietal cell; ar = archesporial cell proper; oi = outer integument; ii = inner integument. Scale Bar = 40 µm in Figures 1 and 2; 50 µm in Figures 3 and 4.

Megasporogenesis follows and the diploid megaspore mother cell undergoes meiosis and gives rise to 4 haploid megaspore nuclei. The first meiotic division of megasporocyte (megaspore mother cell) produces equally-sized dyad cells that are easily identified. Concomitant with this stage, periclinal divisions in parietal cells add to the nonsporogenous layers that are typical of crassinucellate ovules (Figure 6). During this floral stage, megasporogenesis within the developing ovule has also been progressing. A second meiotic division produces a linear shaped tetrad of megaspores (Figure 7).

Only 1 of 4 megaspores, usually the one that is farthest from the micropyle, develops in to embryo sac but in *C. botrys* the chalazal megaspores soon degenerate and micropylar megaspore survives and functions as a functional megaspore. In fact in each ovule examined, the micropylar cell was larger and showed no signs of degenerating. In this functional cell vacuolation begins rapidly (Figure 8). The development of the functional megaspore through meiotic divisions of megasporocyte and degeneration of the 3 chalazal megaspores sets the stage for megagametogenesis (Figures 9-12).

In *C. botrys* in this stage, several changes take place in gross ovule morphology. The micropyle becomes well defined by the final elongation of the inner integument and expansion of integumentary cells in the micropylar area. The outer integument undergoes further elongation and is barely subequal to the inner integument. During this interval, the functional megaspore shows dramatic enlargement and the vacuole expands and occupies most of the cell volume (Figure 9). At the next stage, a mitotic division within the functional megaspore produces 2 nuclei, 1 of which moves towards the chalazal end and other one towards the micropylar pole of the embryo sac (Figure 10). A second mitotic division results in the 4-nucleate stage (Figure 11). The embryo sac now undergoes a dramatic increase in width and length and this growth occurs with slow consumption of nucellus, thus in this stage of ovule development the nucellar tissue around the embryonic sac degenerates slowly (Figure 12).

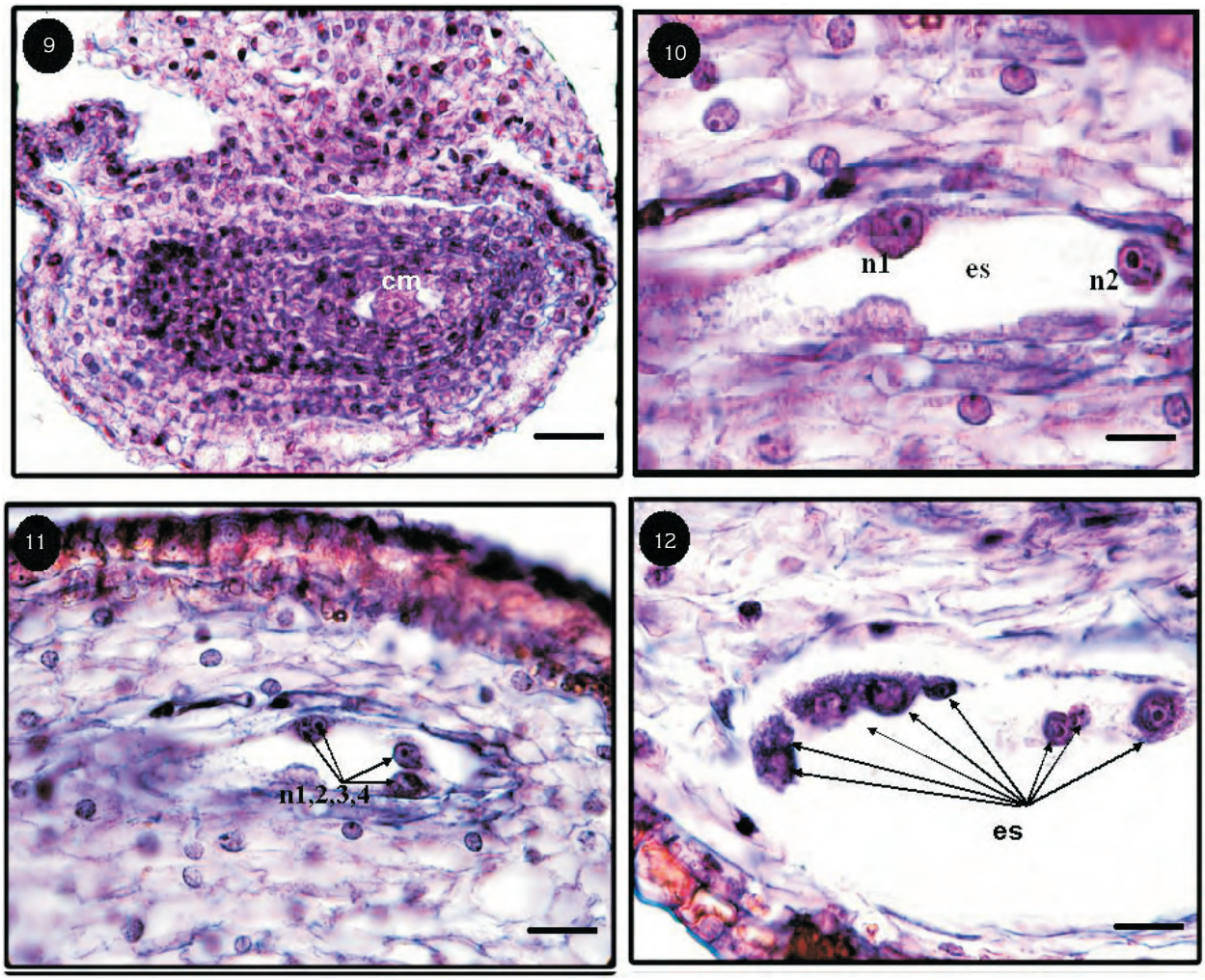
The third mitotic division produces 8 nuclei, thus after the third mitotic cycle, the 8-nucleated gametophyte



Figures 5-8. Photomicrography of longitudinal section of the carpel.

5. Megaspore mother cell that begins megasporogenesis, 2 integuments of ovule are defined, both of them have 2 layers of cells (4000×).
6. Equally-sized dyad cells, produced by the first meiotic division of megasporocyte, divisions in parietal cells add to the nonsporogenous layers (6500×).
7. Linear shaped tetrad megaspores produced by the second meiotic division of megaspore mother cell (5100×).
8. Functional micropylar megaspore when vacuolation begins and 3 degenerating chalazal megaspores (5100×).

meg = megasporocyte; dy = dyad; cm = chalazal megaspores; te = tetrad; fm = functional megaspore. Scale Bar = 50 µm in Figures 5, 7, and 8; 40 µm in Figure 6.



Figures 9-12. Photomicrography of longitudinal section of the carpel.

9. Megaspore mother cell (5100×).

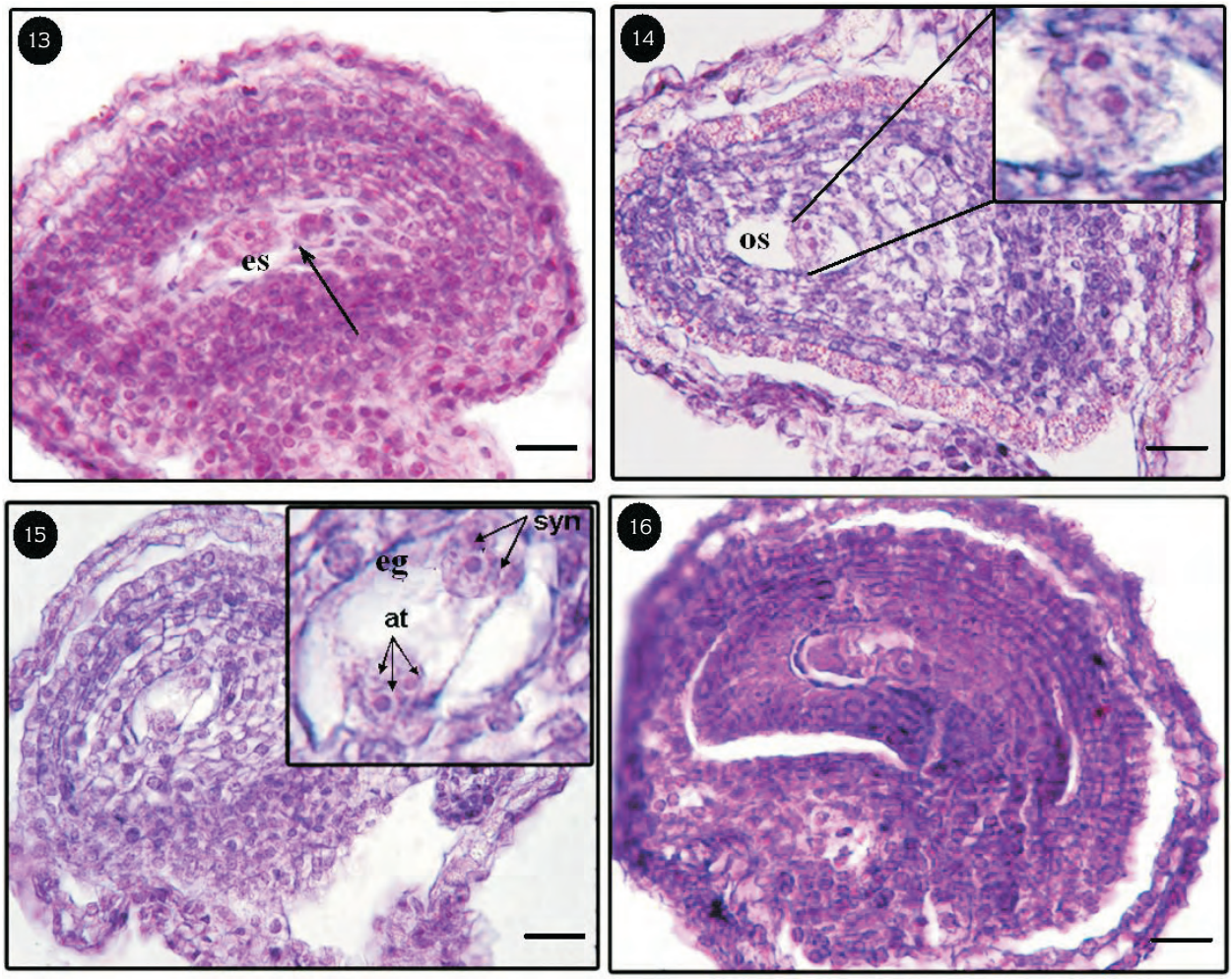
10. Two-nucleated embryonic sac, one of the nuclei stablishes in the chalazal end and other one in the micropylar end of the embryo sac (8100×).

11. Four-nucleated embryo sac (6500×).

12. Eight-nucleated embryo sac, expansion of the embryo sac that occurs with the consumption of nucellus (8500×).

cm = mother cell megaspore; n = nucleus; es = embryo sac, Scale Bar = 80 µm in Figure 9; 30 µm in Figures 10-12.

is presented with 4 chalazal and 4 micropylar nuclei (Figure 12). Development of a 8-celled megagametophyte occurs during the final floral bud stage (Figures 13-16). According to this stage nucellus cells slowly degenerate and their remains are visible around the embryo sac, also 3 of 4 chalazal nuclei, after cellularisation, establish a row of wall-less cells known as the antipodals that degenerate later. Besides, 3 of 4 nuclei at the micropylar end of the embryo sac become organised in to the egg apparatus, consisting of 2 synergid cells and 1 egg cell. The last chalazal nucleus and its micropylar neighbor migrate to the mid-region of the central cell, and compose the 2 polar nuclei. The polar nuclei fuse and form a diploid nucleus that calls the secondary endosperm nucleus (Figure 14). Thus in this stage embryo sac consist of a secondary endosperm nucleus within the central cell, 2 synergids, and an egg cell at the micropylar end and 3 antipodals at the chalazal end (Figures 14,16). Furthermore, all of the cells within the female gametophyte differentiate into polar strictures. In *C.*



Figures 13-16. Photomicrography of longitudinal section of the carpel.

13. Embryo sac in *Chenopodium botrys*, remains of nucellar cell (5100×).

14. Two polar nuclei that fuse and form the secondary nucleus (5600×).

15. Egg apparatus, consisting of 2 synergid cells and 1 egg cell at the micropylar end, and antipodal cells at the chalazal end (5600×).

16. Mature campylotropous ovule in *Chenopodium botrys* (5100×).

es = embryo sac; os = oosphere; at = antipodal cells; syn = synergid cells; eg = egg cell, Scale Bar = 80 µm in Figures 13-16.

botrys, the egg cell nucleus is located toward the chalazal end and its vacuole occupies the micropylar end, by contrast synergid and central cells have the opposite polarity.

Discussion

This report is the first to provide a detailed ovule and embryo sac development in *Chenopodium botrys.* The results presented indicate that many sexual reproductive features expressed in *C. botrys* are similar to those of other members of the Chenopodiaceae. Ovaries of *C. botrys* exhibited 1 locular carpel and 1 ovule within carpel. Current observations showed that patterns of embryo sac development are evidence of standard polygonum type and pattern of the megasporogenesis in this species is monosporic, which is the most common type of development in Angiosperms.

Megasporangium or ovule in *C. botrys* is basipetal and consists of nucellus and 2 integuments. In this species, the ovule is curved, called as campylotropous, which is in agreement with observation reported by Bocquet (1959).

It has 2 integuments, each being 2 thick cells that is in accordance with the findings of Sherry et al. (1933) and Corner (1976). Differentiation of 2 integuments is concomitance but in some cases differentiation of the inner one occurs sooner than that of the outer one.

Depending on the extent of development of the nucellus, ovule in *C. botrys* is crassinucellate type, because in this species there is a well-developed parietal tissue and an archesporial cell is separated from the nucellar epidermis by 1 layer of cell. This finding is in agreement with the finding of Connor (1984).

During the megasporogenesis, as a result of meiotic divisions, a linear tetrad of 4 megaspores is formed. The micropylar megaspore of tetrad serves and gives rise to the female gametophyte, while the remaining 3 megaspores degenerate and disappear; this is a functional megaspore. Our report is also the first about micropylar functional megaspores.

The mature form of female gametophyte or embryo sac of *C. botrys* like embryonic sac of other Chenopodiaceae has 7 cells, 8 nucleate consisting of 3 antipodal cells; a large binucleate central cell and an egg cell adjacent to 2 synergids, that is in accordance with the findings of previous researchers regarding other members of Chenopodiaceae (Marion, 1932; Stebbins, 1974) (Figures 13-16).

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