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ORIGINAL ARTICLE

Development of male and female gametophytes and embryogenesis in the *Arabidopsis thaliana*

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Abstract

Sporogenesis and gametophytes development in *Arabidopsis thaliana* (*Brassicaceae*) was studied. *Arabidopsis thaliana* was selected for this study as a biogenetic model with a small genome and a short life cycle. Flowers and buds in different developmental stages were removed, fixed in an FAA_{70}, stored in 70 % ethanol, embedded in paraffin and sectioned at 7 − 10 μm with a microtome, stained with Hematoxylin – Eosin and analyzed with a photomicroscope. The results showed the anthers are tetrasporangiate, anther tapetum is of secretory type at the beginning and plasmodial at the end of anther development. Also, microspore tetrads are tetrahedral and rarely tetragonal. In addition, mature pollen grains are spherical, tricolporate, scabrate, and tricellular. The gynoecium is bicarpellate and the ovule is amphitropous, bitegmic, and tenuinucellate. Hypostasis was also observed in basis of nucellus. Endothelium is composed of one cell row and cell divisions of megaspore mother cell results in T-shaped tetrad. The chalazal cell is a functional megaspore that survives and functions in megagametophyte development. The embryo sac development belonged to the Polygonum type and it is curved. The mature embryo sac is composed of 7 cells, one central cell containing polar nuclei, two elongated synergids, a triangular oosphere cell, and three antipodal cells that are degenerated immediately by developing the embryogenesis stage in the mature embryo.

Keywords *Arabidopsis thaliana* · *Brassicaceae* · Embryogenesis · Gametophyte

Introduction

The genus *Arabidopsis* belongs to *Cruciferae* family. The members of this family are distributed in all continents of the world. However, north temperate regions, Mediterranean and the Iran – Turan regions show the most population (Rollins 1941). As currently delimited, the *Brassicaceae* comprise 49 tribes, 321 genera, and 3660 species. Of these, 20 genera and 34 species remain to be assigned to tribes. These figures differ substantially from those estimated five years ago, in which 25 tribes, 338 genera, and 3709 species were recognized. Of those 338 genera, 37 are treated herein as synonyms, and 21 genera (10 reestablished and 11 new) have since been added (Al-Shehbaz 2003). There is no exact information about the geographical origin of this plant. (Rédei 1975). *Arabidopsis thaliana* evolved in Southwest Asia (Rédei 1975).

A number of features make *Arabidopsis* suitable for classical experimental genetics. These include their small size, rapid generation time (5–6 weeks under optimum growth conditions), the ability to grow well under controlled conditions (either in soil or in defined media), high productivity (up to 10,000 seeds per plant), easily maintained mutant line e.g. by self-fertilization, and ease with which a mutant line can be out-crossed (Page and Grossniklaus 2002). Since the second half of the twentieth century, when Rédei (1975) introduced *A. thaliana* as a proper herbal model in scientific researches, taxonomists decided to remove *Arabidopsis* from the rank of section and promoted it to the rank of genus (Al-shehbaz 2003). The genus *Arabidopsis* was firstly introduced by *A. thaliana* species. Indiscriminate expansion of the genus, merely due to the similarities in linear fruits and branched trichomes, brings the number of species of genus to 59 species (O'Kane and Al-Shehbaz 1997). Unscientific

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development of the genus provoked the researchers to start extensive studies on the field of molecular, genetic and phylogenetic. Research results led to the removal of 50 species from this genus (Koch et al. 2001). At present, *Arabidopsis* genus includes 9 species and 8 subspecies, 9 species are all native to Europe and 2 species are seen in the north and northeast Asia (Koch et al. 2001). Morphological characters of the ovules and megasporogenesis stages can be used in systematic studies for defining the circumscription of taxa (Rembert Junior 1977). Pollen morphology of 8 species of the genus *Arabidopsis* (*Brassicaceae*) from Pakistan has been examined by light microscopy (Khan 2004) but male and female gametophytes have special characters that show a great variety in different taxa (Johri et al. 1992).

Although morphological and anatomical traits was used as the most important character for separating of the species (Aghajafari et al. 2013) but there are also some reports about taxonomically important of the external morphology and anatomy of the seeds in the *Cruciferae* family such as relationships of seed structure to taxonomy (Vaughan and Whitehouse 1971). During most of this time, systematics and the evolutionary history of the genus *Arabidopsis* were not an issue and they were not much taken into account until recently. Consequently, there is a parallel and often confusing taxonomic history regarding species and genus delimitations (Koch 2019). Historically, many of the researches focused on cytological investigation of seed development following interploidy crosses in *A. thaliana* (Scott et al. 1998; Yadegari and Drews 2004) studied about female gametophyte development in this plant but with molecular and genetic approach. Kasahara et al. (2012) studied about fertilization recovery phenomenon in *A. thaliana*. Koch and Matschinger (2007) studied genetic variation of all evolutionary lineages of *A. thaliana* relatives based on a representative geographic sampling by studying maternally inherited chloroplast DNA (cpDNA) haplotype variation and sequence diversity of the internal transcribed spacer region (ITS) of ribosomal RNA. 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 1966a, b; Rembert 1969a, b, 1971; Prakash 1987; Yeung and Cavey 1990; Johri et al. 1992; Johansson and Walles 1993; Chehregani and Mahanfar 2007; Chehregani and Tanomi 2010). Male and female gametophytes have special characters that show a great variety in different taxa (Johri et al. 1992). However, based on bibliographical studies, there is no report about detailed study of the early stages of flower development in *A. thaliana*. In this paper for the first time we describe sporogenesis, gametophytes and embryogenesis development in *Arabidopsis thaliana* from their first appearance as a buttress on the apical meristem until they open as fully developed flowers in details.

Materials and methods

Arabidopsis thaliana (L.) Heynh. var. columbia Rédei (Somssich 2018) plantlets were grown from seeds purchased from the Iranian National Institute for Genetic Engineering and Biotechnology (Tehran, Iran). The seeds were sterilized and cultured into pots 12 cm in height and 13 cm in diameter filled with the soil composed of perlite, vermiculite, and peat, 1 : 1 : 1. The experimental condition involved 25 ± 2 °C and 15 h day light in a greenhouse. Flowers and buds, in different developmental stages, were removed. The collected plant materials were fixed in FAA (37% formalin, 100% (glacial (acetic acid, and 70% ethanol, 2 : 1 : 17), stored in 70% ethanol, and following dehydration in an ethanol series and embedding in paraffin the specimens were sliced by a Micro DS 4055 rotary microtome (Cut 4060 Co., Mainz, Germany). Staining of serial sections of 8 µm was carried out with Hematoxylin-Eosin, according to the protocol suggested by Yeung (1998). Several slices were studied for each developmental stage using a light microscope (Nikon, Tokyo, Japan) equipped with BEL digital camera (Model BLACKL.3000). At least 20 specimens were studied for each developmental stage and the best ones were taken for photomicrographs.

Results

Anther and pollen grain development

Initially, stamens appear as the stamen primordia under the sepals cover which then grow rapidly (Fig. 1c). There are four middle and two lateral stamens, the former being longer than the later (Fig. 1a, b, h). Anthers are separate from each other (Fig. 1g) and with the four distinguishable pollen sacs (Fig. 1e, f). Pollen mother cells (PMC) are differentiated with large size and high density of cytoplasm in 2–3 rows, and are observed in sporangia (pollen sacs) (Figs. 1f and 2a). The anther walls, from outside to inside, consist of epidermis, endothecium, middle layer, and tapetum, each of them being formed in one cell row (Figs. 1f and 2a).

The tapetum cells with different size and shape as a particular layer surround the pollen mother cells (Fig. 2e), which are rectangular, spindly, one-nucleate, and polynuclear (Figs. 2e and i and 3.d). They are initially fixed in their

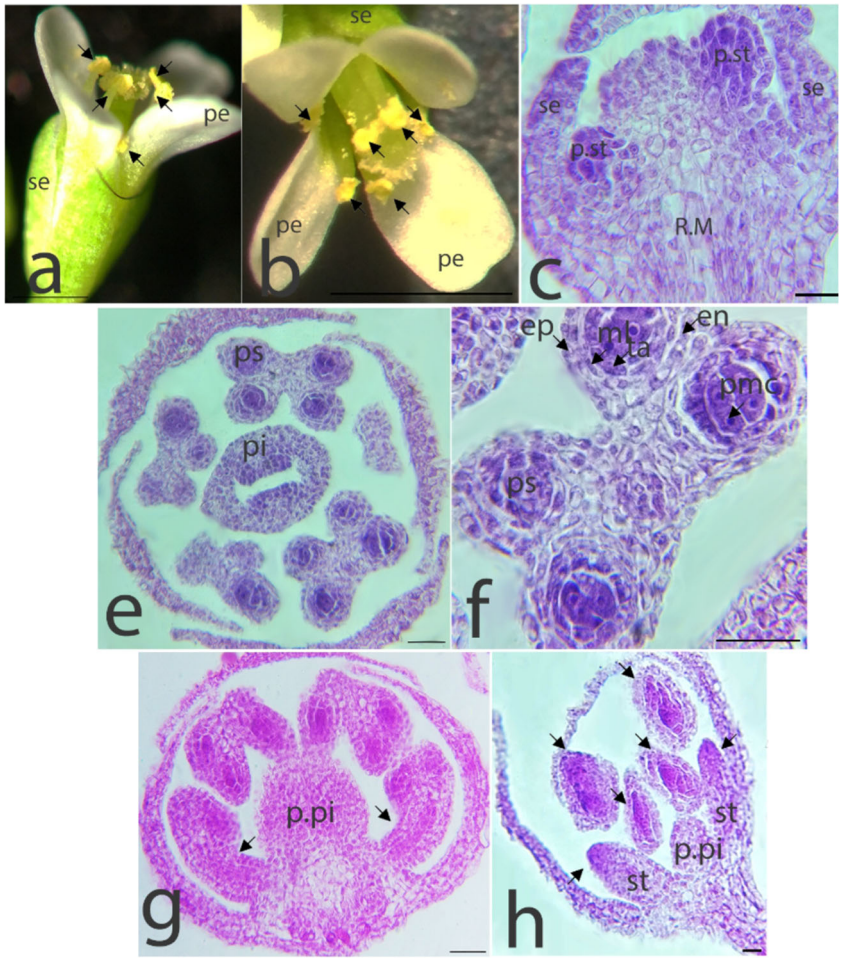

Fig. 1 The flower formation development from the generative meristem staining Hematoxylin-Eosin; **a** and **b** The flower rings (scale bar 1 mm) show 4 middle and 2 lateral stamens. **c** The appearance of stamen primordium; **e** Flower rings; **f** The anther composed of 4 pollen sac and their walls; **g** The anthers development separated from each other; **h** Showing the middle stamens with ( ), 2 lateral stamens with ( ); St: stamen, et: endothecium, ep: epidermis, sty: style, mi: middle layer, ps: pollen sac, pmc: pollen mother cell, ppi: primordium pistil, R.M: receptacle meristem, se: sepal, p.st: primordium stamen; Scale bars are 50 μm

location and are of secretory type (Fig. 2e) and then plasmodial type at the end of anther development (Fig. 3c, g). Initially, tapetum consisting cells are one-nucleate, but even at the stage of meiosis in the microspore mother cell they become two-nucleate as result of mitotic division (Fig. 3d). The middle layer is degenerated gently till the last stage of the mature pollen development. Its remains are seen long and narrow (Figs. 2g and 3g). Anther wall is forced simultaneously and changed by the pollen grain development. Thus endothecium layer evolves and fiber thickening is clearly observed in their wall (Fig. 3k). Starting the meiosis, the callus (special wall) creates a distinct microscopic image as a thick noticeable layer around the microsporocytes (Fig. 2e) and tetrads (Fig. 2h, k, i). Meiosis division in pollen mother cells first results in two cells (Fig. 2c), and then four haploid cells (tetraspore) (Fig. 2i). Each microspore clearly passes through meiosis stages, i.e., prophase I (Fig. 2b), metaphase I (Fig. 2b), anaphase I (Fig. 2d), and telophase II (Fig. 2g). At the end of meiosis, cytokinesis is done simultaneously and then microspore tetrads are created (Fig. 2i, k). No wall is formed between the cells resulted from telophase II. Tetrads are often tetrahedral (Fig. 2h) while tetragonal are rarely observed (Figs. 2j, i), and eventually, microspores were separated from each other (Fig. 2l). Development of microspores in the neighboring pollen sacs is non-synchronized (Fig. 2f, h). At the releasing time, microspores have regular shape and dense cytoplasm with one central nucleus but without vacuole (Fig. 3a). By developing the central vacuole, the nucleus is pushed towards microspore margin (Fig. 3b). Then the cell is divided and creates two unequal cells (Fig. 3d, e, h), a large vegetative cell that leads to pollen tubule formation (Fig. 3g, i) and a small generative cell that results in two sperms of mature pollen (Fig. 3f). The intine is formed after the callus disappears and young microspores are released (Fig. 3c). Each microspore is surrounded by the scabrate exine (Fig. 3c, j). Pollen grains are spherical and triporate at the mature form (Fig. 3c).

Ovule and embryo sac development

The gynoecium is hypogynous (superior) (Fig. 4a, b), with two carpels (separated with a false septum) (Fig. 4c). Each carpel includes many ovules on the axillar

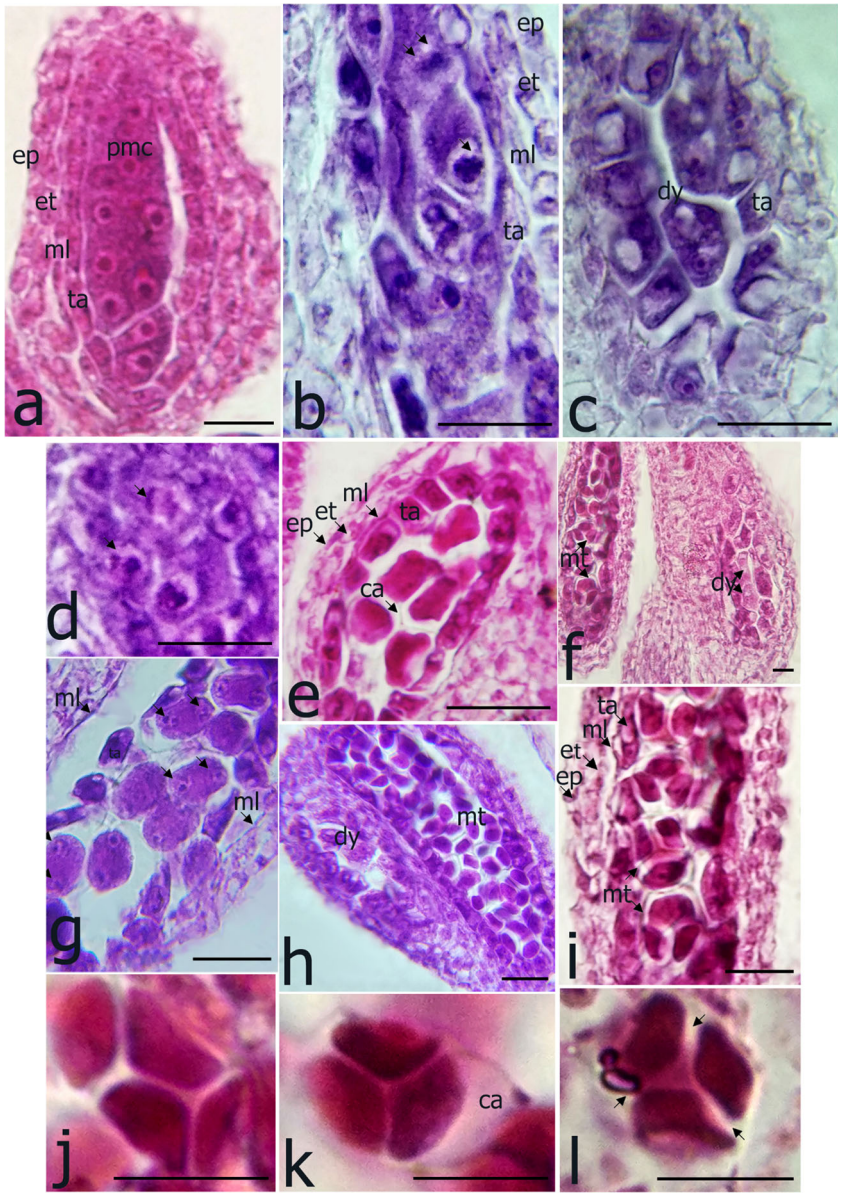

Fig. 2 The pollen grain development; **a** The pollen mother cell within the pollen sac with 4 layers, namely, epidermis, endothecium, middle layer, and tapetum; **b** The initial meiosis stages (prophase I, metaphase I); **c** Dyad cell resulted in PMC; **d** Anaphase I; **e** Microsporocytes surrounded with special wall (callus wall); **f** and **h** Tetraspores and their non-synchronize development; **g** Telophase II; **i** Tetrahedral and tetragonal type; **j** Tetragonal; **K** Tetrahedral; **L** Microspores separation from each other; Ca: callus, dy: dyad, ep: epidermis, et: endothecium, ta: tapetum, pmc: pollen mother cell, mt: microspore tetrads; Scale bars are 50 µm

placenta (Fig. 4a, b). The mature ovule is of amphitropous type (Fig. 4c, l), bitegmic (Fig. 4e) and tenuinucellate (Fig. 4l). At first, an ovule primordium is initiated by periclinal cell division of the ovary wall (Fig. 4d) before development of the style, stigma and ovary superior parts (Fig. 4d). Archespore cells develop in the ovary (Fig. 4g). Archespore cells (with large volume, dense cytoplasm, and voluminous nucleus) are distinguished from the other nucleus cells (Fig. 4g, f). Bitegmic ovule, which is observed clearly when it is rotated (Fig. 4f) during its growth, creates a micropyle narrow gap (falciform-shape) (Fig. 4k). By progressing of ovule bending, the amphitropous type is completely observed at the beginning of embryo sac formation (Fig. 4k). Megaspore mother cell, having voluminous large nucleus (Fig. 4h), enters meiosis division formed dyad (Fig. 4i) and finally produces a T- shaped tetrad (Fig. 4j). Three out of four cells degenerate at meiosis ending and the single cell remained as a functional megaspore forms embryo sac and then undergoes three successive meiotic divisions, that results in two (Fig. 5a, b), four (Fig. 5c, d) and eight-nucleate embryo sacs (Fig. 5e). Thus it consists of 7 cells: one egg cell (oosphere) with spherical, large and remarkable nucleus, two symmetric synergids (with elongated stripe structure) (Fig. 5e), three antipodes, and a central cell with two polar nuclei that most occupied space embryo sac (Fig. 5e). Two central cells migrate towards the egg apparatus, fuse each other, and then result in secondary nucleus (Fig. 5e). The antipodal cells are immediately degenerated before fertilization stage (Fig. 5f), then the

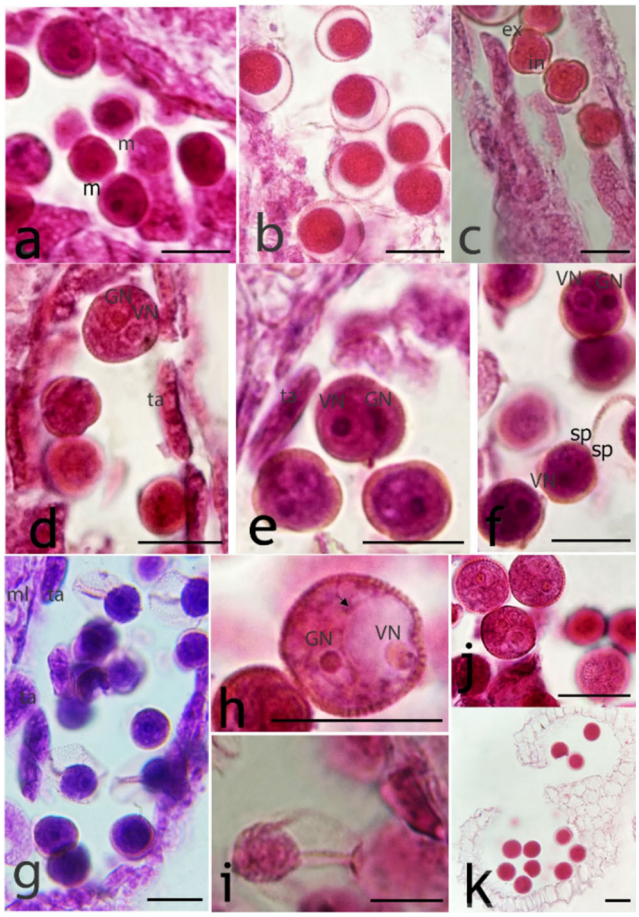

Fig. 3 The mature pollen grain development; **a** Microspores with one central nucleus and secretory tapetum cells; **b** Exine is formed and pollen grains vacuolated considerably; **c** Mature pollen grain with three pores; **d** Binuclear pollen grains (generative & vegetative) and the spindly tapetums, secretory and plasmodial type; **e** Two unequal cells (generative & vegetative) and outer and inner layers of pollen grain; **f** Trinucleus pollen grain (vegetative and 2 sperm); **g** and **i** Growth of pollen tubules from the vegetative cell division; **h** Cytoplasm unequal division in binuclear pollen; **l** Exine pollen grain arrangement; **k** Anther blooming, fibrinous endothecium layer and releasing the mature pollen grains; et: endothecium, ex: exine, in: intine, m: microspore, ml: middle layer, G.N: generative nucleus, sp: sperm, ta: tapetum, V.N: vegetative nucleus, sp: sperm; Scale bars are 50 μm

mature embryo sac is ready for fertilization (Fig. 5h). The embryo sac is surrounded by endothelium cells with distinct nucleus, dense cytoplasm, and high stability and they are present until the embryo is formed (Fig. 5b).

Stages of embryogenesis

Oosphere, is fertilized with one of the pollen tubule sperms and generates a zygote (diploid cell) (Fig. 5h). Combination of the secondary cell with the next sperm establishes a triploid endosperm mother cell (Fig. 5h). It is initially a nucleate endosperm (Fig. 5i, k, l). Subsequently, consecutive division begins in the zygote (Fig. 5i, k, l). Finally, the short suspensor and proembryo are formed (Fig. 6a) the young embryo and nuclear endosperm are created (Fig. 5m). Division acceleration at the proembryo stage is very high, first two cells (Fig. 6b), octant stage (Fig. 6c), and in a short time the globular embryo is produced (Figs. 6f, g and 7a) with several cell columns of suspensor (Fig. 6e). While the basal cells of embryo are concentrated by the narrow root primordium, the cell activities lead to a triangular shape, which is very ephemeral (Figs. 6d and 7b). Cell divisions rapidly expand in apical region and the cotyledons' primordium appear in the lateral location. Therefore, the embryo changes into heart-shaped (Fig. 7c). The shoot promeristem is created between cotyledons' primordium and root promeristem which is connected to the hypophase (Fig. 7c). As the embryo grows, its volume increases, the cotyledons elongate, the endosperm decreases, and eventually, torpedo embryo emerges (Fig. 7d, e). Eventually the expansion of cotyledons, degeneration of suspensor and endosperm, and formation of root and shoot meristems result in the creation of the mature embryo (Fig. 7f, g).

Discussion

We found that in *A. thaliana* stamens appear on the receptacle as forming cellular mass. The anthers were tetrasprangiate as reported previously also by Davis (1962) and with a four-layers wall according to the dicotyledonous-type (Kai-yu et al. 1997), which is similar to the findings reported in *Brassicaceae* (Yurukova-Grancharova et al. 2004). Based on our findings in *A. thaliana*, the anther wall was composed of an epidermal, endothecium, middle layer, and a tapetum. In this plant the epidermis cells were observed rectangular, mononucleated, and developing with outward circle edge. On the other hand, endothecium cells were observed smaller but wider and the middle layer was observed as one layer in the anther. These results are accordance with other reports in *Iberis saxatilis* subsp. *saxatilis*, *Lepidium vesicarium*, *Brassica jordanoffi*) Yurukova-Grancharova et al. 2004; Chehregani and Sedaghat 2009; Yankova-Tsvetkova et al. 2016). The number of middle layers in *Iberis saxatilis* subsp. *saxatilis* (*Brassicaceae*) was reported as 2 rows in some anthers, which is different from *A. thaliana* plants under investigation. Our observations

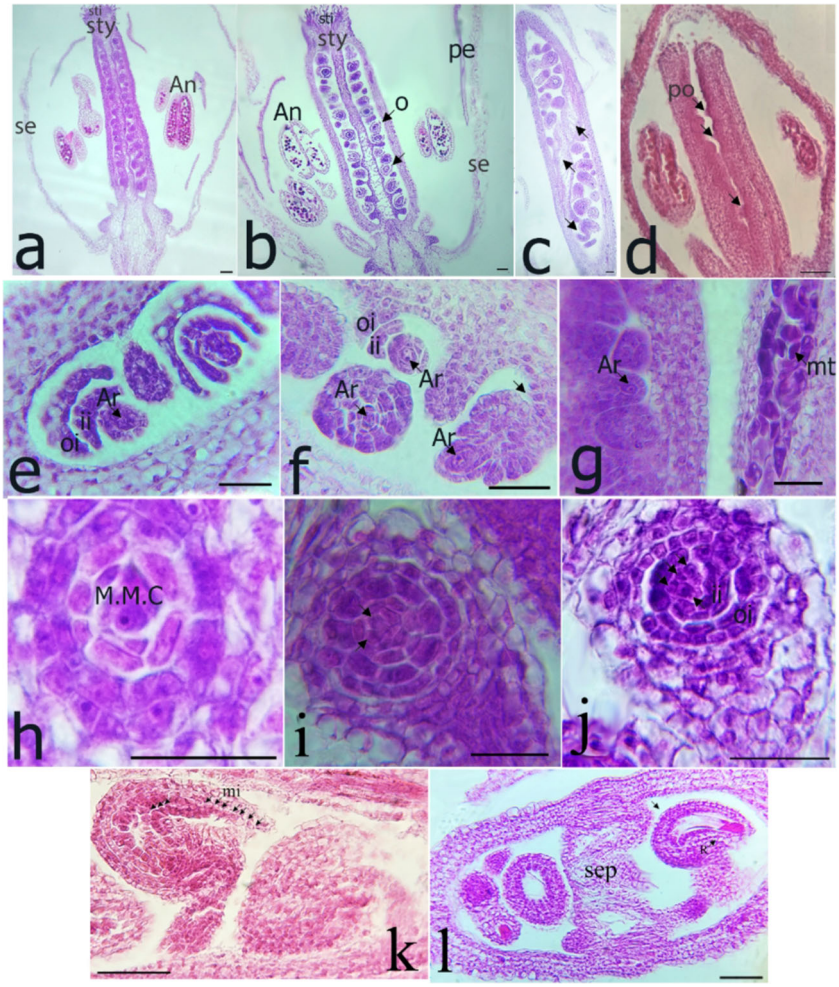

Fig. 4 **a** Superior ovary with 2 carpels and numerous ovules in them; **b** Ovules developing with the false septum, and also blooming of anthers; **c** Ovary with anatropous ovules; **d** Appearance of ovule primordium before style and stigma formation; **e** Bitegmic ovule; **f** Ovule rotation in about 90° and appearance of ovule teguments; **g** finding of ovule archespore cells simultaneously with tetraspores within pollen sac; **h** MMC, **i** The first MMC meiosis (dyad). ovule bending during pollen sac development; **j** The tetrads generated from the secondary meiosis; **k** micropyle duct and the falciform-shaped ovule; **l** Mature ovule is amphitropous; Se: sepal, pe: petal, st: stamen, sty: style, ov: ovule, op: primordium ovule, oi: outer integument, ii: inner integument; Ar-archeospore cell; mt-microspore tetrads; M.M.C-megaspore mother cell; mi-micropylar; Scale bars are 50 µm

were also showed that initially tapetum consisting cells are one-nucleate, but even at the stage of meiosis in the microspore mother cells, they become two-nucleate as result of mitotic division. This finding is agreement with findings of Yankova-Tsvetcova et al. (2016) and similar to several species of *Arabis* genus (Belyaeva and Rodionova 1983). Two basic types of tapetum (the secretory or parietal type and the amoeboid or periplasmodial type(in *A. thaliana* were distinguished in other angiosperms, that is accordance with Pacini et al. (1985). Based on our findings in *A. thaliana*, archespore cells (with dense cytoplasm and prominent nucleus) produced external cells and internal sporogenesis by periclinal division. The pollen sac sporogenous tissue was observed as 2–3 rows with uninuclear rectangular cells. These results are similar to reports of some researchers (Poddubnaya-Arnol'di 1982; Yurukova-Grancharova et al. 2004; Xue and Li 2005) which are multilayered and usually consist of 2–3 layers. Our results indicate that meiosis of microspore mother cells cause to form mostly tetrahedral and rarely tetragonal cells. The presence of tetrahedral cells in *Brassica jordanoffii* (Yankova-Tsvetkova et al. 2016) and tetragonal tetrad cells are similar to the study reported by Chehregani and Sedaghat (2009). In this study, the contiguous sporangia evolution is none-synchronized but differs from the development of pollen sac in *Lepidium vesicarium* as reported by Chehregani and Sedaghat (2009). The special wall (callus) encloses tetrad microspores and is degraded by the activity of callase (complex enzyme) secreted by the tapetum, resulting in individual microspores, this suggests a close collaboration between sporophyte and gametophyte tissues (Chehregani and Sedaghat 2009; Preuss et al. 1994) considers the separation of tetrads into individual microspores to be under sporophyte control. Our results showed that the mature pollen grains include vegetative and generative cells with completely different structures and cell fates at the next stage, that is accordance with Twell et al.

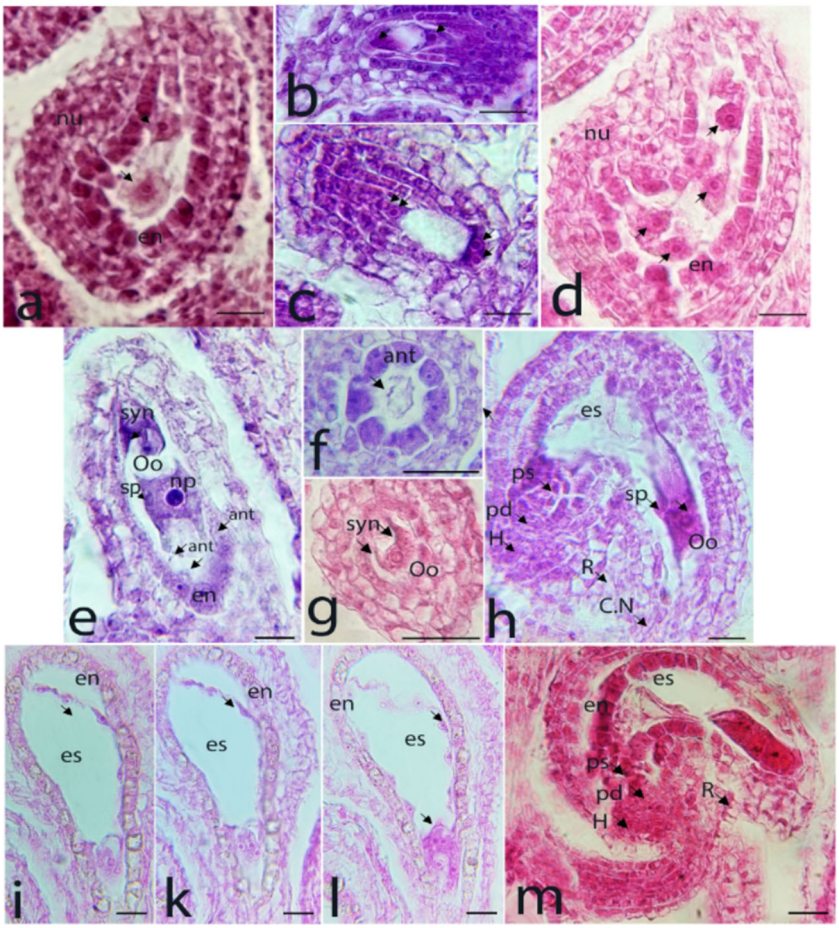

Fig. 5 Embryo sac development; **a** and **b** Binuclear embryo sac; **c** and **d** Four nucleus embryo sac; **e** 7-cell embryo sac, 2 synergids, 1 Oosphere cell, and a central cell with 2 polar nuclei and 3 antipodal degenerated before the fertilization stage; **f** Antipodal cells; **g** One Oosphere and two synergids; **h** Before the formation of zygote; **i**, **k**, and **l** Embryo sac division after fertilization and also endosperm nucleate generated from the endosperm mother cell; **m** Onagrad-type young embryo and nuclear endosperm; mature ovule is amphitropous. EMC: embryo mother cell, en: endothelium, nu-nucleus, syn: synergid, Oo: oosphere, np: nucleus polar, ant: antipodal, R: rafe, es: embryo sac, mi-micropyla, h: hypostasis, pd: podium, ps: postament, CN: cap. nucleus, sp: sperm; Scale bars are 50 µm

(1998). Similar results were represented in some species of the genera *Arabis*, *Bunias*, and *Hesperis* (Belyaeva and Rodionova 1983). At this stage, all anther wall layers are still recognizable. The data differ from those reported by Yankova (2004). Our results were also showed that like in the other studied species, the mature pollen was tricolpate, as a characteristic of the *Brassicaceae* (Chehregani and Sedaghat 2009). We found that in this plant two carpels of ovary were accompanied with numerous amphitropous, bitegmic and tenuinucellate ovules. This finding is agreement with findings of Yankova-Tsvetcova et al. (2016), but against those findings of Chehregani and Sedaghat (2009), ovules were medionucellate. In the *A. thaliana*, the growth of two integuments began simultaneously on the ovule primordium, as were reported by Bouman (1974) in Some Angiosperms, Bouman (1975) in some *Cruciferae* and Shamrov (2002) in *Capsella bursapastoris*. Each of the inner and outer integuments was as two rows cells (Chehregani and Sedaghat 2009).Our findings showed that the archespore cell was observed in the ovule just after tetraspores were formed in the anthers. This was different from the findings reported in *Brassica jordanoffi* (Yankova-Tsvetkova et al. 2016). Our results showed that the archespore cell was located under two layers of nucellus tissue and enclosed completely by the nucellus epidermis layer. Inner and outer integument layers of the ovule were generated from regular cell divisions of the ovular base. Unicellular archesporium was observed in the ovular primordium, but in the genera *Arabis*, *Bunias*, and *Hesperis* two or three-celled archesporium was observed (Belyaeva and Rodionova 1983).

Our findings about this plant showed that the archesporial cell directly differentiated to megaspore mother cell (MMC) during the ovule growth. The MMC was large, uni-nucleated cell with dense cytoplasm that is obviously different from the somatic cells of the ovule. This cell underwent meiosis to arise a T-shape megaspore tetrad that was different from *B. jordanoffi* (Yankova-Tsvetkova et al. 2016) and *Iberis saxatilis* subsp. *saxatilis*

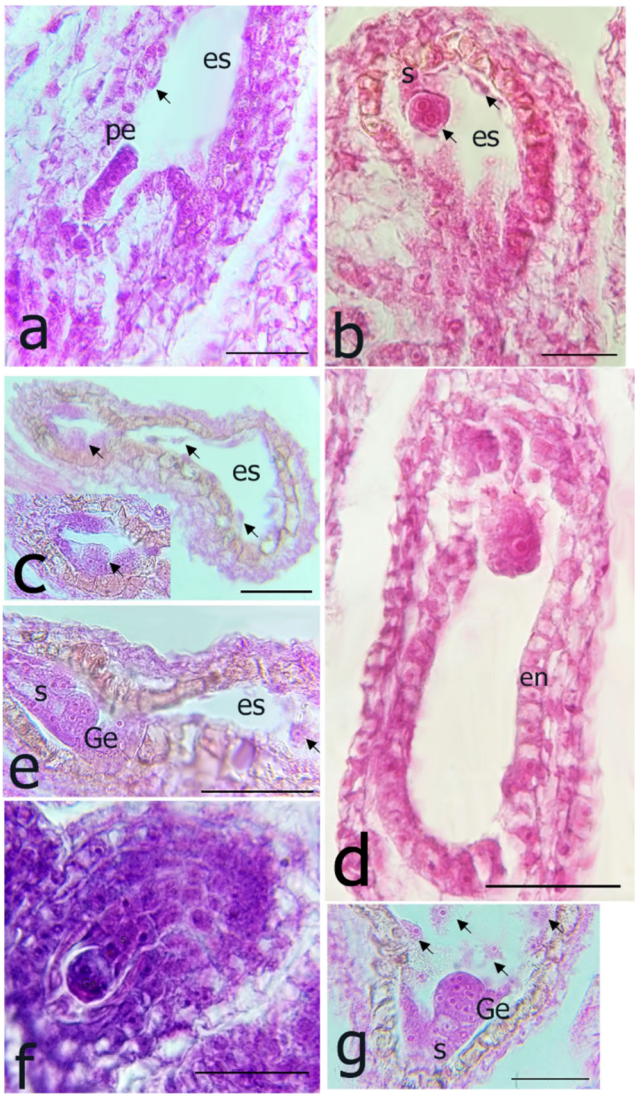

Fig. 6 **a** Proembryo formation; **b** The suspensor caused of basal cell division and the 2-cell embryo derived from apical cell divisions; **c** The 8-cell embryo, nucleate endosperm in the embryo sac; **e**, **f**, and **g** Globular embryo; **d** Triangular embryo with regular endothelium cells; Pe: proembryo, s: suspensor, es: embryo sac, Ge: globular embryo, Te: triangular embryo, en: endothelium; Scale bars are 50 μm

(Yurukova-Grancharova et al. 2004). Our results showed that after that, three daughter megaspores disappeared and subsequently, the functional megaspore entered three successive meiotic divisions. Consequently, an eight-nucleate embryo sac (ES) was formed. The ES development continued as Polygonum (monosporic), which is the distinct type reported both for *Brassicaceae* and most angiosperms (Davis 1966a, b; Poddubnaya-Arnol'di 1982; Belyaeva and Rodionova 1983). We found that, in *A. thaliana* the mature ES contained 7 cells: two synergid cells and one egg cell that, as a whole, are considered the egg apparatus. There was a central secondary nucleus composed of 2 polar free nuclei.

In our investigation of *A. thaliana*, the spherical or ovate egg cell was surrounded by two elongated synergid cells. The triploid endosperm mother cell generated nucleate endosperm till the last stage of globular embryo, then making partition and producing cellular endosperm. The antipodal cells were degenerated quickly at prefertilization stages. This finding was also reported (Jürgens et al. 1991) and is recognizable for the family *Brassicaceae* (Davis 1966a; Poddubnaya-Arnol'di 1982; Belyaeva and Rodionova 1983). These cells do not have any role in fertilization and embryogenesis, but are responsible for nourishing ES (Diboll 1968). Based on our observation the endothelium cells were different from the other cells of ES for their size and regular monotonous shape. They appeared in the binucleate ES form as it was also reported by Ramezani et al. (2018) and Ghimire and Heo (2012) in *Capsicum annuum* L. and *Withania somnifera*, respectively. Based on our findings the cell wall setting direction in proembryo mitoses division indicates that embryogenesis is of the Onagrad type, a typical embryogenesis type for the family *Brassicaceae* that reported previously by some researchers (Davis 1966a; Poddubnaya-Arnol'di 1982; Belyaeva and Rodionova 1983). Observation showed that another aspect of the embryo sac in *A. thaliana* was the existence of hypostasis which is an organized tissue at the base of the nucleus and integument usually formed in chalaza polar after the 2–4 nucleate ES stage as in most *Brassicaceae* taxa (Prasad 1977; Vijayabaghavan and Prabhakar 1981; Shamrov 2002). It has a protective and nutritive role (Sun et al. 2004) and nutrients are transported from vascular bundles through postament and endosperm haustorium to the embryo sac (Rao 1963),

Conclusions

The results showed that, in this plant, the tapetum cells with different size and shape are rectangular, spindly, one-nucleate, and polynuclear. They are initially fixed in their location and are secretory and then plasmodial type at the end of anther development. Meiosis division in pollen mother cells first results in two cells and then four haploid cells (tetraspore). Pollen mother cells release microspores from the tetrads. The most tetrads are tetragonal and the less ones are tetrahedral. The second divisions in all sections occurred only after a wall formed between the microspores. The microspores soon

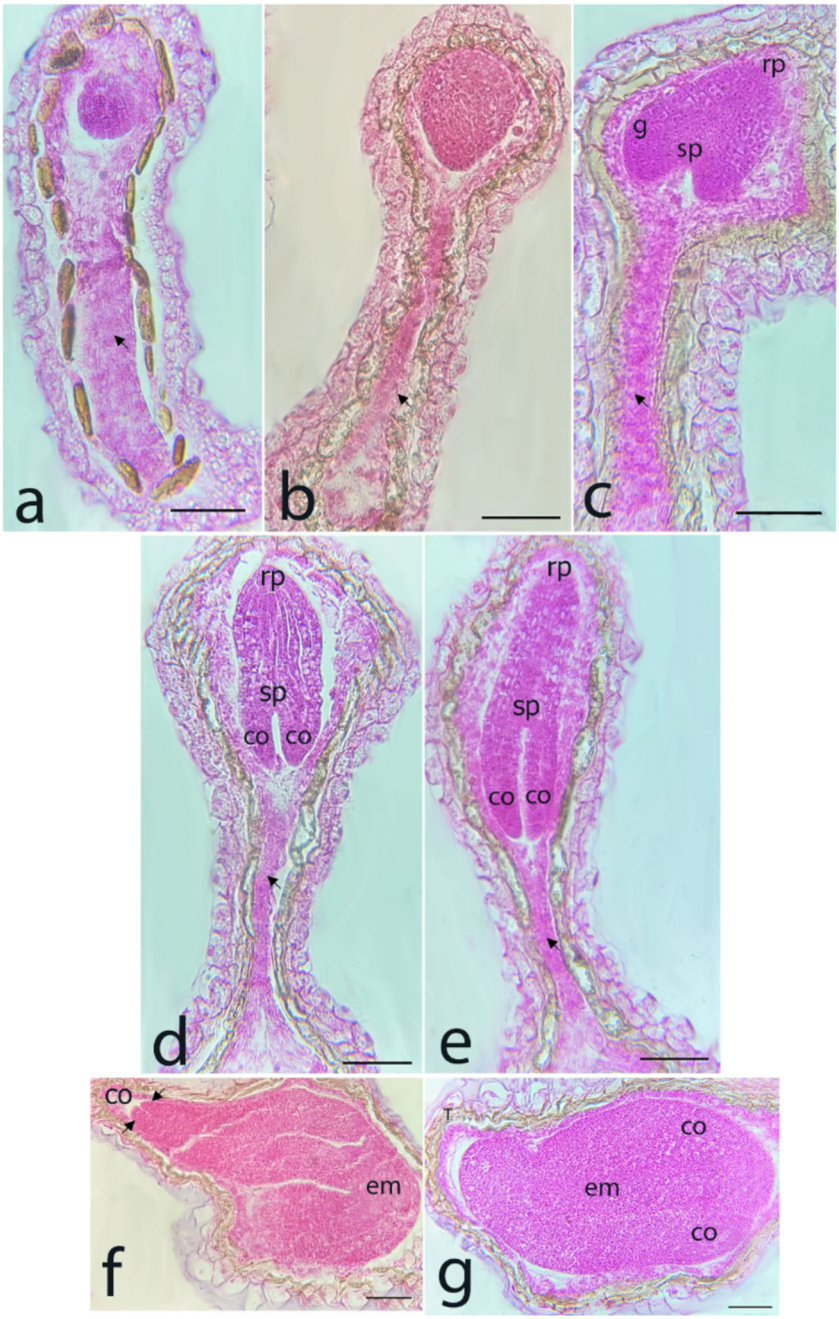

Fig. 7 Embryogenesis; **a** Globular embryo and appearance cellular endosperm; **b** Triangular embryo; **c** Heart embryo; **d** and **e**. Torpedo embryo formation; **f** and **g**. Embryo sac occupied by mature embryo; gr: primordium of ground tissue, rp: root primordium, sp: shoot primordium, co: cotyledonary, em: embryo, T: Teste; Scale bars are 50 μm

separated from each other and were released from the tetrads as free microspores. Pollen mature grains are spherical and triporate. The gynoecium is hypogynous (superior, with two carpels separated with a false septum). The mature ovule is of amphitropous type, bitegmic and tenuinucellate. The embryo sac is Polygonum type and it is curved. The antipodal cells are immediately degenerated before fertilization. Oosphere is fertilized with one of the pollen tubule sperms and generates a zygote. Combination of the secondary cell with the next sperm establishes a triploid endosperm mother cell.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Aghajafari M, Behboodi BS, Pirayesh S (2013) Study on the morphology of genus *Arabidopsis* in Iran. Am J Plant Sci 2013. https://doi.org/10.4236/ajps.2013.412A1004

Al-shehbaz I (2003) Transfer of most North American species of Arabis to Boechera (*Brassicaceae*). Missouri Botanical Garden Press 13, No. 4, Winter, 2003, pp 381–391. https://doi.org/10.2307/3393366

Belyaeva L, Rodionova G (1983) Family *Brassicaceae* comparative embryology of flowering plants. Phytolaccaceae—Thymelaeceae 3: 154–164

Bouman F (1974) Developmental studies of the ovule, integuments, and seed in some angiosperms. Thesis University Amsterdam

Bouman F (1975) Integument initiation and testa development in some *Cruciferae.* Bot J Linn Soc 70:213–229. https://doi.org/10.1111/j.1095-8339.1975.tb01646

Chehregani A, Mahanfar N (2007) Achene micro-morphology of Anthemis (*Asteraceae*) and its allies in Iran with emphasis on systematics International. J Agric Biol 9:486–488

Chehregani A, Sedaghat M (2009) Pollen grain and ovule development in Lepidium vesicarium (*Brassicaceae*). Int J Agric Biol 11:1560–8530

Chehregani A, Tanomi N (2010) Ovule ontogenesis and megagametophyte development in Onobrychis schahuensis Bornm.(*Fabaceae*). Turk J Bot 34:241–248. https://doi.org/10.3906/bot-0908-168

Davis GL (1962) Embryological studies in the compositae, I. sporogenesis, gametogenesis, and embryogeny in Cotula australis (less.) hook F. Aust J Bot 10:1–12. https://doi.org/10.1071/BT9620001

Davis G (1966) Systematic embryology f the angiosperms. Willey, Hoboken

Davis G (1966) Systematic embryology of angiosperms. Wiley, New York

Diboll AG (1968) Fine structural development of the megagametophyte of Zea mays following fertilization American. J Bot 55:787–806. https://doi.org/10.2307/2440968

Ghimire B, Heo K (2012) Embryology of Withania somnifera (L.) dunal (*Solanaceae*). Acta Biol Cracov Ser Bot 54:69–78

Johansson M, Walles B (1993) Functional anatomy of the ovule in broad bean (Vicia faba L.). I. Histogenesis prior to and after pollination International. J Plant Sci 154:80–89

Johri B, Ambegaokar K, Srivastava P (1992) Comparative embryology of angiosperms, vol. 1. Berlin, Heidelberg Springer Berlin Heidelberg

Jürgens G, Mayer U, Berleth T, Miséra S (1991) Genetic analysis of pattern formation in the Arabidopsis embryo. Development 113: 27–38

Kai-yu P, Jie W, Shi-liang Z (1997) Embryological study on Mosla chinensis (*Lamiaceae*). Acta Bot Sin 39:111–116

Kasahara RD, Maruyama D, Hamamura Y, Sakakibara T, Twell D, Higashiyama T (2012) Fertilization recovery after defective sperm cell release in Arabidopsis. Curr Biol 22:1084–1089. https://doi.org/10.1016/j.cub.2012.03.069

Khan R (2004) Studies on the pollen morphology of the genus *Arabidopsis* (*Brassicaceae*) from Pakistan . Pak J Bot 36:229–234

Koch MA (2019) The plant model system *Arabidopsis* set in an evolutionary, systematic, and spatio-temporal context. J Exp Bot 70:55–67. https://doi.org/10.1093/jxb/ery340

Koch MA, Matschinger M (2007) Evolution and genetic differentiation among relatives of *Arabidopsis thaliana*. Proc Natl Acad Sci 104: 6272–6277. https://doi.org/10.1073/pnas.0701338104

Koch M, Haubold B, Mitchell-Olds T (2001) Molecular systematics of the *Brassicaceae*: evidence from coding plastidic matK and nuclear Chs sequences. Am J Bot 88:534–544

O'Kane SL Jr, Al-Shehbaz IA (1997) A synopsis of *Arabidopsis* (*Brassicaceae*). Novon :323–327. https://doi.org/10.2307/3391949

Pacini E, Franchi G, Hesse M (1985) The tapetum: Its form, function, and possible phylogeny inEmbryophyta. Plant Syst Evol 149:155–185

Page DR, Grossniklaus U (2002) The art and design of genetic screens: *Arabidopsis thaliana*. Nat Rev Genet 3:124–136. https://doi.org/10.1038/nrg730

Poddubnaya-Arnol'di VA (1982) Characteristics of angiosperm families in relation to cytoembryological characters. Nauka, Moscow

Prakash N (1987) Embryology of the leguminosae. In: Stirton CH (ed) Advances in legume systematics, 241–278, Part 3. Royal Botanical Gardens, Kew, England

Prasad K (1977) The development and structure of basal body in the ovule and seed of certain species of Cruciferae. Bot Jahrb Syst 98: 266–272

Preuss D, Rhee SY, Davis RW (1994) Tetrad analysis possible in *Arabidopsis* with mutation of the QUARTET (QRT) genes. Science 264:1458–1460. https://doi.org/10.1126/science.8197459

Ramezani H, Chehregani Rad A, Karamian R (2018) Development of the male and female gametophyte in *Capsicum annuum* L. var. California Wonder . Plant Biosyst 152:1172–1181. https://doi.org/10.1080/11263504.2018.1435568

Rao CV (1963) Studies in the *Proteaceae*. 3. Tribe Oriteae Proceedings of the National Institute of Sciences of India. Calcutta 29:489–510

Rédei GP (1975) *Arabidopsis* as a genetic tool Annual review of genetics 9:111–127. https://doi.org/10.1146/annurev.ge.09.120175.000551

Rembert JDH (1969) Comparative megasporogenesis in *Caesalpiniaceae*. Bot Gaz 130:47–52

Rembert JDH (1969) Comparative megasporogenesis in *Papilionaceae*. Am J Bot 56:584–591

Rembert JDH (1971) Phylogenetic significance of megaspore tetrad patterns in Leguminales. Phytomorphology 21:317–416

Rembert Junior DH (1977) Contribution to ovule ontogeny in Glycine max. Phytomorphology 27:368–370

Rollins RC (1941) Monographic study of Arabis in western North America Contributions from the Gray Herbarium of Harvard University:289–485. https://digitalcommons.usu.edu/etd

Scott RJ, Spielman M, Bailey J, Dickinson HG (1998) Parent-of-origin effects on seed development in *Arabidopsis thaliana*. Development 125:3329–3341

Shamrov I (2002) Ovule and seed morphogenesis in Capsella bursapastoris (*Brassicaceae*) in connection with peculiar mode of endothelium formation . Botanicheskij Zhurnal (St Petersburg) 87: 1–18

Somssich M (2018) A short history of *Arabidopsis thaliana* (L.) Heynh. Columbia-0 (No. e26931v2). PeerJ Preprints, Australia. https://doi.org/10.7287/peerj.preprints.26931v3

Sun K, Hunt K, Hauser BA (2004) Ovule abortion in *Arabidopsis* triggered by stress. Plant Physiol 135:2358–2367. https://doi.org/10.1104/pp.104.043091

Twell D, Park SK, Lalanne E (1998) Asymmetric division and cell-fate determination in developing pollen. Trends Plant Sci 3:305–310. https://doi.org/10.1016/S1360-1385(98)01277-1

Vaughan JG, Whitehouse JM (1971) Seed structure and the taxonomy of the *Cruciferae.* Bot J Linn Soc 64:383–409. https://doi.org/10.1111/j.1095-8339.1971.tb02153

Vijayabaghavan M, Prabhakar K (1981) Ontogenetical and histochemical studies on chalazal proliferating tissue in Iberis amara and Alyssum maritimum. Beitrage zur Biologie der Pflanzen 56(1):7–17

Xue C-Y, Li D-Z (2005) Embryology of Megacodon stylophorus and Veratrilla baillonii (*Gentianaceae*): descriptions and systematic implications. Bot J Linn Soc 147:317–331

Yadegari R, Drews GN (2004) Female gametophyte development. Plant Cell 16:S133–S141. https://doi.org/10.1105/tpc.018192

Yankova E (2004) Comparative-embryological study on the bulgarian representatives of grnus Angelica L. (Apiaceae) and revealing the possibility of their in vitro cultivation. Phd Thesis. Sofia(1) (PDF) On the reproductive biology of Angelica archangelica L.

(Apiaceae). https://www.researchgate.net/publication/275040581_On_the_reproductive_biology_of_Angelica_archangelica_L_Apiaceae. Accessed 03 Feb 2021

Yankova-Tsvetkova E, Yurukova-Grancharova P, Vladimirov V (2016) On the embryology of Brassica jordanoffii (*Brassicaceae*)–an endemic species in the Bulgarian flora Phytologia Balcanica. Int J Balkan Flora Veg 22:149–153

Yeung EC (1998) A beginner's guide to the study of plant structure. In: Karcher SJ (ed) Tested studies for laboratory teaching, Vol. 19. Proceedings of the 19th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), pp, 125–141. Purdue University, Lafayette, Indiana. https://doi.org/10.1007/1-4020-2985-3_41

Yeung E, Cavey M (1990) Developmental changes in the inner epidermis of the bean seed coat. Protoplasma 154:45–52. https://doi.org/10.1006/anbo.1994.1114

Yurukova-Grancharova P, Anchev M, Goranova V (2004) Embryological study on diploid and triploid populations in Iberis saxatilis subsp. saxatilis (*Brassicaceae*) in Bulgarian flora. Phytologia Balcan 10(1):69–78