INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
08–224/SBC/2009/11–5–601–605
http://www.fspublishers.org

Full Length Article

Pollen Grain and Ovule Development in *Lepidium vesicarium* (Brassicaceae)

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ABSTRACT

The pollen grains and ovule developmental stages in *Lepidium vesicarium* L. were studied with light microscopy. Young pods and flowers were removed at different stags of development (tiny buds to mature flowers). The anther and ovule development were studied with light microscopy after staining. Results showed that un-differentiated anther is ovoid-shaped and tetrasporangiated. The anther wall development follows the dicotyledonous type, which is composed of an epidermal layer, an endothelial layer, one middle layer and tapetum. The tapetum is secretory at the first and plasmodial type at the end of anther development. Tapetal cells are uniseriate and uni or binucleate. The microspore tetrads are tetragonal. Pollen grains are spherical to ovate, triporate and tricellular at the mature form. The results of this research showed that ovule development, including megasporogenesis and initial stages of megagametogenesis, occurred, while flowers were still in bud. In *L. vesicarium* 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 primordium. In this primordium, an archeosporial cell produces a megaspore mother cell, which undergoes meiosis, forming a linear or T-shaped tetrad. The micropylar cell is functional megaspore that survives and will function in megagametophyte development. The mature gametophyte is composed of 5 cells, one secondary nucleus, two synergids and one egg cell. There are three antipodal cells that are degenerate in the mature embryo sac before fertilization.

Key Words: Development; Anther; Pollen grains; Ovule; Megagametophyte; *Lepidium vesicarium*

INTRODUCTION

The genus *Lepidium* is one of the largest genera of the Brassicaceae consisting of about 175 species. It is distributed world wide, primarily in temperate and subtropical regions; the genus is poorly represented in arctic climates and in tropical areas it grows in the mountains (Al-Shehbaz, 1986a & b). *Lepidium vesicarium* (L.) is a biennial or annual species that distinct in the genus and the family morphologically on account of the nodal swellings (Rechinger, 1968).

There are some reports about taxonomically important characters in the plant family such as, variability in the larger megaspore of tetrads, ovule type, number of archesporial cells, number of parietal layers and the alignment pattern of the integuments (Davis, 1966; Rembert, 1969a, b & 1971; Prakash, 1987; Yeung & Cavey, 1990; Johri *et al.*, 1992; Johansson & Walles, 1993; Chehregani & Mahanfar, 2007; Chehregani *et al.*, 2008). The standard type of male gametophyte and polygonum type of female gametophyte is exhibited by >70% of flowering plants and is the pattern found in many economically and biologically important groups, including Brassicaceae (e.g., *Arabidopsis*, *Capsella*, *Brassica*), Gramineae, Malvaceae, Leguminoseae and Solanaceae (Maheshwari, 1950; Willemse & van Went, 1984; Haig, 1990; Huang & Russell, 1992). But, there are a wide variety regarding embryological characteristics. For example, in *Arabidopsis thaliana* at the chalazal end, the antipodal cells are initially present, but degenerate by the time of pollination in most embryo sacs (Jürgens *et al.*, 1991). Results of Tanawy *et al.* (2004) showed that seed characters are important and significant characters in Brassicaceae family. This family contains many useful and economically important species (Al-Shehbaz, 1986b; Zaitoun & Vorwohl, 2003).

But, based on our bibliographical studies *Lepidium* is not studied regarding embryological characteristics. The objective of this work is to analysis the male and female gametophyte development in *Lepidium vesicarium* (L.) not only for systematic comparisons, but also for the knowledge of micro and mega gametophyte developmental process.

MATERIALS AND METHODS

The experimental material was collected during April, 2007 to July, 2007. The voucher was deposited at the herbarium of Bu-Ali Sina University (BAUH).

To cite this paper: Chehregani, A. and M. Sedaghat, 2009. Pollen grain and ovule development in *Lepidium vesicarium* (Brassicaceae). *Int. J. Agric. Biol.*, 11: 601–605

Inflorescences at all ages (tiny buds to mature flowers) were removed and fixed in FAA 70 (20% formalin, 10% acetic acid, 70% ethanol, v/v), embedded in paraffin and sectioned with a thickness of 7 µm using a Micro DC 4055 microtome. Staining was done with PAS (Periodic Acid Schiff) according to protocol suggested by Yeung (1984) and contrasted with Meyer's Hematoxylin. Several sections for each anther and ovule developmental stage were observed and photographed under a Zeiss Axiostar Plus light microscope. For each stage, at least 20 flowers were studied and photomicrographs were made from the best ones.

RESULTS AND DISCUSION

Microsporogenesis and male gametophyte development. The buds in early developmental stages contained six anther primordia and a central ovular primodium enveloped by sepals (Fig. 1-A). Each young anther was consisted of 4 pollen sacs. Each pollen sac contained peripheral cells forming an un-differentiated wall and a mass of central archeosporal cells (Fig. 1-B). The wall differentiation took place and resulted in to epidermis, endothecium, middle layer and tapetum (Fig. 1-C). At this stage pollen mother cells were well differentiated and large in size with high density of cytoplasm. Each pollen mother cell increased in size and formed a special wall with callosic nature (Figs. 2-D, E). Meiosis caused the formation of tetragonal tetrads that surrounded with special callosic wall (Figs. 2-G, H).

Microspores in the four neighboring sporangia (pollen sac) synchronize in development (Fig. 1-I). The microspore just released from tetrads has no vacuole and somewhat irregular in shape, with a dense cytoplasm and a centrally placed nucleus (Figs. 1-J, K). As a large vacuole developed, cytoplasm together with nucleus was pushed towards microspore margin (Figs. 1-L, M). Then as a result, of an un-equal mitosis, microspore nucleus divided into two nuclei, a large vegetative nucleus and a small reproductive one to give rise a two-nucleate pollen grain (Fig. 1-N). Following second mitotic division in reproductive cell producing two sperms, pollen grain becomes tricellular (Fig. 1-O). The pollen grains shapes ranged from ovule, spherical and tricolporate (Fig. 1-O).

At the stage of microspore tetrad, the anther wall was composed of epidermis, endothecium and tapetum. Mean, while the middle layer was degenerated. During tetrads formation, taptums were differentiated mostly in binucleate or some in uninucleate. Taptum layer was also secretory type i.e., tapetal cells were degenerated *in situ* (Fig. 1-F). Prior to anthesis, the tapetal periplasmodia were consumed completely for the development of pollen grains. At the stage of anther dehiscence, there was one-layer anther wall of endothecium.

Megasporogenesis and female gametophyte development. The gynocium in *Lepidium vesicarium* was consisted of two carpels, a short style and a stigma with differentiated papilliform secretory cells. Two initially separated carpels by postgenital fusion fused to form a single structure. This phenomenon is also reported by Shamrov (2002). Within the ovary two locules were formed by a false septum (replum) with two ovules in each of them. Placentation was formed at parietal position and the ovular primordium was initiated by periclinal cell divisions (Figs. 2-A, B).

Ovule was anatropous, medionucellate, funicular and bitegomic. The mature ovule was about 300 µm long and 110 µm wide that is accordance with the finding of some researchers about other members of Brassicaceae (Maheshwari, 1950; Eames, 1961). At archesporium stage, ovule bending began and both integuments initiated at the base of the nucellus. So it looks that initiation of inner integument was earlier than outer one however, later grew faster than another (Figs. 2-C, D).

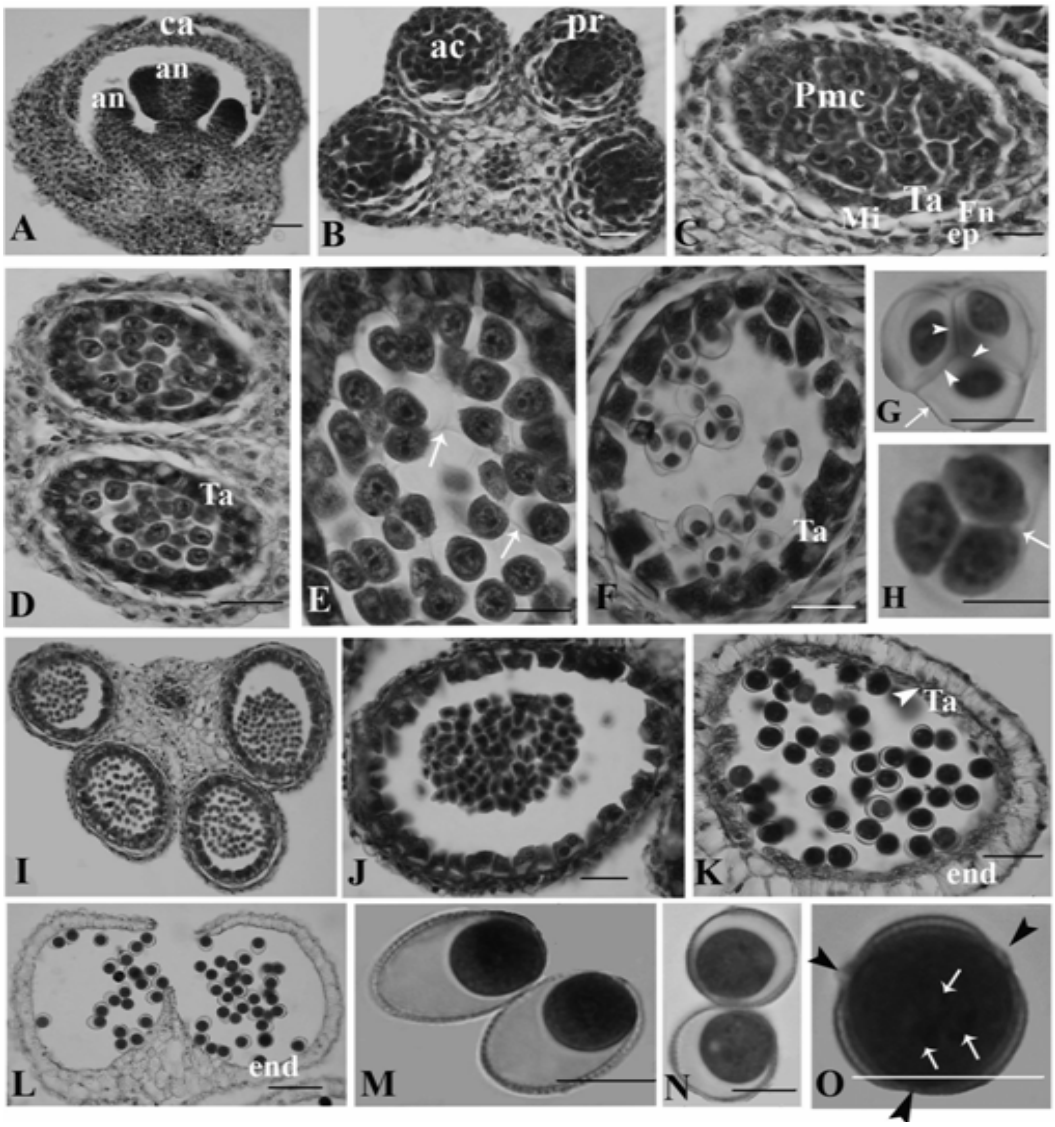
The female gametophyte had a monosporic origin and a polygonum type development. The megasporocyte was originated by enlargement and differentiation of a single hypodermal cell from third cellular layer of nucellus (Fig. 2-B, C). The megasporocyte grew in size and devided by meiosis to give rise linear tetrad (Fig. 2-D, E). T-shaped tetrad was formed with functional micropylar megaspore (Fig. 2-F). Sub-sequently, other three megaspores were degenerated.

The functional megaspore also under went three successive mitotic divisions that resulted to form 8-nucleate embryo sac. Sub-sequent nuclear migration and cytokinesis occurred during megagametogenesis (Figs. 2-G, H) eventually resulting in a mature embryo sac with the typical eight-nucleate form. Thus it consists of 7 cells: 2 synergid cells and one egg cell comprised egg apparatus, 3 antipodal cells and one central cell that contains 2 nuclei (Fig. 2-I). The antipodal cells were degenerated immediately in prefertilization stages (Fig. 2-I), that was also reported for *Arabidopsis-thaliana* (Jürgens *et al.*, 1991). The fusion of two polar nuclei resulted to form a diploid secondary nucleus, occurred adjacent to the egg apparatus and simultaneous with its fertilization by a single sperm nucleus for generating the triploid primary endosperm nucleus (Figs. 2-H, I).

In the mature ovule the inner integument was consisted of 1-2 layers whereas, the outer integument is 2-3 layered. The endothelial cells that differ in their size, form, structure and sub-stances become the endotegmen or pigment layer during seed development (Fig. 2-J). Globular embryo and its associated suspensor were produced resulting cell division of the zygote (Fig. 2-J). The cells of suspensor elongated and vacuolated considerably, but the cells of embryo were small in size and contained condensed cytoplasm without remarkable vacuoles. The embryo was elongated and cell differentiation was taken place during the growth (Fig. 2-L). Mature silique contained two seeds. Each seed was located in a locule and separated by a false wall (Fig. 2-K).

Fig. 1. Developmental stages of anther and pollen grains in *Lepidium vesicarium*; A, longitudinal section of a young bud with anther and ovular primordial that enclosed by sepals; B, Cross section of a young anther with four pollen sac; C, Cross section of a pollen sac that contains pollen mother cells (PMC); D, Cross section of anther with pollen mother cells that are starting meiosis; E, PMC at different stages of meiosis. Each PMC surrounded by a callosic wall. E, cross section of pollen sac that contains tetrads resulting from meiosis. Each tetrad surrounded by a callosic wall. Tapetum layer is secretary type. G and H, tetrad that are separating from together. I and J, Pollen sac that contain young microspores. K, Pollen sac that contains developing microspores. Formation of Exin and degeneration of tapetum layer (↑) are visible. The type of tapetum layer if plasmodial type in this stage. L, Mature anther that are dehiscence and contains mature pollen grains. Anther wall consist of just endothecium layer. M and N, mature pollen grains from different views. Exin are formed and pollen grains vacuolated considerably. O, Mature pollen grain with three pores, a vegetative nucleus and two spermal nuclei. Scale bars are 30 µm.

Abbreviations: An, anther; ca, calyx; pr, pollen sac layers; ep, epidermis; En, endothecium; Mi, middle layer; Ta, Tapetum layer; end, endothecium layer at the mature stage



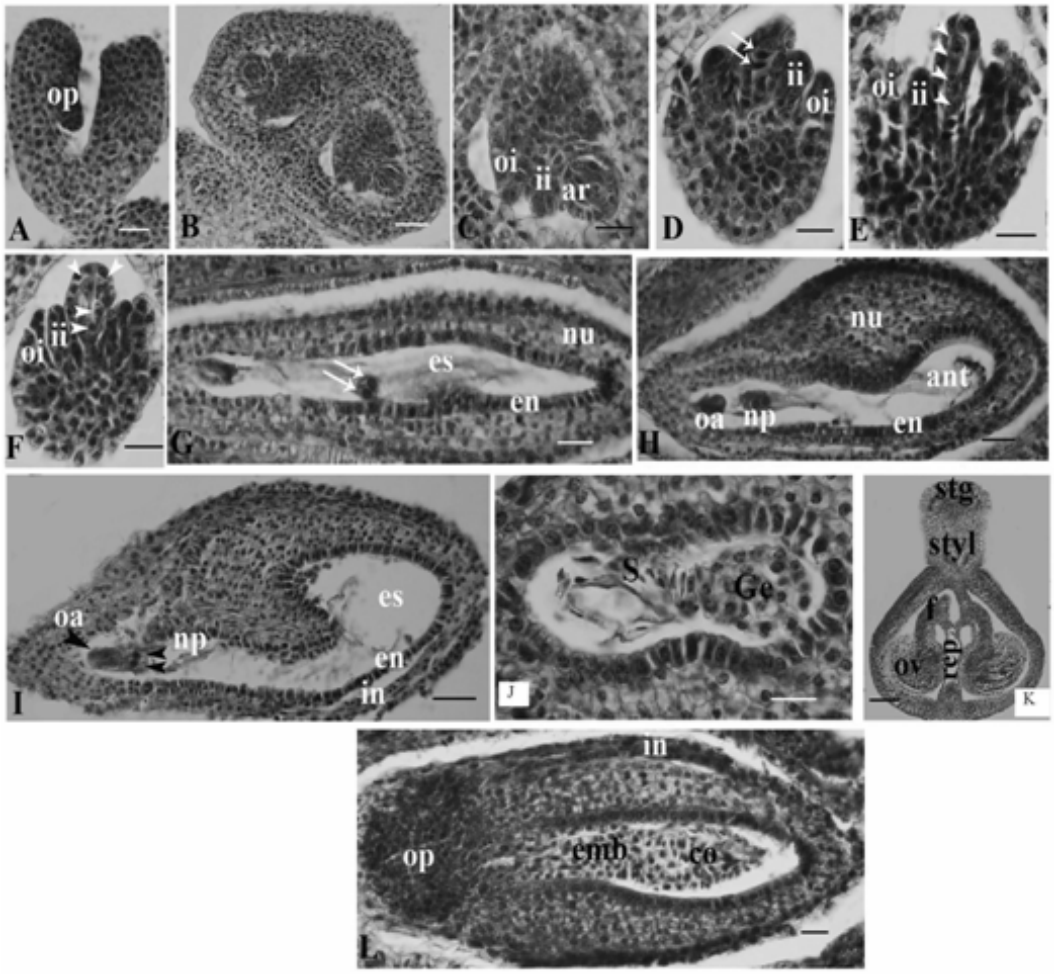
Although our results are partly accordance with the finding of some prior reports about *Arabidopsis thaliana* (Mansfield & Briaty, 1991a & b) and *Capsella bursa-pastoris* (Shamrov *et al.,* 2002), but are the first report about the genus of *Lepidium.*

CONCLUSION

In the studied species, the anther wall development follows the dicotyledonous type, which is composed of an epidermal layer, an endothelial layer, one middle layer and

Fig. 2. Developmental stages of ovule in *Lepidium vesicarium*; A, longitudinal section of a young ovary with an ovular perimordium; B, two locular ovary that are contain a young ovule in each locule; C, Ovule that shows developing integuments and an archeosporial cell; D, Ovule that show first meiotic division of megagametocyte; E, Ovule at the tetrad stage that are arranged linearly; F, Ovule with tetrads that are arranged as T-shaped form; G, developing embryo sac that fusion of polar nuclei (arrows) is visible; H, mature embryo sac. Polar nuclei (np) are migrated toward the micropylar end and ovular apparatus (oa). Antipodal cells (ant) are degenerating; I, mature embryo sac at the pre fertilization stage. Polar nuclei (np) are attached to ovular apparatus (oa). In this stage antipodal cells were degenerated and disappeared; J, young embryo at the globular stage (Ge) that attached to ovary wall through suspensor; K, mature ovary with two locules that separated by a false wall (rep), each locule contains an anatropus ovule; L, mature ovule with a mature embryo (emb) that two cotyledons (co) are visible. Opercolum (op) is visible at the micropylar end. Seed coat is formed by fusion of integuments (in). Scale bars are 30 µm.

Abbreviations: Op, ovular perimordium; ar, archeosporial cells; ii, inner integument; oi, outer integument; es, embryo sac; en, endothelium; nu, nucellus tissue; oa, ovular apparatus; np, polar nuclei; ant, antipodal cells; in, integument; S, suspensor; Ge, globular embryo; Ov, ovule, f, fonicule; stg, stigma; co, cotyledon; emb, embryo; op, operculum



tapetum. The tapetum is secretory at the first and plasmodial type at the end of anther development. Tapetal cells are uniseriate and uni or binucleate. The microspore tetrads are tetragonal. Pollen grains are spherical to ovate, triporate and tricellular at the mature form. The female gametophyte has a monosporic origin and the developmental pattern exhibited is referred to as the polygonum type. Development of ovule starts with the formation of primordium. In this primordium, an archeosporial cell produces a megaspore mother cell, which undergoes meiosis, forming a linear or T-shaped tetrad. The micropylar cell is functional megaspore that survives and will function in megagametophyte development. The mature gametophyte is composed of one secondary nucleus, two synergids and one egg cell. Antipodal cells were degenerate in the mature embryo sac.

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(Received 07 July 2008; Accepted 10 February 2009)